TRAINING MANUAL

- PLANTS HEALTH -

CONTROL ORGANIZATION AND RESIDUES ANALYSIS IN PLANT PRODUCTS

COLEACP

This training manual was produced and designed by the Training, Information and Communication services of COLEACP. This publication was written by Steve Homer, Morag Webb and Nursel Gumusboga.

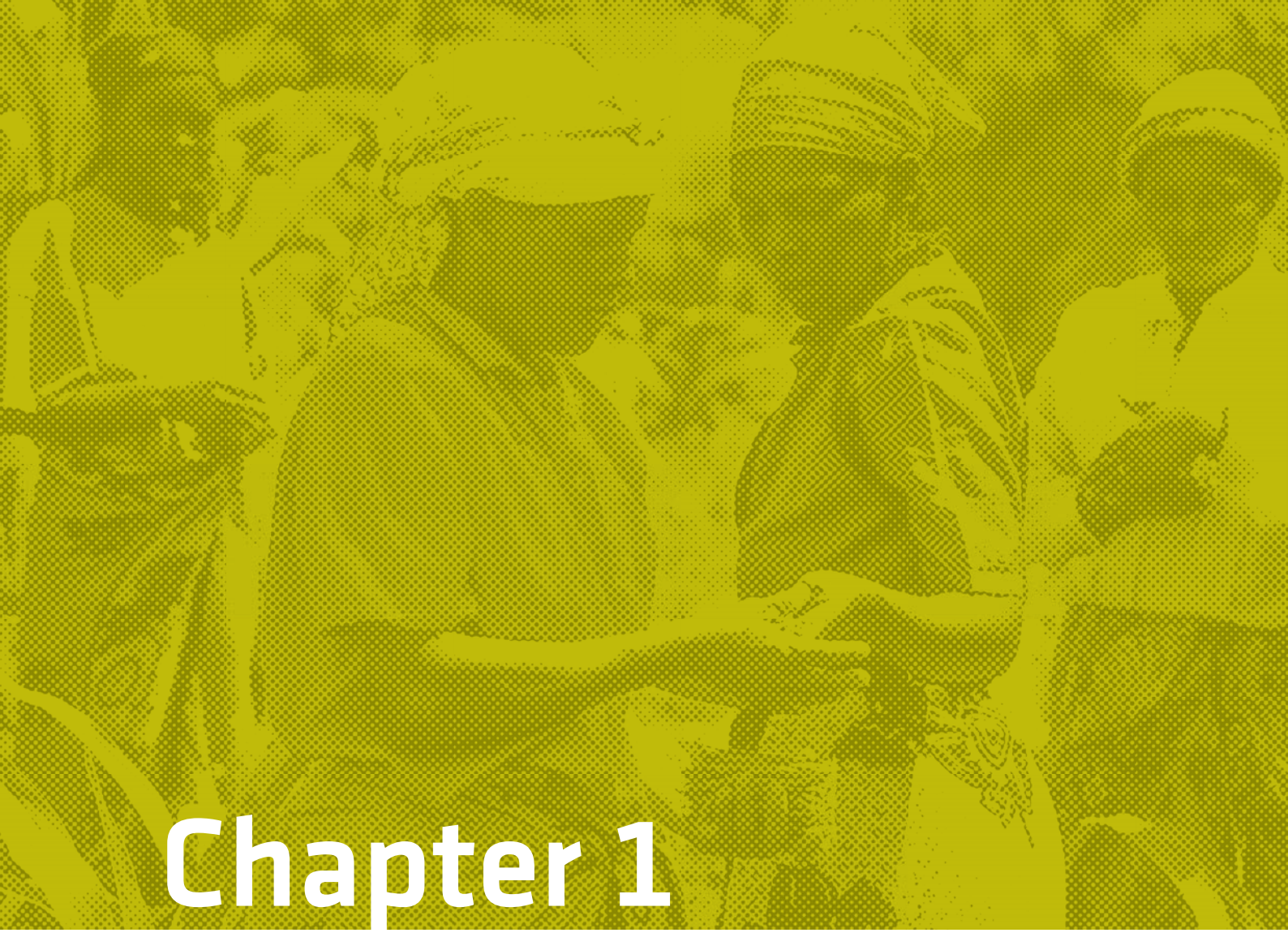
This publication has been prepared by the COLEACP as part of co-operation programmes funded by the European Union (European Development Fund – EDF), the Agence Française de Développement (AFD) and the Standards and Trade Development Facility (STDF).

This publication is an integral part of a COLEACP collection, which is made up of educational and technical tools and materials. All of them are suited to different types of learners and beneficiaries and levels of education found in agricultural supply chains, production and sales.

This collection is available online for COLEACP members and beneficiaries.

FIT FOR MARKET

CONTROL ORGANIZATION AND RESIDUES ANALYSIS IN PLANT PRODUCTS



Chapter 1

Pesticide residues in food and feed: origin and management

1.1. ORIGIN AND VALUE OF PLANT PROTECTION PRODUCTS

Organisms referred to as 'undesirable' (mushrooms, bacteria and pathogenic viruses; pests; competing weeds) are also components of agricultural biodiversity associated with crops. Since humankind began practicing agriculture, we have faced competition from these 'crop enemies' that have threatened our crops. For centuries, farmers have developed cultivation techniques (e.g. crop rotation) to maintain pressure from these bio-aggressors at manageable levels With the mechanisation of agriculture in the 20th Century and the practice of single crop farming, coupled with the use of super productive varieties (from what has been called 'the green revolution'), crops have shown greater vulnerability to biological and climatic hazards, leading to the emergence of major epidemics and the rapid growth of certain pests. Worldwide, financial losses are generally estimated to be around 50 % of production. In addition, these bio-aggressors also seriously affect the food safety and quality of food and feed, through the presence of toxic impurities or mycotoxins, for example. Thus, from time immemorial, farmers have searched for means and methods of combating these enemies.

Chemical control in agriculture has therefore existed for millennia. This is demonstrated in the use of sulphur as early as 1000 BC, and knowledge of the insecticidal properties of certain plant extracts such as rotenone[1] and nicotine in tobacco leaves. From the 19th Century onwards, trade between continents intensified and became the main driver in the import of undesirable insects and plants. One example is *potato blight*, a disease caused by *Phytophthora infestans*, a fungus pathogenic to the potato. Its first epidemics in Europe, in the mid-19th Century, led to severe famines and the death of more than 1 million people, most of whom were Irish,[2] and forced 2 million of them (including the Kennedy family!) into exile in the United States. In 1885, Bordeaux mixture, based on copper sulphate and lime, was the first fungicide to be used in Europe on a large scale. It was an undeniable success, and the product is still widely used to this day, despite restrictions associated with the possible build-up of copper in soils. The development of synthetic organic chemistry and research into chemical weapons during the First World War paved the way for synthetic pesticides.[3]

During the second half of the 20th Century, many molecules were synthesised (more than 1,000 active substances) and marketed by several chemical groups (Bayer, BASF, ICI, Dupont, Rhône Poulenc, Monsanto etc.). Hundreds of thousands of formulations were placed on the market. Crop treatments became widespread and even became routine in most cases (potatoes, cereals, vines, apple trees, citrus fruits, rice, cotton etc.). Advances in chemical plant protection have largely contributed to increased yields per hectare and to the regularity of production, but only because the use of pesticides was combined with varieties selected for maximum yield, the mechanisation of agricultural work, the intensive use

1 Rotenone: And insecticidal substance produced by certain tropical leguminous plants from tropical Asia, or South America.

2 Potato crops at the time accounted for nearly 60 % of Ireland's food supply.

3 Equivalent terms: pesticides for agricultural use, commercial product, formulation, plant protection products or PPP, agro- or phytopharmaceutical product, phytosanitary products, phyto product.

of fertilisers, and irrigation. The use of pesticides in agriculture also helps to improve the commercial, technological and even organoleptic[4] qualities of the products harvested (e.g. the potato variety 'Bintje' continues to be cultivated, despite being highly susceptible to potato blight, because it is the variety considered best by producers of chips, due to its cooking qualities. But this varietal choice requires the use of preventive and systematic fungicidal treatments during the growing season to guarantee sufficient yield).

Today, plant protection products (or pesticides for agricultural use) are used to a degree that depends heavily on the nature of the crops, the climatic conditions, and the presence and frequency of certain bio-aggressors. At national level, consumption of plant protection products is linked to the intensification of agricultural practices. In the so-called emerging economies (China, India, Brazil, Kenya, Nigeria etc.), use of pesticides has therefore risen sharply in recent years, whereas it is tending to stabilise or even decline in Western countries (in the EU, thanks to the 'pesticide and biocide reduction programmes' and in the USA as well, for the majority of products except glyphosate).

From the 1960s onwards, fears concerning the environmental and health impact of pesticides[5] fuelled debates between proponents of industrial agriculture and advocates of a more ecosystem-friendly agricultural model. Since the molecules used are often highly persistent (e.g. organochlorines), highly toxic (e.g. organophosphorus and carbonate pesticides) and not very selective for pollinators and auxiliary insects, the effects of frequent exposure by farmers to the products and consumers to their residues began to cause public concern in the 1970s. Little by little, the debate on 'residues' has gone beyond the specialist field of agricultural practices to become a societal issue, and the rationale for systematic use of chemical products (fertilisers and pesticides) in agriculture is then called into question. European legislation on pesticides is evolving, and regulatory requirements are increasing with every new generation of legislation, affecting the pesticide industry and the agri-foodstuffs sector.

1.2. FORMULATION AND APPLICATION OF PLANT PROTECTION PRODUCTS

1.2.1. Formulation of plant protection products

When an active substance is produced in a chemical synthesis factory (referred to as 'technical material') extracted from plants, fungus, or even when it is a product of fermentation or biological reproduction under controlled conditions (e.g., insect larvae, mites, nematode larvae etc.,) it is rarely usable without any preparation, for several reasons:

1. Because of its physical state: if the substance is present in crystals of varying sizes, in clusters, in flakes, in liquid or in paste, this physical state does not

4 Organoleptic: referring to the taste, texture, smell and visual appearance of something.

5 Publication in 1962 of Rachel Carson's book, *Silent Spring*, in which she decried the indiscriminate nature of pesticides and their detrimental effects on ecosystems.

allow it to be used directly or applied using conventional treatments tools, with the exception of oily substances and liquid technical materials that can be sprayed in very fine drops (ULV) (organophosphorus insecticides are often used, for example, to combat locusts). However, due to their toxicity and environmental impact, these products are gradually being replaced by fungus-based biopesticides.

2. Due to their generally high activity potential (1 to 2 kg in the case of old molecules, but also for some alternative products such as kaolin powder, a few dozen grams, or grams per hectare in the case of pyrethroids or sulphonylurea): it must be diluted to ensure even distribution on plants, objects and treated surfaces.
3. Because of its sensitivity: in a living organism whose activity must be protected and preserved during storage or field application. 'Active substances' of the biopesticide type take various forms, from the living organism or micro-organism, to the purified extract of bacteria, fungi or plants. They are often more sensitive than synthetic substances to environmental conditions (inadequate temperature or humidity, and even UV radiation, which can diminish their activity) during storage or during spraying.

To be used effectively and sprayed on plants, an active substance (whatever its nature and origin) must therefore be formulated. In other words, through a series of processes it will be presented in a form as it is developing, in a specific pest control treatment, optimum biological efficiency while remaining within permissible financial limits. The formulation is of equal importance to synthetic pesticides as to biopesticides and, while the challenges are different, the formulation techniques are exactly the same. In the majority of cases, the formulations have remained the same (WP, WG, EW, SC etc.)[6] for the good reason that manufacturers cannot require farmers to change their application equipment for each type of active substance.

Producing a formulation involves a series of products called co-formulants (or sometimes, incorrectly, adjuvants[7]), which possess specific properties and which must now also have been evaluated and authorised for use in formulations under Regulation (EC) No. 1107/2009, while others have been prohibited: methanol, benzene, formaldehyde etc. In its commercial form, a pesticide therefore consists of:

- the active substance(s): natural substances or chemical molecule(s) that destroy or prevent the organism harmful to the crop from establishing itself;

6 The formulations are designated by an English code containing two letters. WP: wettable powder; WG: wettable granules; EW: emulsion in water; SC: suspension concentrate. There are around 80 different codes.

7 The term 'adjuvant' is used to refer to the product that is added directly to the spray mixture at the time of application, for example to increase adhesion, spreading or penetration (e.g. vegetable oils, humectants etc.). In Europe, adjuvants must be authorised in the same way as commercial formulations.

- co-formulants: a set of products used to secure, facilitate and enhance the action of the active substance(s), such as fillers,[8] thinners, solvents and surfactants, stabilisers, dyes, and emetics, according to a laboratory-developed formulation designed to produce an effective and ready-to-use finished product. The co-formulant is a substance that is normally devoid of biological activity, but is capable of modifying these physicochemical properties, and therefore the effectiveness of a pesticide.

One of the main aims of the formulation of active substances is to facilitate their use: ease of dosing, emptying of the packaging, good distribution in the spray tank (in the form of an emulsion, suspension or solution) ease of spraying (without blocking the nozzles), good adherence to foliage, better penetration in plant tissues etc. Without a good formulation, plant protection products cannot be applied well and treatment cannot be effective.

1.2.2. Application of plant protection products

To act, the plant protection products (herbicides, insecticides, fungicides, and others) must come into contact with the target organism (e.g. the leaf, the larva of an insect or the spore of a fungus). In the vast majority of cases, the products act preventively. This therefore means that they must be present before or at the beginning of any infestation/contamination by a pest. It also means that the parts of the plant that are not protected or not sufficiently protected are susceptible to attack. The products must be distributed on the plants in sufficient quantity (to ensure effective concentration) and sufficient quality (to obtain homogeneous coverage of the plants to be protected).

The **plant protection treatments** that are carried out by the producer therefore consist of depositing quantities of active substance(s), usually as uniformly as possible, and most often by spraying on the target to be protected, regardless of its type (crops, trees, timber etc.). The **active substance** is (most often) a **molecule** whose **toxic** properties[9] allow the prevention, control, or elimination of harmful organisms.

In horticultural crops, plant protection products are usually dispersed in the water of a sprayer (the 'slurry') and spread in two ways:[10]

- **manual spraying:** spraying with a backpack system, with pressure maintained by manual pumping. The drops are relatively large and distribution is average to poor; it depends on the applicator's skills;

8 The term 'fillers' refers to powders that act as an absorbent or thinner (often clays, sometimes synthetic materials, and sometimes even wood or plant flours), but which do not have biological activity by themselves.

9 Toxic: the intrinsic dangerousness of a substance, related to its dose and mode of action in the living organism.

10 For the sake of completeness, we have added chemigration (application through the irrigation system), used in crops under shelter.

- **motorised spraying:** usually with a tractor-mounted device for pressurising a line of nozzles. The drops are relatively fine and the distribution is relatively uniform on the target.

