Guidance on the application of Commission Recommendation 2013/165/EU on the presence of T-2 and HT-2 toxin in cereals and cereal products

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Introduction

The Scientific Panel on Contaminants of the European Food Safety Authority (EFSA) adopted an opinion[1] on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. In view of the conclusions of the scientific opinion and the large year to year variation in occurrence of T-2 and HT-2 toxin, Commission Recommendation 2013/165/EU[2] Member States should, with the active involvement of feed and food business operators, perform monitoring for the presence of T-2 and HT-2 toxin in cereals and cereal products and more information on the effects of food processing and agronomic factors on the presence of T-2 and HT-2 toxin. Furthermore, information on the different factors which lead to relative high levels of T-2 and HT-2 toxin in cereals and cereal products should be collected to help identify potential measures to avoid or reduce the presence of the toxin. In 2015 this information will be assessed.

This Guidance has been developed by the UK Food Standards Agency and UK stakeholders.

Scope and purpose of Guidance

The purpose of this Guidance Note is to provide information and advice to Member State authorities and food and feed business operators to support uniform application of the Recommendation and comparable reporting of the results of investigations. This document provides guidance on the two main requirements of the Recommendation, namely monitoring of T-2 and HT-2 toxin in cereal and cereal products and investigations where repetitive findings above the Indicative level have been found.

The indicative levels detailed in the Annex to the Recommendation are not feed or food safety levels, nor are they maximum or enforcement limits. They are intended to provide orientation on where to focus investigations. The aim of the Recommendation is to collect information that will ultimately support the assessment of trends in levels and exposure to T-2 and HT-2 toxin, and that may contribute to the understanding of the factors affecting levels.

In many cases it may be difficult to establish the agronomic factors unless specific testing is carried out at the farm or at bulk store intake (i.e. at a stage where the lot of grain is directly and uniquely traceable to the farm where grown). Therefore, it may be appropriate for

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[1] http://www.efsa.europa.eu/en/efsajournal/pub/2481.htm

[2] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:091:0012:0015:EN:PDF

Member States to develop, continue or expand specific research or independent studies on agronomic factors.

In 2015 an assessment of the information gathered under the framework of the Recommendation will be undertaken. The monitoring data obtained is intended to enable a better understanding of the year–to-year variance and the presence of T-2 and HT-2 toxin in the wide range of cereal products, the factors resulting in higher levels and the measures which could be taken to prevent the presence or mitigate the presence of T-2 and HT-2 toxin through agronomic factors and/or through processing.

Definitions

For the purposes of the Guidance, the following definitions apply:

Agronomic factors -	variable factors at the farm including, but not limited to, variety, previous crop, soil type and storage which may affect production of T-2 and HT-2 toxin by *Fusarium* spp.
Crop year -	the year in which the grain was harvested.
indicative level -	levels as detailed in the Annex to Commission Recommendation 2013/165/EU. For the purpose of this Guidance it is unnecessary to apply measurement uncertainty to the indicative levels (i.e. measurement uncertainty should not be applied).
Investigations -	investigations to identify the possible causes of repetitive findings above the indicative level.
Processing factors -	physical and / or chemical processes (such as baking, extrusion etc.) that occur during normal manufacture of feed or food products, and which may either concentrate or reduce levels of T-2 and HT-2 toxin.
Repetitive findings -	results from more than one batch (as defined by the Food / Feed Business Operator [FFBO]) of the same product that exceed the indicative level.

Sampling and Analysis - General Note

This Guidance recognises that there are a significant number of existing monitoring schemes, principally aimed at establishing crop year-to-crop year variations in T-2 and HT-2 toxins, coordinated by Trade Associations and Non-Governmental Organisations (TA/NGO Schemes). The sampling and analytical techniques utilised within these schemes do not necessarily produce individual results that are fully in accordance with EFSA mandatory requirements. However, the value of these schemes lies in the significant quantity of data that is gathered - this quantity of data offsets many of the concerns relating to sampling / analytical techniques. The continuation of these schemes is encouraged.

On the other hand, sampling and analysis carried out according to Commission Regulations (EC) No. 401/2006 (food) and (EC) No.152/2009 (feed) ensure that the results are representative and robust.

Both kinds of sampling are of value; the basic sampling schemes provide a substantial quantity of data which would enable Competent Authorities and the European Commission

in making assessments of T-2 and HT-2 toxin prevalence. The refined and representative data from sampling according to legislation is important for the EFSA database and the confidence in the quality of data would be useful in drawing comprehensive conclusions on T-2 and HT-2 toxin levels.