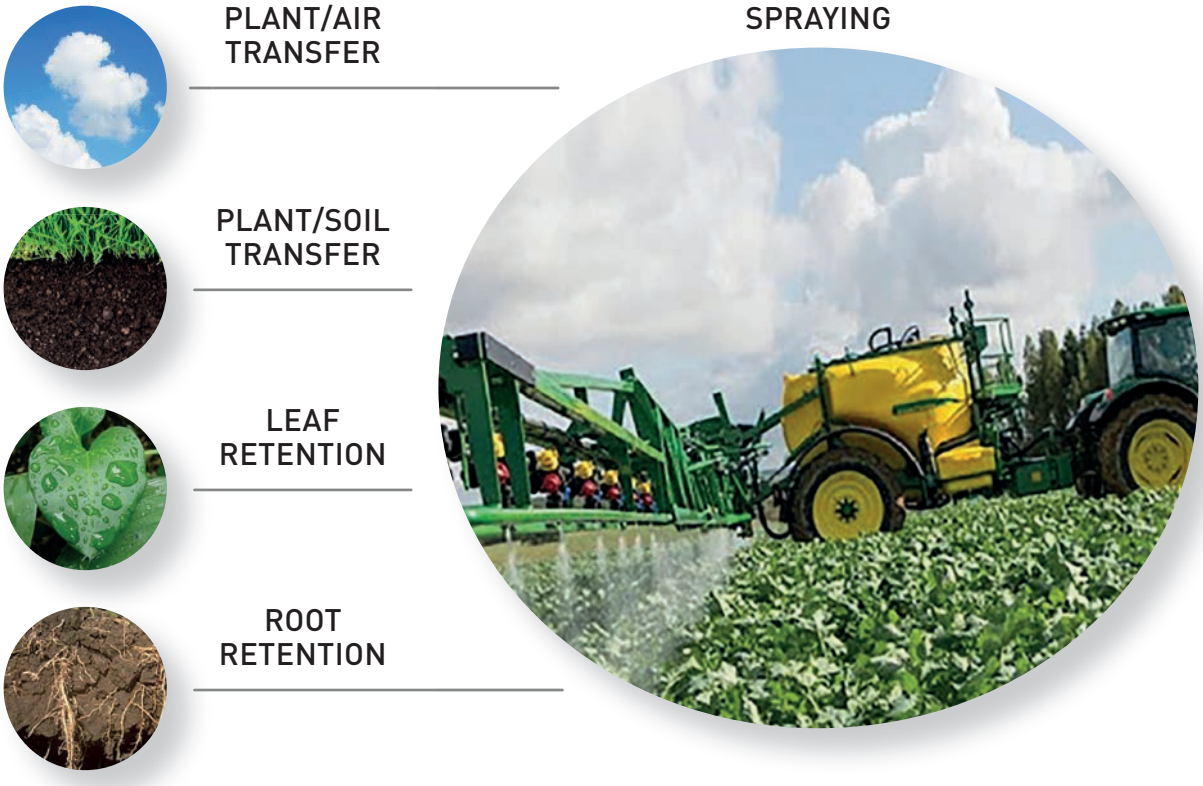
The technique selected largely depends on the target organism, on the surface to be treated and the type of formulation.

1.3. FORMATION OF DEPOSITS AFTER APPLICATION

In agricultural treatments, **the plant protection product is sprayed on the aerial parts of the plant** (leaves, stems, fruit) **or at ground level** (it is mainly herbicides that are deliberately applied at ground level to prevent the germination of weeds).

Foliar treatments involve the distribution of a pesticide solution[11] or suspension[12] using a nozzle system[13] to produce a spray of droplets of varying or controlled sizes.

It must therefore be remembered that **not only do all parts of the treated plant receive a deposit, but so does the soil**, which can generate a 'residue' (or residual quantity of the product applied).



11 Solution: powder fully dissolved in a solvent (generally water).

12 Suspension: powder that remains solid in water but disperses in liquid.

13 Nozzle: rigid large calibre pipe allowing the flow of liquid.

1.3.1. The formation of deposits

The percentage of product intercepted by the plant substrate depends on the characteristics of the plant substrate (stage of development, plant population density) and the application technique. A mist[14] application will tend to spread the slurry evenly over the plant cover. Conversely, application of the pesticide in large volumes[15] causes a greater proportion of the product on the soil, regardless of the target.

The distribution of active substances on the foliage during application involves 3 steps:

- **retention of the slurry on the leaf;**
- **vaporisation** of the aqueous phase of the droplets;
- the **distribution of active substances through the epidermis**.

The cuticle is a hydrophobic epidermal layer that covers the aerial parts of the plants. It is the principal matrix for receiving droplets from the sprayed slurry. This hydrophobic barrier limits the penetration and distribution of plant protection products. The capacity of the slurry to adhere to the cuticle depends entirely on its formulation and on the adjuvants added to the slurry at the time of treatment. The latter perform various functions, the main ones being the spreading and retention of the slurry, penetration of the active substance in the tissues and, if necessary, correction of the water quality.

Properties of some adjuvants used in pesticide formulations:

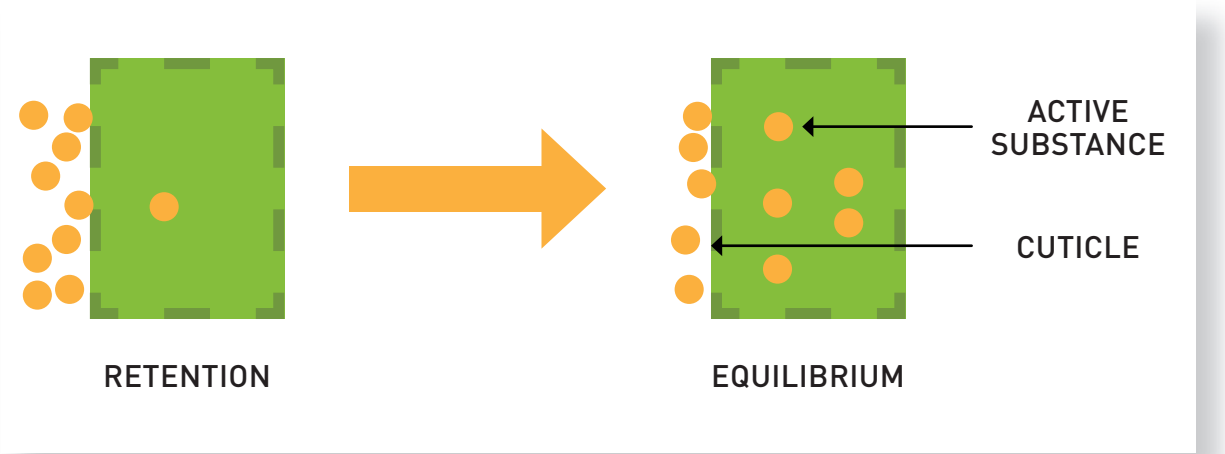
Position	Properties	Examples
Hazard analysis	Reduces surface tensions of the drops and promotes their retention and spreading on the leaf blade	Heliosol, Genamin
Adhesiveness	Promotes maintenance of the slurry after impact	Biofix, Sticman
Humectant	Prevents crystallisation of the active material and evaporation of the slurry	Ammonium sulfate
Acidifier	Maintains an acid pH of between 5 and 7 for active substances that degrade rapidly in a basic environment	X-Change

(Source: Agricultures&Territoires 2012-2013)

The rate of distribution of active substances throughout the cuticle depends on the difference in concentration between the surface deposit and the adjacent tissue. If the epidermal cells are physiologically active, the active materials are redistributed by the water flows resulting from this activity. When the epidermal cells are inactive, the active materials accumulate within them, slowing down the distribution process. Two metabolisation sites must therefore be established; the epidermal tissues and the vascular system.

14 Application in fine droplets (concentrated and undiluted formulations).

15 Application in larger droplets (usually concentrated formulations and formulations diluted in water).

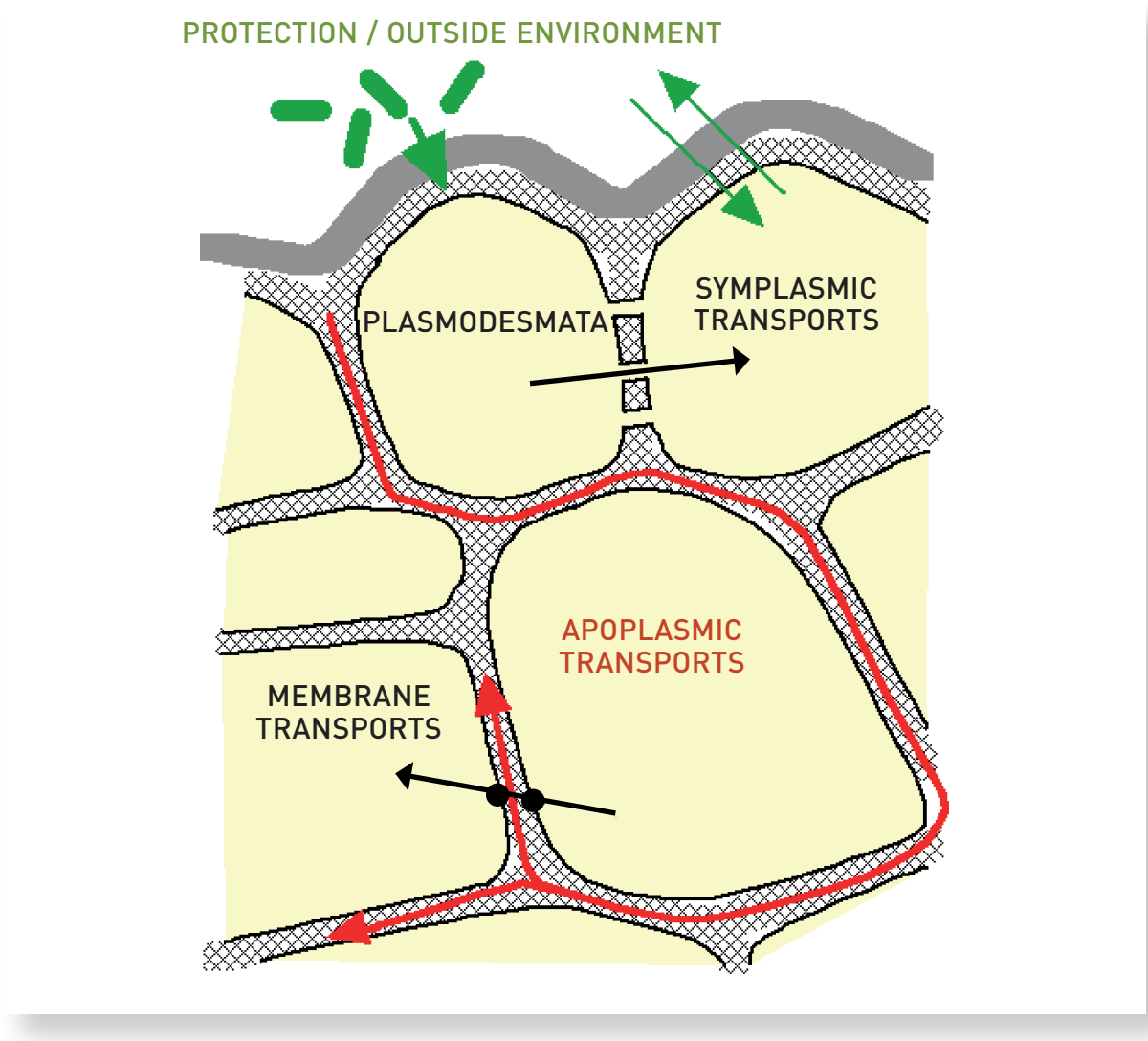


(Equilibrium happens when the epidermal cells are inactive)

In addition to the foliar system, pesticides and their residues can also be absorbed at the root. The distribution of compounds in the plant through ascending (xylem) and descending (phloem) sap flows is traditionally measured by autoradiography after application of the radiolabelled compound. This technique shows the build-up of radioactivity in the plant in detail.

Depending on their direction in the plant, the transfers affect the residual quantities of active substance in the plant or fruit.

- The transfer of compounds **from roots to leaves or fruits**: the compounds are transported in the xylem (with the raw sap) without undergoing chemical transformations. They will accumulate in sites where transpiration is greatest, *i.e.* mature leaves.
- The transfer of compounds **from the leaves to the roots**: in the case of foliar deposits, the compounds must use **the phloem** (with the elaborated sap) to migrate in the plant. The phloem vessels are living cells, which requires the product to again cross a hydrophobic barrier. The more lipophilic a molecule is, the better its capacity to pass through plant membranes. A study of the transport of substances with acid pH (in the case of certain herbicides) shows that these products tend to accumulate in the cytoplasm and phloem of plants.



The circulation of substances at varying distances in the plant can be achieved through distribution in the wall. This is the *apoplastic pathway*. Communication between cells takes place via the wall passively or through 'membrane transporters'.

Another pathway, that of the plasmodesma, allows direct communication from cell to cell. This is the *symplastic pathway*.

1.3.2. The formation of deposits on the soil surface

The soil is a quasi-obligatory crossing point for losses between nozzles and the treated plot.

The soil also plays an important role in the fate of pesticides. Deposited or leached onto the soil surface, the active substances will be carried deeper down by rainwater. The active substances and their degradation by-products initially found on the soil surface can reach the surface waters through seepage, infiltrate the soil or even percolate to the groundwater table.

Certain pesticides remain present in the environment for a long time because they are not very mobile and are eliminated very slowly. They will persist in the soil in their initial form or undergo chemical transformations that lead to the creation of **secondary**

compounds (degradation by-products) following biotic (caused by micro-organisms) and abiotic (ionisation, oxidation reduction, hydrolysis, photolysis), physicochemical transformations that will alter their chemical structure. These phenomena lead to the degradation of the initial molecule into simpler molecules that are often more stable and less toxic. At root level, the absorbent hairs are the entry point for contaminated soil solution.

It should therefore be borne in mind that **absorption of the active substance** (or degradation by-products of this substance) **through the rootlets from the soil solution is possible**, even long after application of this substance. There are interactions between the substance and the colloidal particles of the soil (clay-humus complex) that are responsible for a temporary attachment to their surface (known as 'adsorption'). The clay-humus complexes are formed by the combination of clay and hummus soils, in flocculated state, following the work of the microorganisms in the soil, and earthworms in particular, which can bind these molecules using a special organ in their digestive tract; molecules that are negatively polarised by calcium (Ca^{++}). The mucus of some organisms can also play a role in the formation of these stable and insoluble complexes, which explains the resistance of humus soils to water and erosion, and the maintenance of their structure and exceptional capillarity.

The retention (or adsorption) of pesticides on soil particles is the main mechanism that influences their distribution between the solid and liquid fractions of the soil. The 'soil particles/soil water' **partition coefficient K_d** of an active substance **determines its degree of adsorption** on the colloids. **The higher the coefficient K_d, the greater the absorption.** Herbicides generally have lower coefficient K_d than fungicides and insecticides. This means that they are less adsorbed by the soil particles and are thereforemore mobile.

Values of partition coefficients (K_d) of certain pesticides in soil:

Pesticides	Partition coefficient K_d values
2,4-D	1.59
Simazine	1.93
Diuron	6.29
Disulfoton	25.1

Over time, a gradual release of the substance and of degradation by-products occurs, returning to the soil in solution accessible from the roots. For example, it is not uncommon to find residues of plant protection products that were applied in the preceding year, or even longer ago, in the roots or tubers.

The composition of chlordane residues in vegetative compartments and fruits was examined in courgettes following soil contamination by this molecule. The quantities found varied according to the tissues, from a maximum concentration in the roots to a minimum in the fruits. The results indicated that transport of chlordane by the xylem predominated. The absorption of chlordane by the roots of the plant thus appeared to be faster than translocation from its aerial tissues.

1.3.3. Air contamination

In air, rather than there being a 'deposit', there is to **an airborne contamination** with the possibility of generating a 'residue' either in the form of aerosols (drift of fine droplets caused by the wind and convective movements due to heat and the heating of the soil) or by the formation of 'vapours' (evaporation of part of the deposit from the leaves or soil).

Pesticide drift describes the quantity of slurry carried away from the targets, outside the treated plot. It can be as much as several percentages of the applied quantity and can be used over very long distances (several hundred metres). It is aided by strong winds, large volumes applied, high pressure generating fine droplets, a high spraying height, rapid speed of the apparatus, the presence of surfactants in the slurry etc. It is responsible for an unwanted secondary deposit. Pesticide drift can therefore generate unexpected residues on products even if they are organically grown.

The **vaporisation** of pesticides describes the passage in gaseous form of compounds from the treated soil or plants. In the aerial compartment, pesticides degrade, mainly due to the effects of light radiation, but they can also be transported over long distances before returning to humid form in rain, snow or fog.

1.4. FORMATION OF RESIDUES FROM DEPOSITS

1.4.1. Phenomena that modify deposits

It is possible to identify 4 sets of processes that modify initial deposits:

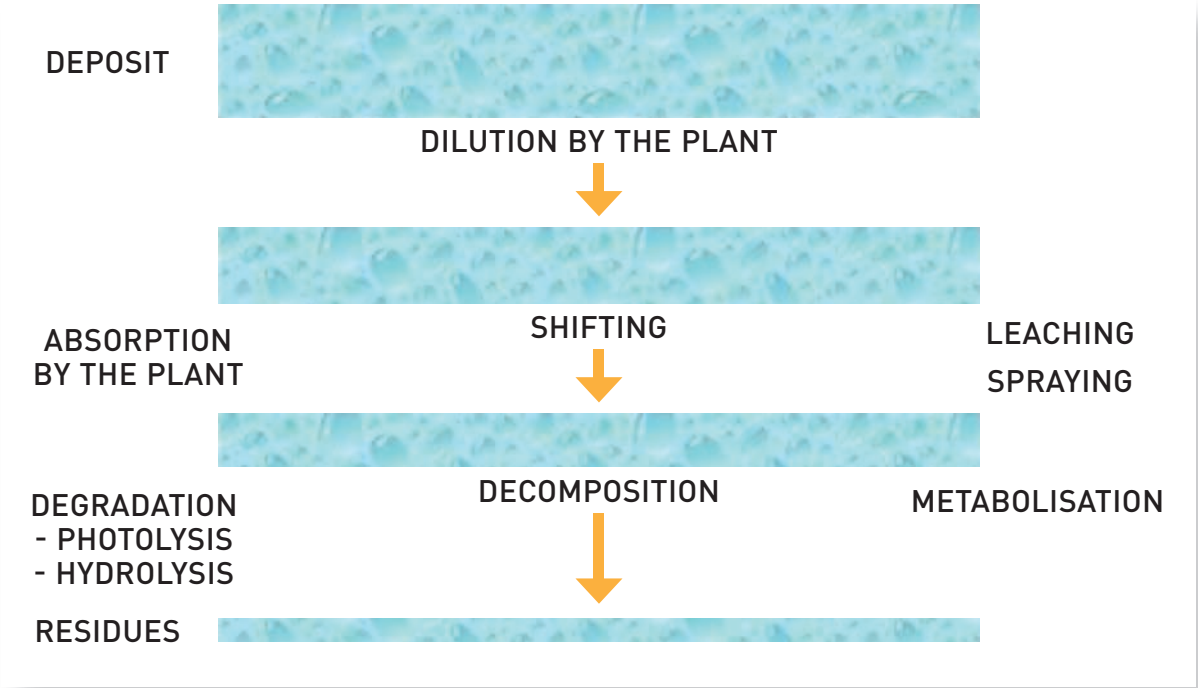
- **dilution** phenomena caused by plant growth (a phenomenon that forces the farmer to treat frequently) and the leaching[16] or lixiviation[17] of active substances by rainwater (transfer from the plant to the lower levels and the soil;
- the **volatilisation** of active substances in the atmosphere (transfer from the plant in the air);
- **biological phenomena**, mainly related to the **action of microorganisms** present on the surface of leaves, stems and fruits on active substances;
- **transformation phenomena** responsible: (a) for the production of 'metabolites' and (b) the physicochemical degradation of active substances (by moisture and light) and the production of 'degradation by-products'.

It is important to emphasise that the concentration of active substance never remains constant over time, even if degradation can sometimes be slow. Since the majority of pesticides are xenobiotics, they are not found naturally in ecosystems, which means that the biological mechanisms of degradation of these molecules are not systematically active. Repeat treatments with the same substances allows effective microorganisms to multiply, making degradation faster.

16 Leaching: extraction of soluble elements by the soil solution.

17 Lixiviation: extraction of non-soluble elements by the soil solution.

The evolution of the deposit can be illustrated in the following diagram:



1.4.2. Major pesticides degradation phenomena

Biological (**biodegradation**, **metabolisation**), and physicochemical (**hydrolysis**, **photolysis**) processes are the main degradation mechanisms. Compounds whose degradation time is particularly long are called persistent. The latter combine in the environment without undergoing any transformation. The degradation of active substances is a process that leads to **the creation of intermediate molecules** that in most cases **do not have the same properties** yet are no less toxic to humans (on the contrary!) or less polluting for the environment. If mineralisation is the only process that leads to the total elimination of these active substances, these physicochemical transformations do not routinely result in the formation of inorganic compounds.

The degradation of active substances is a process measured by **their half-life time, or DT_{50}.** The half-life represents the time required for 50 % of the mass of the substance to disappear from the soil or from the water as a result of transformations. It is generally shorter in the field than in the laboratory.

Degradation and DT_{50} must be **studied and measured by the manufacturer** in the laboratory and in the field **when assessing risk** for placing the substances on the market (the product registration procedure or 'type approval documentation'). The manufacturer must provide the following information:

- **The degradation pathway:** identification of all of the metabolites and degradation by-products formed from the original molecule (what we call the 'pathway'). From these compounds, identify those that are potentially of concern due to their toxicity (those that exhibited a certain activity, for example biochemical affinity for the target).

- **The degradation kinetics:** measure the potential persistence and rate of transformation of the molecule, of its metabolites and degradation by-products (once these constitute more than 10 % of the starting mass of the active substance).
- **The organs** (of the plant or of the animal/human if there is plant to animal/human transfer via feed) in which residues tend to persist and/or build up (e.g. the external surface of the leaves, fruits, seeds, tubers etc.).
- **The process involved:** role of microorganisms (which) and physicochemical phenomena (sensitivity to light, water, soil pH etc.).

These studies are carried out by the manufacturer in the laboratory through the use of isotopic markers.

The active substance molecule is 'marked' by the presence of an isotope with low radioactivity (usually C^{14}), which makes it possible to track the transfer of the latter from the soil or leaves. The distribution of radioactivity can be seen by autoradiography by placing the plant on a photographic plate sensitive to radiation: the accumulation zones appear darker. It is then possible to understand the behaviour of the initial and newly formed compounds (accumulation, transfer upwards or downwards to the roots etc.).

These studies are in turn confirmed in the field trial.

1.4.2.1. Microbial decomposition (biodegradation)

Biodegradation refers to the transformation of a substance by microorganisms. In the environment, biodegradation may be affected by a number of factors, including the presence or absence of oxygen, and nutrient availability. The soil contains the largest source of microorganisms, but they are also found on the cuticle surface. No active substance is a priori resistant to their action, especially if they contain the groupings OH, COOH, NH_2 and NO_2. Only organochlorines showed a degree of resistance to microbial biodegradation, which largely explains their persistence in the soil (traces of DDT are still found in some cultivated soils).

1.4.2.2. Chemical decomposition by hydrolysis

During hydrolysis, an RX compound is split by contact with water. It undergoes a chemical reaction in which part of the substance's molecule is replaced by an OH group This process depends, to a large extent, on the acidity (pH) of the environment. An example of this are triazines, with the loss of an aromatic chlorine.

$$RX + HOH \rightarrow ROH + HX$$

1.4.2.3. Chemical decomposition by photolysis

Photolysis is the breaking down of a component as a direct result of exposure to radiation. Several pesticides have a structure that **absorbs light energy in the ultraviolet range** (e.g., parathion, chlordane and diuron). They are therefore susceptible to undergoing photolysis in a natural environment. This process can take place in the atmosphere, on the leaves of the plants, or even in the first few centimetres of soil. The energy absorbed must be sufficient to cause the breakdown of chemical bonds, rearrangements, and oxidation and reduction reactions.

1.4.2.4. Decomposition through metabolisation in the plants

Plants can modify a fairly large number of pesticides using a series of metabolic pathways, including oxidation reduction processes, synthesis of conjugated products (*i.e.* products bound by sugars, for example) or hydrolysis.

1.4.2.5. Decomposition by metabolisation in animals from the soil

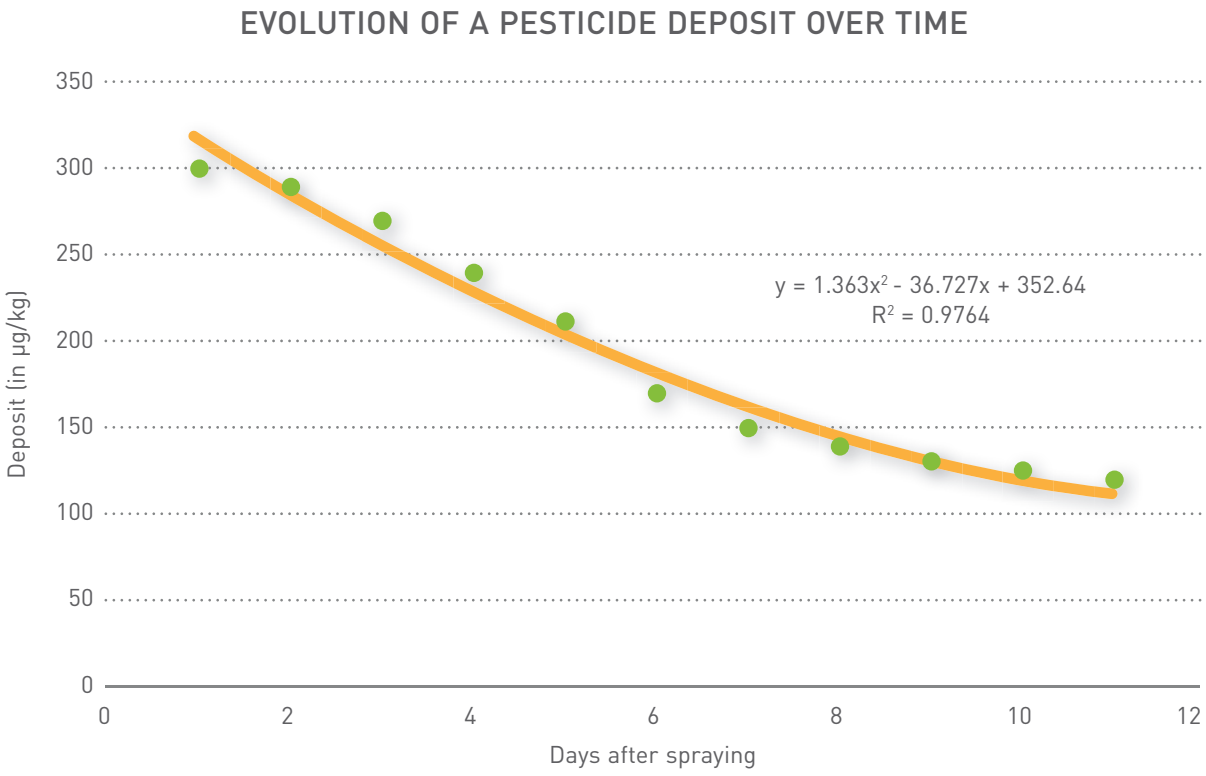
In soils, a there is also a biotransformation of active substances by the animals. There is the example of soil invertebrates such as earthworms, major actors in soil fertility. Metabolites identified in these invertebrates are similar to those found in animals with more advanced nervous and enzymatic systems. We know that DDT (dichlorodiphenyltrichloroethane) tends to convert

to DDE (dichlorodiphenyldichloroethylene) in aerated soils or to DDD (dichlorodiphenyldichloroethane) in water-saturated soils. However, in earthworms, the main metabolite of DDT is DDD.

1.4.3. The decay curve of deposits

The initial deposit decreases over time due to the combined action of these various phenomena. If regular analyses of deposits are performed on the plant, decay can be recorded and measured. From this data it is possible to establish the decay curve equation. Through this calculation, **it is possible to predict the theoretical value of the residue** from the active substance at time 't+n days'.

The figure below shows the typical decay of an initial deposit:



1.4.4. Definition of residue

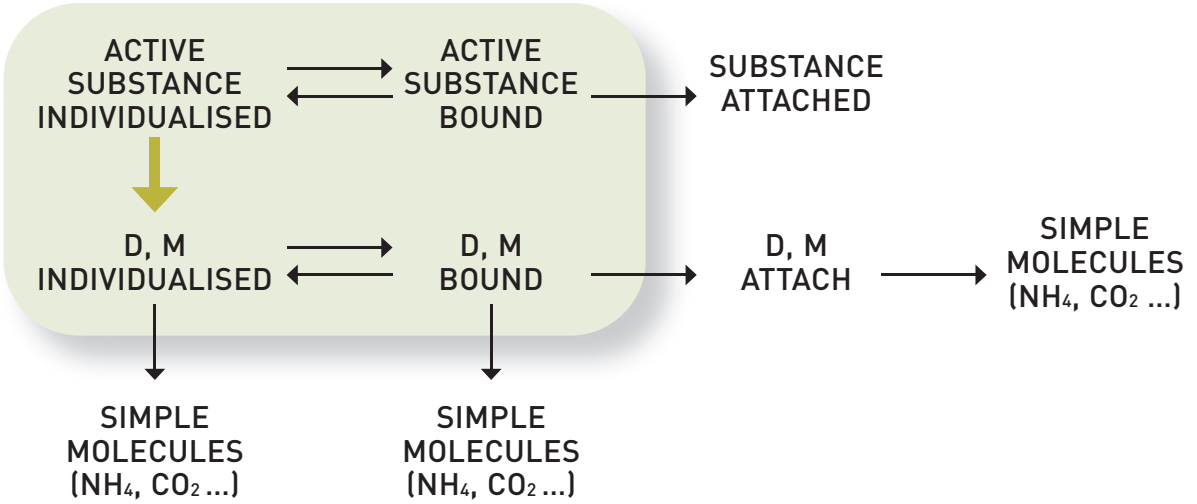
The term '**pesticide residue**' refers to all of the deposits distributed between the plant, the soil and the atmosphere.

It is the **persistent fraction** of the sprayed product containing:

- The **active substances** themselves (when they are persistent). In the cells they combine, conjugate, with various compounds, including sugars. They can bind to organic matter and evolve to become simple molecules.
- Their **metabolites** (**M**): stable, identifiable compounds derived from the biochemical transformation of an initial molecule by metabolism (of plants, microorganisms or animals). These metabolites can bind, attach or degrade gradually into simple molecules.

- The **by-products resulting from their degradation (D)** and/or the reaction of these active substances. These products can also conjugate, attach or degrade gradually into simple molecules.

'Biochemical residue' is everything that derives from the application, except for the simple molecules that form at the end of the degradation and/or metabolisation process, and those that are attached because they are no longer extractable and measurable by analysis.



With: D = Degradation by-product; M = Metabolite

Example 'pathway' for the **carbofuran** systemic insecticide (carbamate family, used as a soil insecticide, it penetrates through the roots and is distributed in the aerial parts of the plant). Since it is the N-methyl carbamate group that reacts with acetylcholinesterase (an essential enzyme for the functioning of the nervous system in humans and animals), all compounds that possess this group therefore potentially act on the nervous system by inhibiting the acetylcholinesterase enzyme. In this example, 3 compounds are therefore potentially toxic: carbofuran, 3-hydroxycarbofuran (main metabolite formed in plants) and 3-keto-carbofuran (a particularly elusive metabolite).

The 'residue' to be sought is therefore the sum of carbofuran and of 3-hydroxycarbofuran.

Diagram of degradation of carbofuran and formation of its metabolites

1.4.5. Simple products and complex products

As can be seen through this example, 3 scenarios can therefore arise:

- **Residue = parent active substance** (undecomposed product or '*simple residue*')
- **Residue = single metabolite** (parent compound entirely decomposed into a single product or *'metabolite'*)
- **Residue = parent active substance + metabolites and/or degradation by-products that have formed** (mixture or '*complex residue*'))

We therefore distinguish 'simple products' and 'complex products'. This distinction is important to understand, particularly **during analysis of residues carried out on foodstuffs.** The analyst must know what type of products they are dealing with: should they simply measure the concentration of the starting product (the substance) or also of the metabolites? Through what methods? How should the result of the analysis be expressed?