Sampling

The Recommendation requires sampling and analysis to be either:

- Carried out in accordance with Commission Regulation No. 401/2006 for food and Commission Regulation (EC) No.152/2009 for feed;
- Food and/or feed business operators may deviate from these requirements (as appropriate) provided sampling is representative of the lot. For example,
    - Sampling that follows a recognised national plan. A non-exhaustive list of such schemes is given in Appendix 4;
    - Sampling that is carried out as part of a TA/NGO scheme monitoring plan;
    - Sampling of consumer products at retail point-of-sale. These typically will be individual samples taken at random because, given the nature of cereal milling operations and supply chain logistics, they would be regarded as being "sufficiently representative" for the purpose of this Guidance.

Analysis - General Requirements

General Analysis Requirements and Definitions are given in Commission Regulation (EC) No 401/2006. For specific requirements refer to recitals (3) and (4) from Recommendation 2013/165/EU.

Methods of Analysis used should comply with the provisions of items 1 and 2 of Annex III to Commission Regulation (EC) No 882/2004, in as far as possible any laboratory used should be accredited to ISO 17025. However lack of accreditation should not be a barrier to producing and submitting data (especially in the case of TA/NGO monitoring schemes) providing minimum quality parameters and method performance criteria are met. Where possible, fully quantitative methods that allow determination of individual T-2 and HT-2 toxin should be used. However, screening methods that produce a result as the sum of T-2 and HT-2 toxin may be used provided any exceedance of an indicative level is verified by a confirmatory method.

Samples should, wherever possible, also be tested for deoxynivalenol and zearalenone (note: maize samples should additionally be tested for fumonisin B_1 and B_2) - this is to enable analysis of the co-occurrence of these *Fusarium* toxins with T-2 and HT-2 toxin.

Where analytical methods permit, samples should additionally be tested for masked mycotoxins, in particular the mono- and di-glycosylated conjugates of T-2 and HT-2 toxins.

Method Validation

Any method used should preferably be accredited to ISO17025 but this is not an absolute requirement.

Methods must be at least in-house validated to a recognised procedure or protocol, e.g. IUPAC Harmonised guidelines for single-laboratory validation of methods of analysis, or other similar suitable protocol. References[3],[4],[5],[6]given are examples and are not an exhaustive list. For the purpose of this Guidance, measurement uncertainty need not be considered.

Performance Criteria (From Regulation (EC) No 401/2006)

Performance criteria from Regulation (EC) No 401/2006 are applicable for deoxynivalenol, zearalenone and fumonisins B_1 and B_2. The performance criteria for T-2 and HT-2 toxin in Regulation No 401/2006 do not currently cover the range of levels for T-2 and HT-2 toxin stipulated in Recommendation 2013/165. Regulation No 401/2006 is currently under revision and performance criteria for these toxins in a suitable range are being established[7]. A table of suggested values is given below (Table 01).

Table 01: Acceptable Performance Criteria for T-2 and HT-2 toxin

Level µg/kg	T-2 and HT-2 toxin		
	RSD_r %	RSD_R %	Recovery %
15 - 250	≤ 40	≤ 60	60 to 130
> 250	≤ 30	≤ 50	60 to 130

Notes to the performance criteria for the mycotoxins

The detection limits of the methods (LOD/LOQ) of methods used for deoxynivalenol,

[3] Thompson, M., Ellison, S.L.R and Wood, R., 2002. Harmonised guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Appl. Chem., 74, 5, 835-855.

[4] Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC)

[5] Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed Document N° SANCO/12495/2011

[6] CEN Technical Report Food Analysis – Performance criteria for single laboratory validated methods of analysis for the determination of mycotoxins. NPR-CEN/TR 16059:2010.

[7] SANCO-10556-2013 Version 3, Draft COMMISSION REGULATION amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots and spices, performance criteria for T-2 and HT-2 toxin and screening methods of analysis

zearalenone and fumonisins B_1 and B_2 are not stated as the precision values are given at the concentrations of interest.

- The precision values are calculated from the Horwitz equation([8]), i.e.:

$$RSD_R = 2^{(1-0.5\log C)}$$

where:

-- RSD_R is the relative standard deviation calculated from results generated under reproducibility conditions [(s_R / $\bar{x}$) x 100]

-- C is the concentration ratio (i.e. 1 = 100g/100g, 0.001 = 1000 mg/kg)

This is a generalised precision equation which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

Recommendation 2013/165/EU sets out maximum target limits of quantification for T-2 and HT-2 toxin. These are summarised in Table 02.