1.4.5.1. Simple residues

In the case of simple residues, the products that make up the 'total residue' can be expressed as additional **to the parent compound, optionally** taking into account the molecular weight of the metabolites**. We talk about simple products in the following instances:**

- In the **absence of metabolites** (e.g. copper sulphate, an anti-potato blight fungicide).
- When metabolites are present at significant levels but the analytical technique measures the **total residue in a single compound** (e.g. maleic hydrazine: free or conjugated, is expressed as maleic hydrazine).
- In the presence of metabolites, but **without toxicological significance** (e.g. prosulfocarb).
- Where the parent compound is **entirely converted into another chemical compound** (e.g. fungicides of the maneb and mancozeb dithiocarbamate families: caused by the moisture they produce from carbon disulphide. The analysis method is based on carbon disulphide titration. The analysis report will therefore not record a difference between maneb and mancozeb. 'Total dithiocarbamates' will be indicated.

Degradation of EBIS (Ethylene-bis-dithiocarbamates, maneb and manozeb type).
The *formation of CS_2 (carbon disulphide) is observed. Note the possibility of generating a particularly toxic residue, ETU (ethylene thiourea).*

- Where are the toxicologically relevant compounds are present at significant levels but the **analysable residue is the metabolite only** (e.g. benomyl is only dosed as its metabolite, carbendazim).

1.4.5.2. Complex residues

In the case of complex residues, the products that make up the 'total residue' are the **parent compound + metabolites and/or degradation by-products that have formed**. **The molecular weight of the metabolite(s)/products formed** must therefore be taken into account in order to express the residue content.

- Where the compounds from the total residue are dosed together and **can be expressed as additional to the parent compound**, taking into account the molecular weight of the metabolites. For example, this could include:
  - Isoproturon (a herbicide): sum of isoproturon and metabolites containing the group 4-isopropylaniline.
  - Methiocarb (an insecticide and anti-slug product): sum of methiocarb, methiocarb sulfoxide and methocarb sulfone.

Biotransformation in the liver of methiocarb into sulfoxide and sulfone

- When the compounds of the total residue have **different toxicological profiles**, are present in significant quantities and **are dosed separately** (e.g. benfuracarb: benfuracarb + carbofuran + 3-hydroxycarbofuran must be dosed).

1.4.5.3. Analysis report

On the analysis report, the residue will be 'defined' according to its official definition (as listed on the European Commission pesticides and pesticide residues database[18]).

The formula for calculating the residue will also be indicated. For example, in the table below, the value of methiocarb residue is calculated as follows:

*methiocarb (9.6301 * 1.0000) + methiocarb sulfone (0.0000 * 0.8760) + methiocarb sulfoxide (0.4081 * 0.9340) = 10.011 mg/kg*

Active substance	Residue defined on the analysis report	Measured value of the residue (with calculation formula)
Prochloraz	Prochloraz	0.014 mg/kg
Methiocarb	Methiocarb (sum of methiocarb and methiocarb sulfoxide and sulfone, expressed as methiocarb)	10.01 mg/kg [methiocarb sulfone (0.0000 * 0.8760)] + [methiocarb sulfoxide (0.4081 * 0.9340)] + [methiocarb (9.6301 * 1.0000)]
Thiametoxam	Thiametoxam (sum of thiamethoxam and clothianidin expressed as thiamethoxam)	0.830 mg/kg (clothianidin (0.0000 * 1.1680)] + [thiaméthoxam (0.0830 * 1.0000)]

1.5. RESIDUE RISK MANAGEMENT PRINCIPLES

1.5.1. The presence of residues in horticultural products is almost routine

Because fruit and vegetables are foodstuffs that are particularly sensitive to bio-aggressor attacks, many plant health treatments are carried out during production and sometimes conservation. These treatments lead to the almost routine presence of residues on the surface or in the consumable part of the treated plant.[19] Only products that have been farmed organically, which by definition prohibits any use of synthetic chemical products and reduces the use of pesticides to strictly monitored conditions, and baby food, which is subject to specific legislation, may be considered free from traces of pesticides.

Residue analysis measures the nature, rates and persistence of any chemical contamination in food. The latest European Food Safety Authority (EFSA) report from 2014 found that of 83,000 food samples from the 28 EU Member States, 97% were either free from pesticide residues or contained detectable but non-measurable traces (when concentrations were below the limit of quantification for analytical methods or LOQ), *i.e.* around half the products harvested contained measurable concentrations that were nevertheless below the authorised maximum residue limits (MRLs).

18 Database available at: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN.

19 But also in surface and underground waters through seepage and lixiviation, as explained in previous pages. Because this chapter is dedicated to residues in foodstuffs consumed by humans (and possibly animals), aspects relating to residues in the environment will not be covered here.

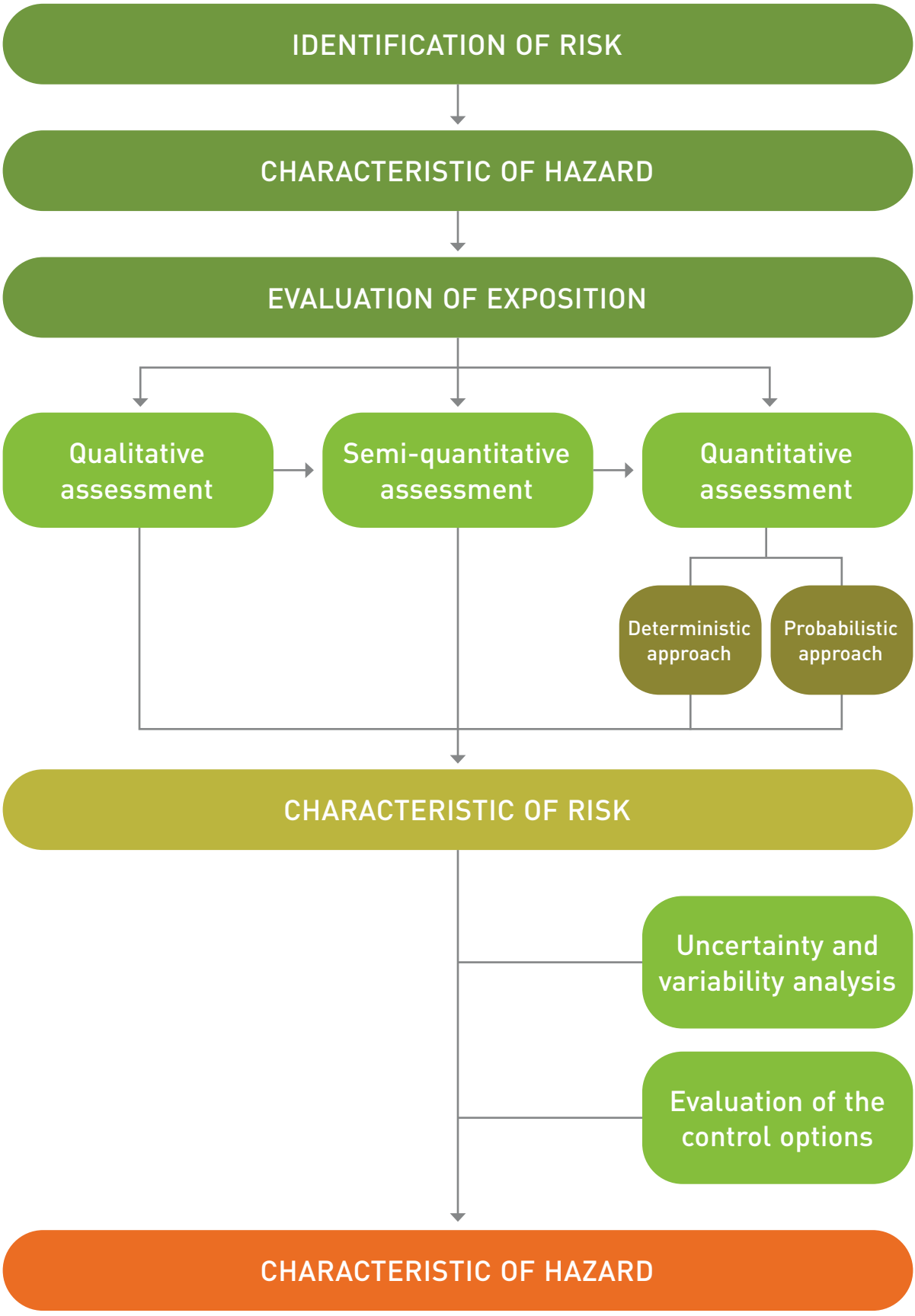
The agri-food industry tends to play on words, using the phrase 'zero pesticides' in advertising (in some Italian pastas, for example) for products that are in fact based on cereals produced with the use of synthetic pesticides, but for which, through strict compliance with Good Agricultural Practices (GAP) and a limited number of treatments with non-persistent pesticides, residue analysis does not detect quantifiable traces of residues using routine analytical methods (those for which the mean LOQ is around 0.01 mg/kg of foodstuff).

The systematic presence of residues raises a number of problems: it raises **health concerns** on the one hand, and **financial risks** for producers on the other. These small quantities of pesticides with several traces mixed together, present in the diet for long periods (in principle throughout one's lifetime, at each meal) pose a potential health problem for consumers (e.g., carcinogenic or mutagenic effects). In addition, the non-conformity of foodstuffs with regulations (EU or other regulations, depending on the destination markets) on **Maximum Residue Limits** is subject to criminal (fines) and commercial penalties (seizure of batches and possible destruction, import restrictions, enhanced monitoring of origins etc.).

This is why **an assessment and management of risk** relating to the presence of residues in foodstuffs is essential. Residues in foodstuffs are subject to specific legislation.

1.5.2. Assessment of the 'residue' risk to health

The procedure for assessing risk can be represented schematically as follows:



The risk is dependent on two factors: the **concentration** (of toxic residues, determined by laboratory analysis) and **consumption** of the food during a meal (in practice, we analyse consumption in grams per day, established through food surveys of consumer groups). The result of these two factors represents **the risk of consumer exposure**.

Two instances must be distinguished:

- In cases of **high concentrations** of a toxic substance (or its metabolites) (sometimes from several substances): acute intoxication is possible after ingestion of the food in sufficient quantity to obtain the effects. However, these cases remain exceptional, although the risks are greater for vulnerable groups, including children (who have a lower body mass).
- In the most frequent cases, with **relatively low concentrations** of residues in habitually consumed foods, intoxication and chronic side effects are possible (the suspected manifestations being chronic diseases, such as cancers).

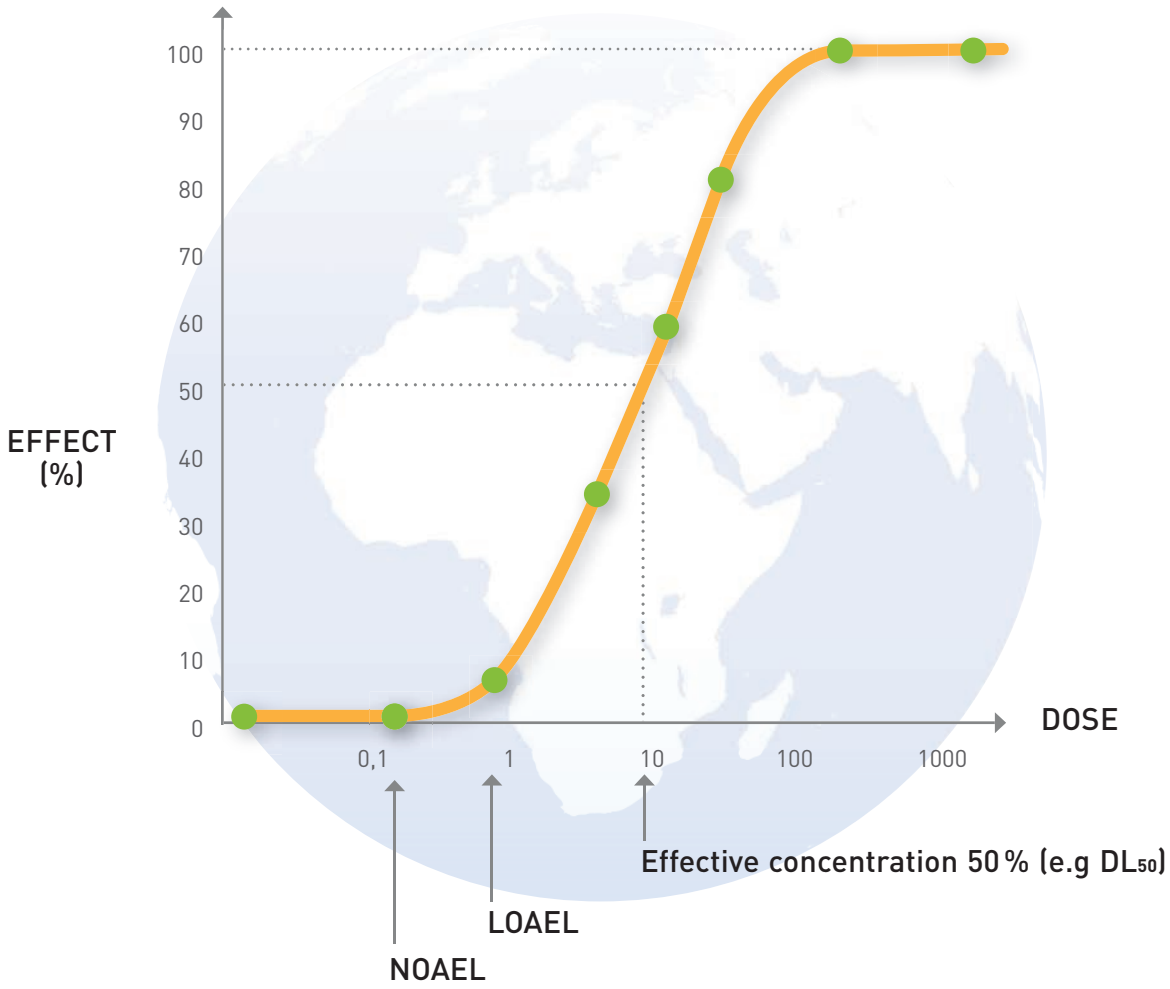
For these two scenarios, an objective risk assessment is required.

Risk assessment is a process that involves the following 4 steps:

1. **Identifying the hazard:** what is the residue? It must be defined as 'simple product' or 'complex product' (see above). Consultation of scientific literature or databases is necessary. This has been discussed in detail.
2. **Characterisation of the hazard:** what are the effects of the residue on health if exposed? This refers to the toxicity of the substance and its metabolites, and to defining the toxicological values that should be referred to. The European and international health authorities (WHO, EU) must ensure that their residue levels in foodstuffs remain below **reference values** known as **Toxicological Reference Values** (TRV).

   These TRVs are toxicological indices that establish a link between the dose of a contaminant A and the emergence of a toxic effect B. The pertinence of TRVs is based on the quality of the studies developed by the health authorities. These studies are based, for example, on processes of assimilation, distribution and degradation of substances within animal test organisms. They are established for **toxic effect, pathway and duration of exposure**. Laboratory tests are the principal source of toxicological data and their results make it possible to extrapolate the risks of consumer exposure to foods.

   To **measure toxic effect**, high doses of a chemical contaminant are administered to laboratory animals (usually rats), which allows signs of toxicity to be observed on the tested animals. In most cases, the intensity of the toxic effect observed is proportional to the increase in dose. There is a critical value called '**no observed adverse effect level**' (or **NOAEL**), the dose at which no adverse effects are observed or expected in animals.



Schematic representation of the dose to effect relationship and determination of *thresholds:*

- *LOAEL or 'lowest observable adverse effect level': this is the lowest dose of a chemical for which a harmful effect can be observed during in a toxicity study (sometimes DMENO in French, which stands for 'dose minimale avec effet nocif observé/minimum dose with adverse effect observed').*
- *NOAEL or 'no observable adverse effect level': this is the highest dose of a chemical that produces no observable adverse effects in a toxicity study. In French, they refer to no observable adverse effect dose (DSENO), or 'dose sans effet/no toxic effect dose', 'dose maximale sans effet/maximum dose without effect' or 'dose maximale sans effet néfaste observable/maximum dose without observable adverse effect'.*

For the **exposure pathway**, first the acute toxicity of the chemical contaminant is assessed by oral exposure pathway, then cutaneous and pulmonary pathways.

For the **duration of exposure**, intense exposure over a short period of time is distinguished from prolonged exposure:

- **Acute toxicity or short-term exposure**: the effect is induced by the ingestion of a single large dose of a chemical substance, or cumulative doses over a short period. A practical way of characterising the toxicity of a substance is to determine its median lethal dose (LD_{50})[20] **or its acute reference dose (ARfD)**.
- **Chronic toxicity or long-term exposure**: this occurs only after prolonged exposure to a low dose of a chemical substance. This does not refer to a 'threshold dose' of toxicity, but the **absence of risk** to the consumer. Chronic toxicity is defined by the observation of, among others, carcinogenic effects (1 to 2 year studies in rodents), effects on the nervous or immune system (in adults or foetuses) effects on reproduction and effects on the genetic material of mammalian cells (e.g.: *in vitro*, human blood cells).

There is not necessarily a link between acute toxicity and chronic toxicity: a pesticide with high acute toxicity can have low chronic toxicity and vice versa (e.g.: the fungicide captafol).

By determining the NOAEL, by applying **safety factors** (linked to the scientific uncertainty of the animal method and the need to extrapolate data to humans), a number of TRVs could be proposed, such as:

- **ARfD** (Acute Reference Dose) = LD_{50} / safety factors. Pesticides with a very high LD_{50} may be the cause of severe food poisoning. ARfD is the quantity of an active substance A that the consumer may ingest during a single meal or day without significant risk, expressed as substance A mass per kilogram of bodyweight, per day (mg/kg/w/d).
- **ADI** (Acceptable Daily Intake) = NOAEL / safety factors. This is a chronic reference dose corresponding to the daily administered dose of this active substance below which there is no health effect, expressed as substance mass per kilogram of bodyweight, per day (mg/kg/w/d).

The safety factor (SF) is most commonly 100:

- a species factor of 10: it is assumed that the human species is 10 times as sensitive as the most sensitive animal species tested;
- an individual safety factor of 10: in a human group, not all individuals have the same sensitivity, some may be more sensitive than average (children, pregnant women, the elderly etc.).

The safety coefficient is by convention 100 for a two-year study and for non-carcinogenic compounds, 500 for a 90-day study and, if there is the slightest doubt, this coefficient is raised to 1,000.

20 LD_{50} or median lethal dose: Quantity of a substance ingested that can cause the death of 50 % of the population, expressed as substance mass per kilogram of bodyweight.

The ADI values are either equal to or lower than the ARfD values. ADIs and ARfDs are set either by the European Commission (EFSA) or by international bodies (FAO/WHO).

Examples of relationship between LD_{50} and the ARfD:

Active substance	LD_{50} (mg/kg)	ADI (mg/kg/j	ARfD (mg/kg/j)
Carbendazim	> 10000	0.02	0.02
Chlorpropham	4200	0.05	0.50
Chlorpyrifos	64	0.01	0.10
Cyromazine	3387	0.06	0.10
Deltamethrin	87	0.01	0.01
Difenoconazole	1453	0.01	0.16
Dimethoate	245	0.001	0.01
Imazalil	227	0.025	0.05

3. **Exposure assessment:** estimating **dietary intakes** according to the most exposed groups of consumers (children, pregnant women, the elderly or sick, as well as vegetarians who consume more plant products than others). This estimate may be qualitative, semi-quantitative or quantitative. It can be conducted through a *deterministic* or *probabilistic* approach.

   - In a **deterministic approach**, exposure is calculated based on a single piece of consumption data (for example, the percentile 97.5) and a single piece of concentration data (for example, the median concentration). The result, a single value, is obtained by simply multiplying these two values.
   - In a **probabilistic approach**, exposure is determined by using all of the consumption levels of each individual of a given population and all of the concentrations obtained through analysis: a distribution of exposure values is thus obtained.

     To carry out this assessment, data must be available:

   a. *On the contamination of the foodstuff*

      The concentration at the time of consumption must be known. Managing chronic risk requires a large amount of data from concentrations usually found in the foods.

   b. *On the consumption of the foodstuff*

      For the calculation, data on eating habits (consumer surveys) will be needed. The estimate is based either on the median (in grams per day) consumption per day of the population as a whole or, to take account of 'major consumers', on the percentiles (P97.5, P90) of consumption per day. Thus, to estimate the consumed portion of a given food product (water, plant or animal product), a curve is established for distribution of daily consumption within a population. The consumption value is usually taken as 97.5 percentiles (or P97.5)

of the consumption distribution, *i.e.* the major consumers (also known as 'LP'" or large portion consumers).[21]

Examples for consumption (in grams) of certain plant products:

Food	Average	P25	P50	P75	P97.5
Beans	83.4	57	77	103	175
Tomatoes	110.3	88	108	129	178
Bananas	143.9	118	134	160	267
Grapes	144.1	94	129	175	337

(source: *Institute of Public health*, consumer survey conducted in Belgium)

Certain **specific categories** of the population also need to be taken into account, where possible, and where justified (e.g. **adults and children** for whom the level of risk may differ according to differences in consumption and bodyweight).[22] Consumption data must take into account socio-economic and cultural influences (e.g. vegetarians), factors relating to the seasons, age differences, consumer behaviour (e.g. ethnic groups, religious prohibitions) etc.

4. **Risk characterisation:** is the risk acceptable? It must be checked that intakes are not excessive and do not exceed the value ranges considered acceptable.

   Risk characterisation is an estimate based on the integration of all data obtained in the preceding steps. It aims to determine the probability of a hazard arising, as well as the magnitude of the undesirable effects associated with it. Risk characterisation translates, quantitatively and/or quantitatively, the probability and severity of the adverse health effects that may arise in a given population: hazard x occurrence x effects.

   Risk characterisation can be expressed qualitatively (high, medium or low risk) or quantitatively (e.g., as a percentage of ARfD for a group of consumers or as a percentage of ADI for the population).

   The risk characterisation must explicitly take into account variability, uncertainties (incomplete data, partial knowledge), as well as assumptions made, with the aim of providing an idea of the reliability of the risk estimate.

21 Unfortunately, there is no reliable consumption survey for ACP countries, which hinders a reliable risk assessment for local populations. In the absence of data, reference is made to the values of the GEMS/FOOD Regional Diets, WHO 2003.

22 In particular, vulnerable groups (called YOPIs: *young, old, pregnant and immunosuppressed*).

1.5.3. The two situations in which a risk assessment is carried out

The risk assessment process will be performed in **two situations** that should be clearly distinguished here:

- **Situation 1**: laboratory analysis reveals that a ready-for-consumption foodstuff[23] contains a pesticide residue whose concentration exceeds the current standard (residue in mg/kg > MRL).

  The questions asked are as follows:

  a. Is there a risk to the consumer if this food is ingested? If the response is yes, market exclusion measures must be taken as soon as possible to prevent the marketing and consumption of the batches from which this product originates (preventive seizure, withdrawal or recall of batches).

  b. What measures should be taken? For example, permanent seizure of the batches after verification of the analysis reports (and, potentially, counter analysis to confirm) and destruction or recycling of the batches (biomass or animal feed).

This situation is comparable to managing a crisis. It requires a rapid review and immediate response from the risk manager (the local authorities in charge of food chain safety). The **value to refer to here is the ARfD**, since this poses an immediate risk (acute exposure).

- **Situation 2**: the risk manager (local authorities in charge of food chain safety in liaison with producers and their professional bodies, and perhaps other stakeholders such as laboratories and consumer associations) have various data from analyses of residues performed on a **panel of foodstuffs** collected from various locations (fields, fish auctions, warehouses, markets, shop stalls, etc.) and at various times (months, seasons, years) by several laboratories. Some of these analyses indicate that MRLs have been exceeded, but not the majority (a 'normal' situation is, for example, a frequency of exceeding the MRLs by between 2 and 5 %, for all foodstuffs).

The questions asked are as follows:

a. Is there a risk to the local population exposed daily to these residue levels?

b. What might be the effects of repeated exposure, that is, are the standards adopted the standards adopted sufficient to protect the population?

c. How can the risk be managed effectively (monitoring, targeted controls?)?

d. What are the most 'high-risk' foods and how can the risk be prevented for these specific foods?

23 Food (foodstuff): Any substance or product, whether processed, partially processed or unprocessed that is intended to be, or reasonably expected to be, ingested by humans. This term covers drinks, chewing gum and all substances including water that are intentionally added to foodstuffs during their manufacture, preparation or treatment.

e. How can we communicate to stakeholders about the risk, and the control measures required to reduce the risk?

This situation involves management of a known and accepted risk. It requires a thorough and regular review (e.g. every year or with every new 'control programme' decided on by the authorities) to check whether the risk management measures are still appropriate to maintain the risk at a level considered acceptable. It is also within this framework that the standards (MRLs) are set. The **value to refer to here is the ADI**, since this is a long-term risk (chronic exposure over a lifetime).

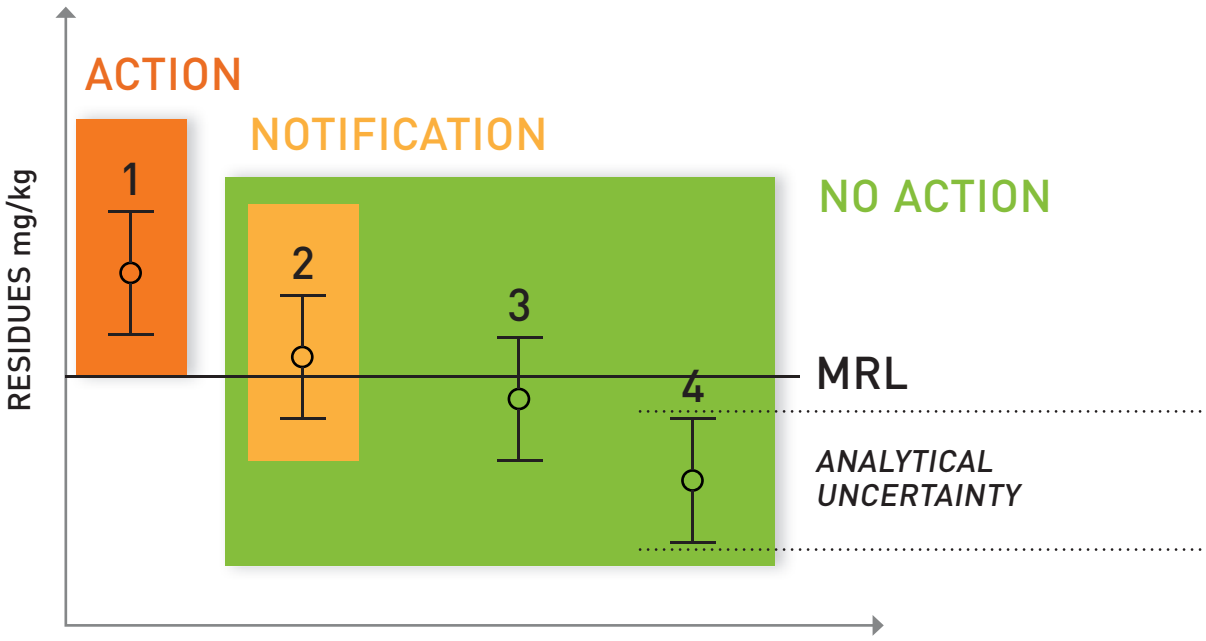
1.5.4. Acute risk management and assessment (situation 1)

To find out whether a situation is high-risk, the **'action limits'** must first be established.[24] Even if the analyses are carried out, preferably, in a laboratory accredited to ISO17025 standard for pesticide residues in plant and/or animal matrices, the values delivered are shrouded in a degree of 'uncertainty' that is essentially generated by the sampling (which cannot be 100% representative) and by the analysis itself (the method and the aptitude to perform it, *i.e.* the laboratory's performance). In the desired concentrations, close to the MRL values, this uncertainty is around +/- 50% of the value found.[25] In practice, this means that the situation will only be of concern if the value given by the analysis report exceeds the MRL by a value equal to the MRL + 0.5 x MRL.

For pesticide residues in a foodstuff, the following 4 cases illustrated in this figure can be considered (according to technical document SANCO/10232/2006 of DG HEALTH, EC):

24 The so-called 'action limit' is the threshold value from which an action must be initiated. In this case, it is the assessment of risk, with calculation of the PSTI.

25 Considering the results obtained from EU inter-laboratory tests for fruit and vegetables, using multi-residue methods, it was considered that an uncertainty figure of 50% (corresponding to a confidence level of 95%) would cover most of the variation in the results of these laboratories. This uncertainty rate of 50% has therefore been recommended by the EU's regulatory authorities for cases where the MRLs are exceeded. This recommendation is consistent with the recommendation of the Codex Committee on pesticide residues (CCPR 2005, ALINORM 05/28/24). A prerequisite for using a 50% default expanded uncertainty is that the laboratory proves its own calculated expanded uncertainty to be less than 50%. However, in cases where a MRL being exceeded at the same time results in exceeding the acute reference dose, an expanded uncertainty with a lower confidence level may be applied as a precautionary measure (source: Document No. SANCO/10232/2006, *Quality Control Procedures for Pesticide Residues Analysis*).



Source : Document n° SANCO/10232/2006
"Quality Control Procedures for Pesticide Residues Analysis"

- Case 1: the MRL (value and its uncertainty) is **exceeded by more than 0.5 times its value**. There must be a calculation of PSTI (*Predictable Short-Term Intake*) to determine if there is a risk to consumers. This is a non-conformity that entails criminal and/or financial penalties for the producer.
- Case 2: the MRL is exceeded, but by less than 50 % of the value of the MRL. Judged to be a non-conformity, however **no action** other than a simple notification to the producer (warning).
- Case 3: the mean value of the residue does not exceed the MRL, but the uncertainty around the median overlaps the MRL. Judged to be in conformity.
- Case 4: conformity. Neither the mean value of the residue nor the uncertainty exceed the MRL value.

In 'case 1', the toxicological risk to consumers (adults and children) should be estimated by a calculation of intake during a meal/day, according to the PSTI formula (WHO and DG SANCO document 3346):

$$PSTI = \frac{((U * OR * v) + (LP-U) * OR) * Pf}{bw}$$

where:

U = units (unit weight of the food) in kilograms (see data table)
OR = observed residue, **concentration determined at analysis** (n mg/kg > MRL)
v = variability factor = 0, 5 or 7 (if U < 25g: v = 0 - 25 < U < 250g: v = 7 - U > 250g:

v = 5). This variability factor is linked to the unit weight of the food. It represents the difficulty in sampling this food (e.g. sampling cereals is easy: v = 0).

Pf = processing factor (washing, peeling, baking etc.) due to lack of data in general =1

bw = bodyweight of the group considered (adult: 76 kg; children: 14.6 kg[26])

The PSTI formula therefore adapts according to the type of food:

Sampling conditions	Examples	Formula (By default, Pf = 1)
U < 0.025 kg (v=0)	Cereals Strawberries, Pepper Cherries	$PSTI = \frac{LP * OR}{bw}$
U > 0.025 kg et U < LP	Apples Oranges Mangosteen Dried fruit	$PSTI = \frac{(U * OR * v) + (LP - U) * OR}{bw}$
U > 0.025 kg et U > LP	Watermelon Pineapples Melons	$PSTI = \frac{LP * OR * v}{bw}$

The conclusion from this risk assessment is the **characterisation of risk** by comparison between the estimated exposure value and TRV:

- If PSTI > ARfD, there is a **proven risk** to the consumer.
- If PSTI < ARfD (as long as an ARfD value has been set), there is **no toxicological** risk to the consumer.