[8] *M. Thompson, Analyst, 2000, 125, 385-386.*

Table 02: Maximum Limits of Quantification (from Commission Recommendation 2013/165/EU)

	Target Limit of Quantification‡ (LOQ) µg/kg		
Product / analysis method	T-2 toxin	HT-2 toxin	Sum T-2 and HT-2 toxin
Cereals, cereal based foods. Quantitative or confirmatory method	5	5	10
Unprocessed cereals. Quantitative or confirmatory method	10	10	20
All cereal foods – screening method	-*	-*	25
Cereals and cereal products intended for feed and compound feed. Quantitative or confirmatory method	10	10	20
Cereals and cereal products intended for feed and compound feed - screening method	-*	-*	25

‡Analytical techniques with an LOQ as detailed in the recommendation are not currently widely available and this should not be a barrier to testing or collection of data. Therefore it may be appropriate, based on appropriate assessment and validation, to use accredited techniques with a higher LOQ.

**It is accepted that some screening methods may not be capable of measuring T-2 and HT-2 toxin individually, but any method should be able to determine both toxins if they are present. In this case the target LOQ applies to the sum of T-2 and HT-2 toxin and not either/or, i.e. a method that measures only T-2 or HT-2 toxin is not applicable.*

Screening interpretation

Screening techniques may be used to ensure that a range of data is collected. However screening methods may only be used if they are validated. Any results exceeding the indicative level should be verified by a confirmatory analysis.

Where possible screening methods should meet the target LOQs in recitals (3) and (4) of Recommendation 2013/165/EU and as outlined in Table 02 above.

Screening methods can be interpreted to be any method that may not result in a fully quantitative or confirmed result. Examples of screening methods may include, but not be limited to:

- Commercial test kits – e.g. ELISAs, Lateral Flow Devices or other rapid formats.
- Instrumental methods designed for rapid throughput – e.g. LC-MS/MS methods with single point calibration

In the case of test kits, any result greater than the indicative level should be confirmed by an alternative confirmatory method. In the case of instrumental screening, confirmation may be carried out using the same technique, provided a fully quantitative measurement is made.

Data Collection

Data on levels of T-2 and HT-2 toxin in product categories detailed within the Annex to the Recommendation may be collected by Member States with the active involvement of Feed and Food Business Operators.

Member States should identify the responsibilities for sampling, testing and reporting in accordance with the categories detailed within the Annex to the Recommendation in collaboration with Feed and Food Business Operators and / or relevant sector stakeholders (including Trade Associations and Non-Governmental Organisations). The responsibilities and level of data collection, along with data submission requirements, should be reviewed and agreed per crop year to ensure data are representative. Where relevant, existing TA/NGO monitoring plans may be included – typically, data from these schemes will be submitted annually. Appropriate consideration must be given that where TA/NGO monitoring schemes operate on a European level (i.e. across Member States) that, where appropriate, data are only submitted once (i.e. not at both European and National levels).

Data should be submitted to the Member State national authorities according to the agreed reporting schedule using an agreed template (see Appendix 2 for an example).

Note on the Annex to the Recommendation

The Annex to the Recommendation is not completely clear with regard to the categorisation of oats and oat products. To ensure a consistent approach across Member States the following categorisation is suggested:

	Category	**Description**
1.	**Unprocessed cereals**	
1.2	Oats (with husk)	Oats with husk
2.	**Cereal grains for direct human consumption**	
2.1	Oats	Oat groats (i.e. oats without husk)
3.	**Cereal products for human consumption**	
3.1	Oat bran and flaked oats	Oat bran, flaked oats, whole rolled oats, wholegrain oatmeal, wholegrain oat flour
3.2	Cereal bran except oat bran, oat milling products other than oat bran and flaked oats and maize milling products	Oatflour with bran removed
4.	**Cereal products for feed and compound feed**	
4.1	Oat milling products (husks)	Animal feed: oat milling products (husks)

Repetitive Findings

Where any individual result exceeds the relevant indicative level, further samples should be tested. Depending on the nature of the product (for example - raw cereal grain, processed cereal or retail sample), further samples must be taken from different lots representing different production batches of the same product (unless this has already been done as part of the original sampling programme, e.g. in the case of TA/NGO monitoring schemes, and no additional results exceeding the indicative level were identified).

Whilst the appropriate level of additional testing will be dependent on a number of factors (such as the number of batches available, frequency of production batches, etc.) typically a minimum of four additional samples would be expected. The responsibility for additional testing would normally rest with whoever carried out the initial tests.

If none of the additional samples exceeds the Indicative level then no further investigation is required, and the data may simply be added to the overall data submission.

If any one of the further samples exceeds the Indicative level this would be deemed a ‘Repetitive Finding’. An investigation should be carried out by the FFBO and the results

from the further testing should be shared with the Member State national authorities (see below).

Investigations

In the case of Repetitive Findings, an investigation should be carried out as soon as is practicable. As part of the investigation it may also be appropriate to study similar products with levels significantly lower than the indicative level - this may assist in identifying approaches to reduce or avoid the presence of T-2 and HT-2 toxin.

Investigations may be carried out either by the Member State, by Feed and Food Business Operators, or by both in partnership. Where investigations are carried out by Feed and Food Business Operators the results of the investigation shall be shared with Member States.

The investigation should seek to identify possible factors resulting in repetitive exceedance of the indicative level. The results of the investigation may be documented following an agreed checklist (see Appendix 3 for an example).

Data submission

A database has been developed that will allow Food and Feed Business Operators to submit data on T-2 and HT-2 toxin occurrence. As referred to in the General Note on Sampling and Analysis, there are two types of data that are collected. Information from rapid, basic methods of analysis which generate extensive data is useful to Competent Authorities and the European Commission. These should be submitted to the Member State using an agreed system. The data from sampling carried out in compliance to legislation is to be submitted to the EFSA database. The specifications for this database can be found on the EFSA website.[9]

Review of Data / Investigations

Data

At the end of each crop year the Member State national authorities, together with relevant stakeholders, should review the data collected and assess where there may be gaps or areas that require more emphasis in following years.

Investigations

On an annual basis (as a minimum), there should be a review of all investigations per crop year to identify any consistent factors or trends. Should any consistent factors be identified this may warrant:

[9] http://www.efsa.europa.eu/en/datex/datexsubmitdata.htm

- Further monitoring over subsequent crop years (including additional monitoring in other Member States where relevant)
- Detailed research to identify and quantify the impact of an agronomic factor or food process on the level of T-2 and HT-2 in cereals and cereal products

Further Information

Further information is available at the following links:

UK Stakeholders

Food Standards Agency www.food.gov.uk

Association of Cereal Food Manufacturers http://www.fdf.org.uk/fullmembers_acfm.aspx

Agricultural Industries Confederation http://www.agindustries.org.uk/home/

British Oat and Barley Millers' Association http://www.fdf.org.uk/fullmembers_bobma.aspx