In the absence of an ARfD, comparison with the ADI is possible, but consulting a toxicology expert is then necessary to establish whether this makes sense or not. However, substances that do not have ARfD values are rare: they are either old substances (which are no longer permitted) or products with non-existent acute toxicity.

1.5.5. Chronic risk management and assessment (situation 2)

1.5.5.1. Chronic risk assessment

Consumption of food products with traces of pesticides that vary in size (residues from treatments applied + accidental contamination from drift or from previously treated soil or contaminated irrigation water), day after day, at each meal, potentially represents a long-term health risk if these residues reach some of our organs

26 Note that bodyweight value (bw) varies by source. The WHO uses a body mass of 60 kg for adults.

and harm some of our vital functions. It is therefore normal to be concerned about this risk, to assess it and manage it as well as possible (unless a radical step such as doing without pesticides altogether is taken).

To assess chronic risk, there must be an estimation of daily exposure not to a contaminated food, but to a set of foods (a diet) in which a residue from the same active substance may be present if at least one product formulated from this substance is permitted on the crop. After this estimate, this value should then be compared with the acceptable limit, *i.e.* ADI, which is the TRV for chronic toxicity.

According to this approach, it is therefore necessary to begin by identifying **all of the agricultural products on which the active substance *z* is permitted for use**. On this basis, the **Theoretical Maximum Daily Intake** (or TMDI, in mg per day) may be calculated. The TMDI is the theoretical maximum quantity of a given active substance that an individual is likely to ingest daily throughout their lives via their total food intake (in milligrams of active substance per day). The TMDI is a **maximalist approach to exposure** because it takes into account systematic contamination of all foods at the regulatory threshold of the MRL.[27] If the agricultural product is not consumed in its raw state, consideration should also be given to any processing that may reduce the initial levels of pesticide residues. This is referred to as estimated daily intake (or EDI).

The TMDI of an active substance

$$z = \Sigma$$

[Consumption per day of food x MRL of the food]

Take the example of an insecticide permitted on tomatoes (MRL: 0.05 mg/kg), cucumbers (1.00 mg/kg) and aubergines (0.05 mg/kg). Consumption (in kg per day) is given in the consumption tables for these foods for an adult.

Tomatoes:	0.05 mg/kg x 0.130 kg/j = 0.0065
Cucumbers:	1.00 mg/kg x 0.056 kg/j = 0.0560
Aubergines:	0.05 mg/kg x 0.087 kg/j = 0.0043

TMDI = 0.0668 mg/d

27 Beyond this threshold, it would not make sense, since a commodity whose residue is greater than the MRL would not be marketed or consumed. It is therefore the 'theoretical maximum'.

The TMDI is then expressed in mg/kg of body weight per day, **to be compared with the ADI. For the chronic risk to be acceptable**, the TMDI (like the EDI) must be less than the ADI. **In our example**, if the value of the ADI is equal to 0.5 mg/kg, the TMDI represents 13.4% of the ADI.

1.5.5.2. Chronic risk management through the MRLs

Managing day-to-day risk means, in practice, ensuring that most of the foods placed on the market do not contain excessive quantities of residues. To achieve this goal, at least **5 conditions** must be met:

1. To have, prior to its authorisation on a crop, **assessed the dangerousness** of a substance (define its toxicity) to **establish the usage conditions** of the commercial product **(GAP[28]) and studied the risk associated with the use** of this product (which is carried out within the framework of Regulation (EC) No. 1107/2009, which sets out the conditions for the placing of active substances and commercial products on the market). Only permitted active substances will therefore have a tolerated residue level (MRL). As soon as the active substance is withdrawn, its MRL is automatically deleted (and brought into line with the threshold, the LOQ).
2. To have defined a **regulatory framework** for setting acceptable standards for pesticide residues in foodstuffs (achieved under Regulation [EC] No. 396/2005).
3. To have defined a **scientific process** for establishing standards (MRL).
4. To implement a monitoring system based on a sampling plan.
5. **To inform the producers** of the conditions for using the product (Good Agricultural Practices to be adhered to) through labelling, information and training.

The first stage for a permitted active substance is therefore to establish the Maximum Limit applicable to pesticide residues (MRL) **for each** 'active substance – foodstuff' **pair.**

The **MRL**[29] **is a regulatory standard** for a permitted concentration of a given pesticide in a food, because if this residue was consumed daily by a human population over their lifetime, it would not have undesirable side effects on the health of the individuals. The MRLs are by no means reference toxicological values, but **plant health quality standards** that apply to agricultural producers and, to some extent, to the agri-foodstuffs industry. In other words, they concern an **agronomic threshold**. An importer or producer who exceeds the MRL may be charged severe fines for having placed a non-compliant product on the market. Exceeding the MRL does not, therefore, necessarily mean there is an immediate

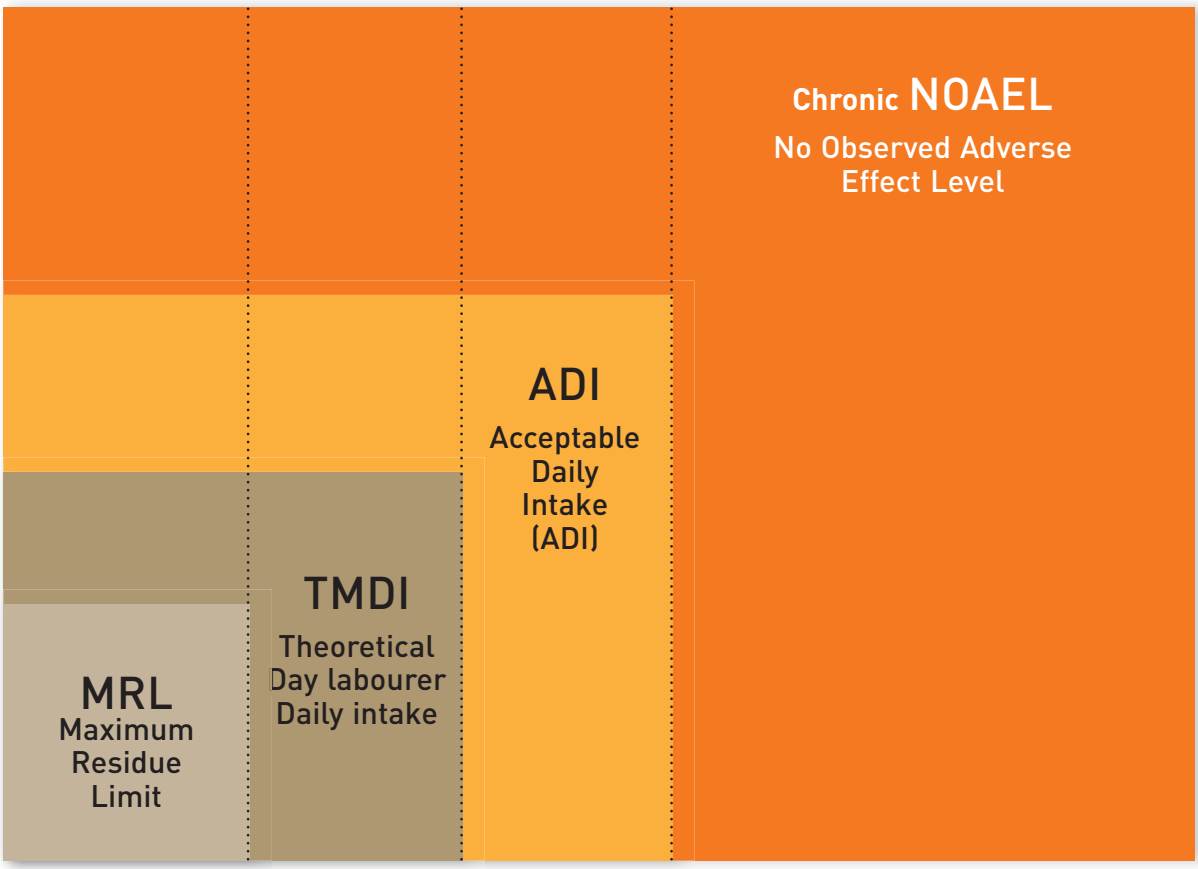
28 Good Agricultural Practices.

29 MRL: maximum concentration of a pesticide residue (expressed as mg/kg) legally permitted in or on food commodities and animal feeds (*Codex Alimentarius*).

hazard (*cf.* above, situation 1). The MRLs are not absolute data. They are subject to revision in the context of the authorisation and reauthorisation records for the active substances.

MRL's serve a dual purpose: to protect the health of consumers and monitor adherence with authorised agricultural practices or 'Good Agricultural Practices' (GAP).

These agronomic thresholds are governed by Regulation (EC) No. 396/2005. Identifying them requires advice from experts from the European Food Safety Authority (EFSA). This authority establishes a threshold according to the lowest residue concentration of the active substance that can be measured and recorded through routine monitoring of the agricultural products. They are calculated in an agricultural product so that a theoretical maximum daily intake (TMDI) of residues from a given pesticide is less than the value of the ADI of that pesticide. The following diagram provides a simplified view of the safety thresholds in the process of establishing MRLs:



Simplified diagram of relationships between TRV and agricultural thresholds

Even if definition at the chronic NOAEL threshold means there would be no effect on health, the maximum threshold is that of the ADI (ChronicNOAEL/SF, safety factors, usually 100). On the margins of the ADI-TMDI zone, there may be a hazard

to public health. Assessments must be carried out on a case-by-case basis before preventing the sale of the food in question. In the TMDI-MRL zone, there is little risk to the consumer, but the product is non-conformity for sale.

Setting an MRL requires a procedure. They are established based on field trials, applying Good Agricultural Practices. For EU MRLs, there are 4 steps:

- ***Step 1: Define a critical Good Agricultural Practice***

To carry out the field trials, the manufacturer must establish a 'GAP'[30] that includes:

1. the dose of the active substance per hectare;
2. the maximum number of applications;
3. the pre-harvest interval;
4. the application methods (apparatus, volume of slurry per hectare).

Because in the EU products may be used differently in different countries, what is referred to as the 'critical GAP' must be established. All 'critical' conditions for the MRL will be considered: high applied dose, high frequency of plant health treatments and the shortest pre-harvest interval. The shorter the interval between treatments and pre-harvest intervals, the more residues there will be.

Establishment of a critical GAP for a crop y treated with the pesticide z in three European countries:

Country	Recommended dose per hectare	Number of applications	Pre-harvest interval (PHI)
Belgium	120 g	2	14 days
Germany	200 g	3	14 days
Spain	200 g	2	7 days

In this example, the residue tests for pesticide z on the crop y will be carried out with the following GAP: 200 g dose per hectare, 3 treatments and a 7-day PHI.

- ***Step 2: Residue testing in the field and residue analysis***

Residue tests are conducted under GLP[31] in the field. These tests have only two subjects: the treated (with a product containing the active substance and according to a critical GAP that has been set) and untreated plots (with the substance). Depending on the type of foodstuff,[32] the number of tests required will vary, but

30 Good Agricultural Practices.

31 Good Laboratory Practices These principles are defined in the OECD (Organisation for Economic Co-operation and Development) Monographs They require the establishment of 'study plans' and an internal quality control system before, during and after the GLP study. These GLP studies can only be carried out by laboratories that are 'GLP-certified for residues' (including for field trials and multi-sites).

32 A distinction is drawn between 'major crops' and 'minor crops', depending on the agricultural area cultivated in the EU and the annual production of this crop. The number of trials required for a major crop (e.g. wheat) is twice that of the so-called minor crops (e.g., radishes). Note that crops under cover are subject to an additional assessment to be carried out under these conditions (no extrapolation from the field to greenhouses, or vice-versa).

repetitions over time are necessary. These tests are conducted in different climatic zones over at least two cropping seasons.

In the example below, 9 trials are carried out in the field according to GLP principles (the active substance content in the formulation used, the concentration and homogeneity of the slurry during the trial are also checked). The residue content is analysed for GLP (with a validated method) at the time of harvest, respecting the pre-harvest interval (PHI) proposed in the critical GAP.

Example of residue analysis results obtained in 9 GLP field trials:

Residue test result		
Trial number	**Residue (mg/kg) at harvest**	**Parameters (mg/kg)**
Trial 1	< 0.03	Mean = 0.252 Standard deviation = 0.182 Highest concentration = 0.62 STMR (median residue) = 0.23
Trial 2	0.06	
Trial 3	0.11	
Trial 4	0.15	
Trial 5	0.23	
Trial 6	0.29	
Trial 7	0.33	
Trial 8	0.45	
Trial 9	0.62	

- *Step 3: Calculation of TMDI*

From the set of data obtained from the 9 trials carried out, **two calculations will be made.**

A **calculation of TMDI** incorporating the median residue value of the MRL (STMR) in the place of the MRL, to verify that the supplementation does not result in the ADI being exceeded.

As a reminder TMDI = Σ [Consumption per day of food x MRL of the food]. If the test was conducted on courgettes, for example, the TMDI will be calculated to compare it to the ADI of the substance as follows:

Tomatoes: 0.05 mg/kg x 0.130 kg/d = 0.0065
Cucumbers: 1.00 mg/kg x 0.056 kg/d = 0.0560
Aubergines: 0.05 mg/kg x 0.087 kg/d = 0.0043
Courgettes: **0.23** mg/kg x 0.092 kg/d = 0.02116

TMDI = 0.08796 mg per day

If the value of the ADI equals 0.5 mg/kg, the TMDI represents **17.6 % of the ADI.** There is therefore no additional risk to the consumer in permitting this substance on the crop if the GAP normally recommended is respected.

This calculation can also be performed using the EFSA's PRIMo model. At Community level, the EFSA's PRIMo scheme compiles a set of national food models.

For the sake of completeness, note that the calculations can be made for both acute exposure (NESTI)[33] and for chronic exposure (NEDI)[34] using calculation spreadsheets developed by the PSD (Pesticides Safety Directorate, UK). Several nutritional models based on different food consumption data are used to assess a population's exposure to pesticide residues. Consumer exposure estimates will differ, depending on the nutritional models chosen. This is why the WHO has established 5 regional model diets (GEMS/FOOD[35]) that have been adapted to different regions of the world (Europe, including the USA and Canada, Latin America, Africa, the Middle East and Far East). These models are based on respecting a nutritional balance that is broadly suited to these five regions. They will be used by international assessment authorities to estimate exposure to pesticides.

- ***Step 4: Proposing an MRL according to OECD methodology***

A calculation using various approaches to **propose an MRL to be set** for this active substance-crop pair. (According to the Lundhen document: SANCO Guidance Doc -7039/VI/95).

Based on this data, several calculations will be carried out using three calculation methods recommended by the OECD to provide a statistical basis for a proposed MRL:

- Parametric method *No. I*:
  R_{max} = mean + 4 *x* standard deviation, *i.e.* 0.252 + 4 * 0.182
  R_{max}= **0.98** mg/kg
- Parametric method *No. II*:
  R_{max}' = 3 * average * CF (correction factor)[36],
  i.e. 3 * 0.252 * (1-(2/3)*(1/9))
  R_{max}'= **0.70** mg/kg
- Non-parametric method *No. III:*
  HR (highest value of the residue) = **0.62** mg/kg
  The proposed MRL will be the highest value provided by these three methods, in this case: **0.98 mg/kg**.

33 Acute: An Excel spreadsheet from the PSD may be used to calculate the *National Estimates of Short-Term Intakes* (NESTIs) at percentile 97.5 of consumption.

34 Chronic: An Excel spreadsheet from the PSD may be used to calculate: (a) Individual commodity National Estimates of Dietary Intakes (NEDIs) and (b) Total dietary intake calculations (Total NEDIs).

35 Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme.

36 This correction factor will make it possible to assimilate non-quantifiable data because they are below or above the LOQ. CF = (1 - 2/3* [proportion of censured data]).