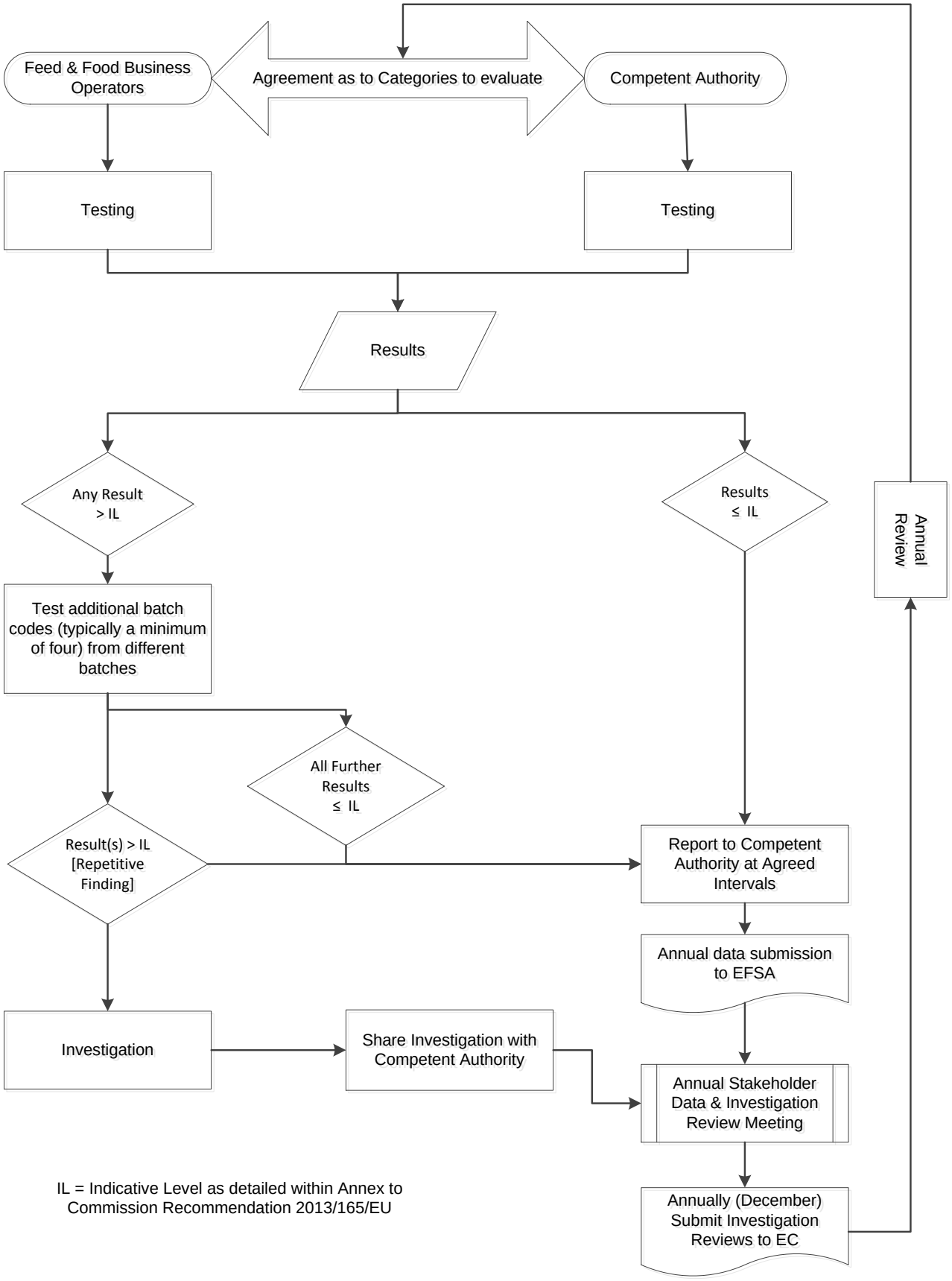
CEEREAL,The European Breakfast Cereal Association
http://www.ceereal.eu/asp2/welcome.asp

Food and Drink Federation http://www.fdf.org.uk/

Malsters' Association of Great Britain http://www.ukmalt.com/

Harper Adams University http://www.harper-adams.ac.uk/

Appendix 1 - Example Flow Chart Illustrating Data Collection & Investigation Rationale/Processes



IL = Indicative Level as detailed within Annex to Commission Recommendation 2013/165/EU

Appendix 2 – Information to collect for data submitted to the Commission, rather than EFSA - predominantly from industry testing plans/ regimes.

Information to collect; In order to ensure maximum benefit from data submissions it is recommended that the following data be recorded.

- Product/ Crop type
- Intended Use (eg. food, feed)
- Crop Year
- Region of Production (if available)
- Sampling Plan used
- Mycotoxins tested for
- Analysis details/ type of test kit used
- Results; including units and the result is expressed in a dry weight matter basis

Appendix 3 - Investigation summary checklist

Areas to be addressed	Guidance
1. Identify the product	Depending on the point in the chain this may be field number, storage bin, batch or lot number
2. Identify intended use	Is the product concerned intended for use in the food or feed chains. Is the intended market a specific sub-sector eg. infant food, ruminant or non- ruminant feed
3. Identify processes carried out	Is the product unprocessed grain or has it been subject to processing (not including drying and cleaning). Include details of processing time, temperature
4. Detail raw material information	What percentage of the product in stock has high levels, is this isolated to a particular raw material – if so then identify the supply chain including (where possible) country of origin and crop year.
5. Detail storage	Record details of storage including (where available) length of storage, temperature and moisture levels of product in store, relative humidity levels.
6. Record other relevant quality parameters	Depending on the position in the chain, including level of processing, detail other relevant quality information (eg. fibre, pH, protein etc.)
7. Detail available agronomic detail	Record available information on variety, previous cropping, region of production, year of production, details of crop establishment and crop management

Appendix 4 - Non-Exhaustive list of recognised national sampling schemes

ISO 24333:2009 Cereals and cereal products - sampling

HGCA *grain sampling worksheet*
(http://www.hgca.com/document.aspx?fn=load&media_id=6144&publicationId=7908)

GRAIN SAMPLING AND ASSESSMENT: SAMPLING GRAIN IN LORRIES (HGCA Project Report)
(http://www.hgca.com/publications/documents/cropresearch/339_complete_final_report.pdf)