1.6. REGULATORY FRAMEWORK FOR RESIDUES

1.6.1. For the European Union

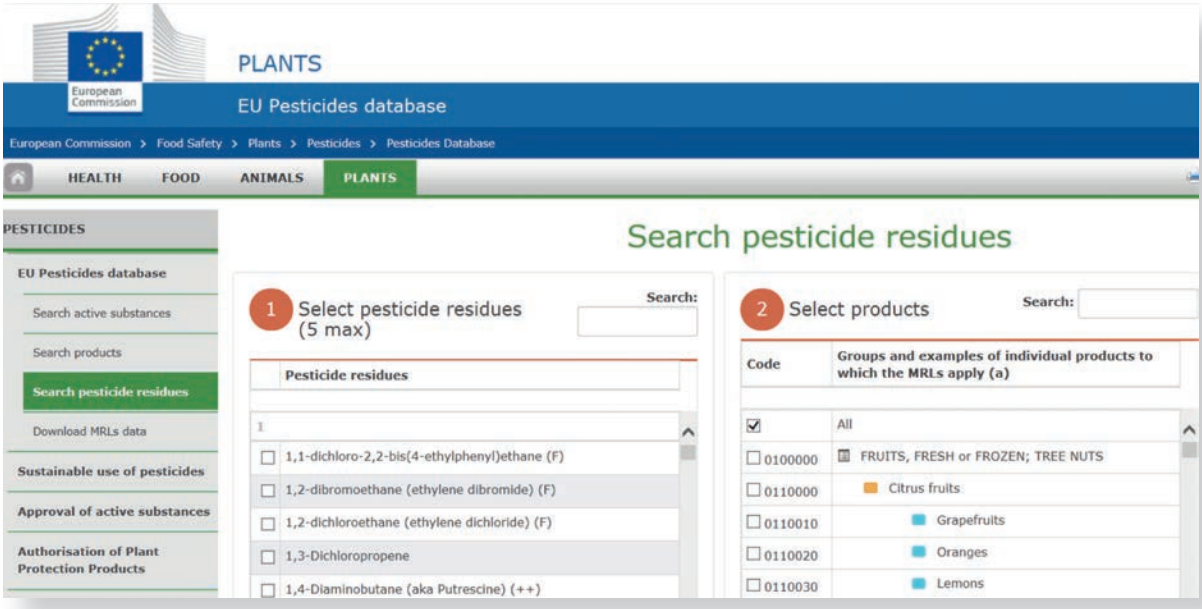
Prior to 1 September 2008, legislation on pesticide residues was a joint responsibility of the European Commission and of the Member States. National MRLs varied from country to country, and could be below or above European thresholds. This situation caused confusion as to the applicable MRL and complicated trade. Certain thresholds were set as non-tariff barriers and concealed a desire to protect markets from imports of certain products. In a single European market, the situation was no longer acceptable. Legislation on pesticides MRLs has therefore become harmonised between the Member States. Today, for the EU, the main regulatory framework is Regulation (EC) No. 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin, amending Council Directive 91/414/EEC.

This legislation also applies to **imports of food** from third countries. It therefore indirectly influences the rules of production in ACP countries. Foodstuffs intended for human and animal consumption produced in ACP countries and exported to the European Union must therefore comply with the European MRLs, as specified in the annexes to Regulation (EC) No. 396/2005. When a substance is not listed in any of the aforesaid annexes (and is not therefore subject to any MRL), it can still be used on crops for export to the EU if the residue levels do not exceed the default MRL of 0.01 mg/kg (equivalent to the LOQ).

There is a database specifying the MRL applicable to each crop and to each pesticide on the European Commission's public Web site:

http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN

European MRL database homepage:



Where normal use of a substance results in the de facto presence of residues greater than the MRL, an application for setting an **import tolerance** value may be filed. An import tolerance is an **MRL that meets the European food safety standards** set for an active substance used on a product (or foodstuff) imported into the EU. An import tolerance application must contain information on residues, toxicology and risks to consumers, as well as a certificate of authorisation for the product in the country of production and a proposed MRL established according to the same rules as those set out above. The assessment is carried out in advance by a rapporteur Member State, before being forwarded to the EFSA. The process can last 18 months, depending on the timescales set out in Regulation (EC) 396/2005. Where there is an MRL value set by the Commission for the *Codex Alimentarius* residues, the import tolerance value will be based on this Codex MRL.

An MRL (or IT) can also be granted for a group of crops, and in this case applies to each crop from this group (the 'groups' are defined in the regulation).

The **authorities of the Member States** are responsible for monitoring compliance with the MRLs on a permanent basis and for ensuring that they are applied both to food produced within EU territory and to imported products. The **Commission** (via the FVO) **carries out inspections in Member States to assess and review their monitoring activities.** Three types of controls are carried out.[37]

- The **monitoring programmes:** these are the regular official controls carried out by the Member States to ensure compliance with the MRLs in their territory (monitoring, sampling and analysis using methods validated at Community level);
- The **national control programmes:** these are the targeted controls, based on risk to the consumer.
- The **Community control programmes**: these are controls decided on by the European Commission, which take place in all Member States. They relate to a pre-established list of commodities to be sampled and substances to be analysed. A multi-annual report issued by the Commission for the attention of the European Committee on pesticide residues.

The Member States must publish the results of their residue monitoring online, on an annual basis. They usually produce an annual report. If an MRL is exceeded, Member States may publish the names of distributors, vendors and producers of these foodstuffs. The EFSA must also publish a report on pesticide residues annually.

Note the specific point concerning drinking water. Commission Directive (EU) 2015/1787 complementing Directive 98/83/EC on the quality of water intended for human consumption, sets out the standards for drinking water.[38] It aims to protect human health from the harmful effects of the contamination of water intended for human consumption by guaranteeing the health, cleanliness and sanitation of such water. A quality limit of 0.1 µg/L (and 0.5 µg/L is accepted for all pesticides). This value corresponds to the detection thresholds of the analysis methods.

37 All of these points relating to the control plans will be discussed further in another chapter.

38 Water intended for human consumption: all water, whether treated or untreated, intended for drinking, cooking, preparing food or other domestic purposes. The water may come from the tap, a tanker lorry or tanker vessel, or may be supplied in bottles or containers.

1.6.2. For other regions of the world

At international level, the *Codex Alimentarius* Commission, established in 1961 by the United Nations (UN) and the World Health Organisation (WHO), has an expert committee on pesticide residues for the establishment of maximum residue limits. These MRLs cover a very wide range of uses and 'good agricultural practices'. They are generally higher than the European MRLs. A database contains the maximum residue limits for pesticides and the extraneous maximum residue limits adopted by the *Codex Alimentarius* Commission until its 38th session (July 2015), including:

www.fao.org/fao-who-codexalimentarius/standards/pestres/pesticide-detail/en/?p_id=246

Le lien ne fonction

In the database, users can find information on the Codex maximum residue limits (MRLs) and the extraneous maximum residue limits (EMRLE) for one or more pesticides, and for a product or group of products. Names and definitions of commodities can be found in the *Codex* Classification of Foods and Animal Feeds. Most ACP countries use the Codex MRLs as the basis for their regulations and monitoring systems. The Codex's MRLs are therefore applicable to crops grown for local and regional markets.

MRL search page in the *Codex Alimentarius* database:

Russia, the USA, Canada, Japan, *i.a.*, also have their regulations on MRLs.

1.7. ANNEX: GLOSSARY

Acute toxicity: Refers to the adverse (acute) effects resulting from exposure to a single high dose of a product or single exposure to it, usually used to describe the effects observed in experimental animals.

Approval: Certification of a product's conformity with a standard or regulation.

Autoradiography: Technique that aims to mark out a specific molecule with radioactivity. The marking facilitates the discovery of the molecule's location at cellular organelle level.

ne pas

Bioagressor: Also called crop-destroyers, these are living organisms such as fungi, bacteria and weeds that affect the health of the cultivated plants.

Carcinogenic (effect): Toxicity manifested by the onset of cancer.

Chronic toxicity: Refers to a harmful effect resulting from repeated doses of a substance, or exposure is thereto, over a relatively long period. Usually used to describe the effects observed in experimental animals.

Contaminant: Any biological or chemical agent, foreign matter, or other substances not intentionally added to food which may compromise food safety or suitability.

Cuticle: Protective layer covering the aerial organs of plants. It consists of successive deposits of wax coated in a layer of hydrophobic fatty acids, the cutin.

Epidemiology: Science studying the frequency of illnesses (incidence), their causes, distribution in society, risk factors and deaths related to these illnesses.

Exposure: Contact of a target with a chemical or physical agent during a certain period of time. Exposure is quantified by the quantity of substance coming into contact with the organism's barriers of exchange and available for potential adsorption.

Food (or foodstuff): Any substance or product, whether processed, partially processed or unprocessed that is intended to be, or reasonably expected to be, ingested by humans.

Geochemical: As it relates to the Earth, this discipline aims to understand the cycles by which most chemical elements are conducted alternately on the surface and deep within the Earth.

Hydrolysis: Breakdown of a chemical by water.

Ionisation: Process by which a neutral molecule or atom becomes a carrier of a positive or negative electrical charge.

Metabolic pathway: Series of chemical reactions that take place in a living cell, catalysed by a series of sequentially-acting enzymes.

Metabolisation: Biochemical transformation within the metabolism, that is, the process of organic synthesis and degradation in the living being.

Mineralisation: Transformation, within a biologically active environment, in particular soil, of organic matter, leading to the release of mineral substances (ammonia, water, carbon dioxide, nitrates, phosphates, sulphates).

Mutagenic (effect): This is an agent that changes its genome (usually the DNA) of an organism, raising the number of genetic mutations above the natural background rate.

Organoleptic: Refers to the taste, texture, smell and visual appearance of something.

Oxidation Reduction: A chemical reaction based on the transfer of one or more electrons between two reagents called oxidising agent and reducing agent, respectively. The oxidising agent then undergoes a reduction, that is, it gains electrons. The reducing agent, on the other hand, undergoes oxidation by losing electrons.

Partition coefficient: Distribution of the pollutant between the solid and liquid phase of the soil.

Pesticide (for agricultural use): Pesticides are products mostly obtained through chemical synthesis and whose toxic properties make it possible to combat pests. From a regulatory perspective, there is a distinction between pesticides used mainly for the protection of plants, which we call plant protection products (Directive 91/414/EEC).

Pesticide residues: One or more substances present in or on plants or products of plant origin, edible animal products or elsewhere in the environment and resulting from the use of a plant protection product, including their metabolites and products resulting from their degradation or reaction.

Phloem: Conductive tissue of the elaborated sap, which is a solution rich in glucides such as sucrose, sorbitol and mannitol in plants.

Photolysis: Any chemical reaction in which a chemical compound is broken down by light.

Plant health product: Synonym of pesticide for agricultural use, phytopharmaceutical product, agropharmaceutical product, plant protection product (PPP), commercial product, formulation, phyto product.

Radioisotope: Contraction of radioactivity and isotope; these are atoms whose nucleus is unstable. This instability can be due to an excess of protons, neutrons, or both. Radioisotopes exist naturally or are produced artificially by bombarding small quantities of material with neutrons, usually produced in a nuclear reactor. They are widely used for diagnosis or research purposes.

Residual action: Where certain chemicals remain in the environment over time after spreading or pouring.

Sampling: Process that involves choosing a part, or a number of units of a product, that best represents a study population (batches of foods etc.)

Sorption (or adsorption): Surface phenomenon by which gas or liquid molecules bind to solid surfaces, adsorbants.

Spectrometry: Set of spectral analysis methods for accessing the composition and structure of the material.

Spray nozzle: Rigid, wide conduit in which the formation of droplets is effected by the passing of pressurised fluid through a narrow orifice applied in the stopper or nozzle.

Synergist: Substances or preparations which can give enhanced activity to the active substance(s) in a plant protection product.

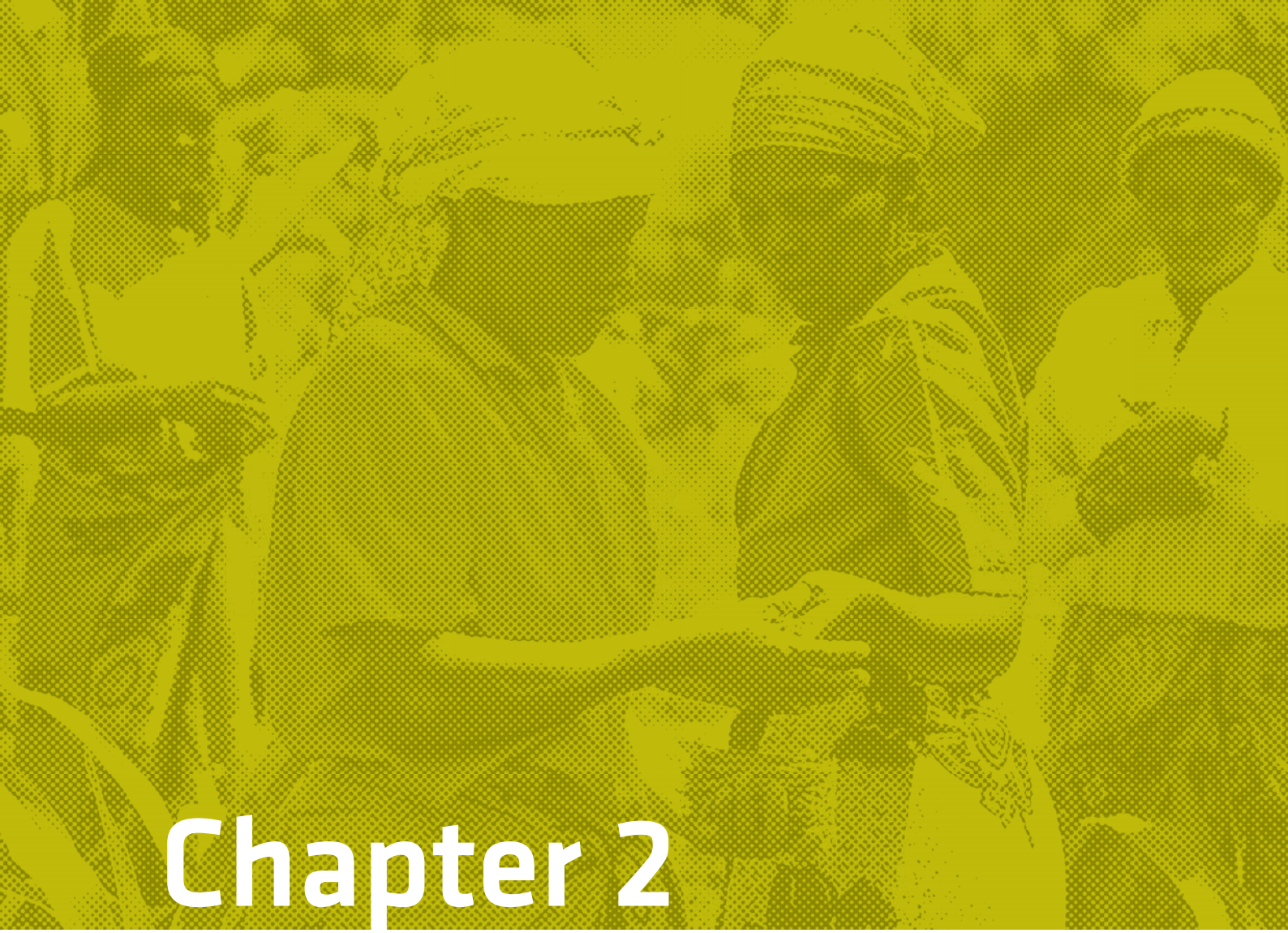
Toxicological Reference Value: Toxicological index that makes it possible, through comparison with exposure, to qualify or quantify a risk to human health.

Translocation (botany): Process of transferring organic compounds and other substances of varying solubility from the leaves to other organs of the plant, for example the growing organs (buds, flowers etc.) and the reserve organs (roots, tubers etc.). This transfer takes place through circulation of the sap into the phloem and xylem tissues.

Volatilisation: Passage of a substance from a liquid state to a vapour state.

Xenobiotic: Substance or molecule extraneous to the biosphere. These are usually pollutants, agrochemical and/or pharmaceutical residues or contaminants.

Xylem: A set of dead cell clusters aligned and surrounded by lignin. They have the ability to transmit large quantities of water and nutrients from the soil to the leaves.



Chapter 2

Official control of residues and environmental contaminants

2.1. RESIDUE CONTROL

2.1.1. Risk analysis

FAO/WHO has published a guideline[39] to help national authorities improve their food control system. In this publication, the objective of monitoring and the use of risk analysis are outlined:

"The objective of reduced risk can be achieved most effectively by the principle of prevention throughout the production, processing and marketing chain. To achieve maximum consumer protection it is essential that safety and quality be built into food products from production through to consumption. This calls for a comprehensive and integrated "from farm to fork" approach in which the producer, processor, transporter, vendor, and consumer all play a vital role in ensuring food safety and quality".

The *Codex Alimentarius* Commission defines risk analysis as a process composed of three components:

- **Risk assessment:** a scientifically based process consisting of the following steps:
  - hazard identification;
  - hazard characterisation;
  - exposure assessment;
  - risk characterisation.
- **Risk management:** the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.
- **Risk communication:** the interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk analysis must be the foundation on which food control policy and consumer protection measures are based. While not all countries may have sufficient scientific resources, capabilities, or data to carry out risk assessments, it may not even be necessary in all cases to generate local data for this purpose. Instead countries should make full use of the international data and expertise as well as data from other countries that are consistent with internationally accepted approaches. Risk assessments carried out at the international level by JECFA, JMPR, and other expert bodies are particularly useful. Developing countries should take a pragmatic approach and develop a cadre of scientists to interpret such data and assessments, and to use this information for the development of national food control programmes.

39 ftp.fao.org/docrep/fao/009/a0601e/a0601e00.pdf. Le lien ne fonctionne pas

2.1.2. Objectives of residue control programmes

Residue control programmes are generally implemented to achieve one of two objectives:

- either to assess the compliance of consumable products on the market with rules and regulations;
- or to provide information that can be used in the process of evaluating the exposure of the population to the compounds investigated.

But the residue programmes may aim to combine these two objectives.

Assessment of compliance includes testing for forbidden substances, unauthorised use of authorised substances as well as testing for compliance with established limits (e.g. maximum residue limits/levels, action levels, target levels) for the concentration of substances in the object of investigation.

Before planning a residue control programme, the objectives to be met must be clearly defined, since these objectives define which type of samples should be taken, the sampling strategies that should be used, where samples should be taken and how the sampling should be done.

Example 1:

Objective: provide information for exposure calculations

Type of sample: commodity with high consumption and/or high average concentration

Sample matrix: part eaten

Where to sample: where consumer buys the commodity

Sampling strategy: objective

Example 2:

Objective: control for illegal use of a forbidden veterinary drug

Type of sample: animal where a relevant use for drugs exists, or feed, water, etc., if relevant

Sample matrix analysed: organ where residue can be found in highest concentration

Where to sample: where illegal use can be suspected

Sampling strategy: selective or suspect

2.1.3. Sampling programme

2.1.3.1. Sampling strategies

The sampling strategy used for the sampling programme can have direct influence on the results achieved in a surveillance programme.

The 'sampling strategy' can be defined as the approach used to select the units of the target population subject to controls: businesses, animals, foodstuffs, etc. It is worth noting that the comparability and interpretation of the results relies on the sampling strategy but as well on other parameters like the analysis methods, analysis matrices, preparation of samples, methods of calculation of the results, etc. (Eurostat – Typology of sampling strategies):[40]

- **Objective sampling:**

  Strategy based on the selection of a random sample from a population on which the data are reported. It includes also other random samplings as "stratified" in subpopulations and sampling with proportional criterion, multistage sampling, etc.;

- **Selective sampling:**

  Strategy based on the selection of a random sample from a subpopulation (or more frequently from subpopulations) of a population on which the data are reported. The subpopulations are determined on a risk basis or not. The sampling from each subpopulation is not proportional: the sample size is proportionally bigger for instance in subpopulations considered at high risk;

- **Suspect sampling:**

  Selection of an individual product or establishment in order to confirm or reject a suspicion of non-conformity. It's not a random sampling. The data reported refer themselves to suspect units of the population.

2.1.3.2. Where to sample?

The place of sampling can be critical for the legal use of an analytical result e.g.:

- when testing for compliance of pesticide residues in food with MRLs, the samples must be taken after the lot/consignment has entered the market, not on farm;
- when testing for forbidden veterinary drugs, the sampling can be done at the farm or later in the production chain as long as the origin of the animal and absence of cross-contamination can be trusted.

2.1.3.3. How to sample?

The sampling techniques used are paramount to the analytical results that can be achieved.

40 circabc.europa.eu/sd/d/2fc47bd9-237a-4c79-93e0-6a4665cf3591/201_Typology_sampling_strategies.pdf.

For official sampling, sampling must be done in adherence to procedures laid down in legislation.

Guidelines could be designed in a tiered approach with general guidelines resting at the sampling institution and specific guidelines being distributed by the requesting laboratory together with the request for samples.

Guidelines should be detailed and targeted to the specific sampling situation in order to provide all necessary information for carrying out the sampling as well as packing, storing and transportation of samples and readily available for the relevant personnel (*i.e.* inspectors and sampling officers) in the local language.

For some substances and/or matrices, specific information on sampling must be available – including specifications for the type of packing, requirements for storage before transportation and time limits and conditions for transportation. Such information could be provided by the laboratory responsible for requesting the samples.

Inspectors and sampling officers should have a clear knowledge of the purpose of sampling, including the sampling strategies to be used. Depending on the actual organisation, this information could be part of a sampling guideline; general knowledge about sampling strategies, etc., could be subjects for training of sampling personnel.

2.1.4. Data management

Data collection and maintenance is an important task of any monitoring programme and a fundamental component of risk assessment.

Data collected during sampling and analysis should be stored either manually or electronically in such a way that data will not be compromised by either tampering or loss.

For enforcement, the most important information (apart from the analytical result) is the data needed to identify the sample and the responsible producer, while for monitoring and exposure programmes, a clear and systematic description of the sample type becomes more important. For mixed programmes, care must be exercised to gather and store both types of data.

The data structure should be tailored to the actual situation; if the data system must store data from many different chemical domains, a general structure may be useful. The EFSA standard sample description includes a list of standardised data elements (items describing characteristics of samples or analytical results such as country of origin, product, analytical method, limit of detection, result, etc.), controlled terminologies and validation rules to enhance data quality. These can be used to describe analytical samples for evaluation purposes.[41]

41 www.efsa.europa.eu/en/efsajournal/pub/1457.htm.

2.1.5. National and international database with residue data

The *EFSA Journal*[42] is an open-access, online scientific journal that publishes the scientific outputs of the European Food Safety Authority. EFSA's various output types are devoted to the field of risk assessment in relation to food and feed and include nutrition, animal health and welfare, plant health and plant protection, e.g. the European Union Report on Pesticide Residues in Food.[43]

Also the United States Department of Agriculture publishes annual summaries of their Pesticide Data Program.[44]

The World Health organization (WHO) homepage information on chemical risks in food[45] can give information, for example, on POPs and melamine. Since 1976, WHO has implemented the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food),[46] which has informed governments, the *Codex Alimentarius* Commission and other relevant institutions, as well as the public, on levels and trends of contaminants in food, their contribution to total human exposure, and significance with regard to public health and trade. The programme was implemented by the WHO in cooperation with a network of more than 30 WHO Collaborating Centres and recognized national institutions located all around the world.

2.1.6. Rapid Alert System for Food and Feed and exchange of information

The EU Rapid Alert System for Food and Feed (RASFF)[47] is used to provide food and feed control authorities with an effective tool to exchange information on measures taken responding to serious risks detected in relation to food or feed. This exchange of information helps authorities to act more rapidly and in a coordinated manner

42 www.efsa.europa.eu/en/publications/efsajournal.htm.
43 www.efsa.europa.eu/en/efsajournal/pub/2430.htm.
44 www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5091055.
45 www.who.int/foodsafety/chem/en/.
46 www.who.int/foodsafety/chem/gems/en/.
47 ec.europa.eu/rasff.

Le lien ne fonctionne pas

in response to a health threat caused by food or feed. Whenever an EU member state has any information relating to the existence of a serious direct or indirect risk to human health deriving from food or feed, this information is immediately notified to the RASFF. RASFF notifications usually report on risks identified in food, feed or food contact materials that are placed on the market in the notifying country or detained at an EU point of entry at the border. The notifying country reports on the risks it has identified, the product and its traceability and the measures taken.

According to the seriousness of the risks identified and the distribution of the product on the market, the RASFF notification is classified as alert, information or border rejection notification. A 'border rejection notification' concerns a consignment of food, feed or food contact material that was refused entry into the EU for reason of a risk to human health and also to animal health or to the environment if it concerns feed.

The contamination of food by chemical hazards is a worldwide public health concern and is a leading cause of trade problems internationally. Contamination may occur through environmental pollution of the air, water and soil, such as the case with toxic metals, PCBs and dioxins, or through the intentional use of various chemicals, such as pesticides, animal drugs and other agrochemicals.

2.2. RESIDUES OF VETERINARY DRUGS

Food-producing animals may be treated with medicines to prevent or cure disease. This can leave residues in the food products from the animals. The legislation on residues of veterinary medicinal products used in food producing animals should provide for a scientific evaluation before respective products are authorised. If necessary, maximum residue limits (MRLs) must be established and in some cases the use of substances prohibited, in order that proper use of authorised substances do not leave residues that are compromising consumer safety.

The description in the present chapter of controls with residues of veterinary drugs in food products of animal origin will be described with reference to EU legislation. Special focus will be on the surveillance system that must be in place to ensure fulfilment of EU export requirements for animals and products of animal origin.

2.2.1. Legislation concerning residue controls

With regards to food safety, Article 11 of Regulation (EC) No. 178/2002 require that *"food and feed imported into the community for placing on the market within the Community shall comply with the relevant requirements of food law or conditions recognised by the Community to be at least equivalent thereto or, where a specific agreement exists between the Community and the exporting country, with requirements contained therein"*.

The present manual is widely based on legislation and practices required for or implemented by EU Member States. According to the legislation cited above, an exporting country can implement the EU requirements otherwise as long as this implementation is equivalent with the EU requirements.

Several legal acts should be taken into consideration, when the national residue control plan (NRCP) is prepared and implemented for export of animals and products of animal origin to EU. All of this legislation is publicly available and can be accessed via the European Commission's EUR-Lex Web site.[48]

EU countries must monitor food of animal origin for the presence of residues and draw up respective residue monitoring plans. How these plans need to be designed and implemented is outlined in the following legislation:

- Regulation (EC) No. 178/2002/EC[49]: general principles and requirements of food law;
- Directive 96/23/EC:[50] sampling frequency and level, controlled substances for each food;
- Decision 97/747/EC:[51] rules for milk, eggs, honey, rabbits and game meat;
- Decision 98/179/EC:[52] official sampling and treatment of samples;
- Decision 2005/34/EC:[53] standards for testing residues in products of animal origin imported from non-EU countries.

Presently, the requirements for residue control in the Member States are regulated by the rather stiff prescriptions in Directive 96/23/EC. This legislation includes requirements for control of pesticide residues (either authorised for use as veterinary drugs or present as contaminants from feed...) and environmental contaminants.

48 eur-lex.europa.eu/en/index.htm.
49 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32002R0178:EN:NOT.
50 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31996L0023:EN:NOT.
51 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31997D0747:EN:NOT.
52 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31998D0179:EN:NOT.
53 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32005D0034:EN:NOT.

It seems very likely, however, that future regulations[54] will be more flexible, putting more weight on a risk based sampling program laid down by the member state, while still maintaining a core of coordinated control for the whole EU. Such change in legislation could also influence the requirements for residue control in countries, exporting food of animal origin to the EU.

2.2.2. Planning and implementing residue control

The task of setting up and implementing the national residue control should be assigned to a central public department or body. This institution should draw up the plan and coordinate the activities of central and regional departments involved in the implementation of the plan, including inspections, sampling, analysis, reporting and follow-up activities.

The following sections describe the elements necessary for the implementation of the residue plan. Section 2.3 describes how to set up a national residue control plan (NRCP).

2.2.2.1. Sampling strategy

The residue control plan should be aimed at detecting all illegal treatment (*i.e.* use of unauthorised substances or products or misuse of substances authorized for other use or purposes) and controlling the compliance with the maximum residue limits (MRLs) for residues of veterinary drugs. The control plan should also be aimed at surveying and revealing the reasons for residues in food of animal origin.

To optimise the control of residue levels, the sampling should be targeted at detecting the presence and highest levels of those substances that the samples are to be analysed for. This implies that the sampling may not be representative for food on the market – and may include matrices not included in the diet of the consumer, e.g. offal, urine, feed and water.

The sampling must be unforeseen, unexpected and effected at no fixed time and on no particular day of the week. Sampling shall be carried out in variable intervals spread over the whole year. In this context it has to be considered that a number of substances are administered only in particular seasons.

Some examples of criteria for targeted sampling on farm and on primary processing establishment (*i.e.* slaughter houses) are given in the Annex to Commission Decision 98/179 (CD 98/179):

54 Presently (2012), it seems that such changes will not be implemented before 2016.

Figure 1 - On farm targeted sampling (Annex to CD 98/179)

Figure 2 - Targeted sampling at e.g. slaughterhouse (Annex to CD 98/179)

In addition to the sampling defined by the NRCP, samples for follow-up investigations must be foreseen in budgets for sampling institutions and laboratories, and procedures for such follow-up actions should be laid down.

2.2.2.2. Substances to monitor

The present legislation in the EU (Directive 96/23, Annex I) classifies residues in two main categories.

Group A contains most of the substances which are prohibited from use in food producing animals in EU. Group B contains residues of many pharmacologically active substances which may be authorized for use in food producing animals in the EU. It also includes pesticides and chemical contaminants. Some overlapping exists; *i.e.* Group B also includes some substances without approved use (e.g. while all corticosteroids belong to Group B2f, only a few – e.g. betamethasone, dexamethasone, methylprednisolone, prednisolone – may have an authorised use).

Group A – having anabolic effect and unauthorised substances

1. Stilbenes, stilbene derivatives, and their salts and esters
2. Antithyroid agents
3. Steroids
4. Resorcylic acid lactones (including Zeranol)
5. *ß*-agonists
6. Compounds included in Table 2 in Regulation (EU) No. 37/2010 (including later amendments) on pharmacologically active substances and their classification

Group B – Veterinary drugs[55] and contaminants

1. Antibacterial substances, including sulphonamides, quinolones
2. Other veterinary drugs
   a. Anthelmintics
   b. Anticoccidials, including nitroimidazoles
   c. Carbamates and pyréthroids
   d. Sedatives
   e. Non-steroidal anti-inflammatory drugs (NSAIDs)
   f. Other pharmacologically active substances
3. Other substances and environmental contaminants
   a. Organochlorine compounds including PCBs
   b. Organophosphorus compounds
   c. Chemical elements
   d. Mycotoxins
   e. Dyes
   f. Others

55 Including unlicensed substances which could be used for veterinary purposes.

Annex II to Directive 96/23 lists for each commodity which Group A and Group B subgroups must be monitored for in the respective commodities (Figure 3). However, this table has been elaborated into the table shown in Annex 1, which shown the present requirements for an acceptable plan.

Figure 3 - Annex II from Directive 96/23/EC

Substances in Group A are of greatest concern to the EU as they are either banned or restricted. Non-EU countries must monitor compounds in Group A1 – A6 in the relevant commodities. If testing for the relevant substances is not in the residue monitoring plan, it may not be approved and the country would not be eligible to export these commodities to the EU.

There are several other substances banned in animal production in the EU not currently listed in Group A e.g. malachite green (for treatment of fungal disease in fish) and several growth promoting antibiotic substances banned from animal feeding stuffs in the EU because of known chemical risks e.g. olaquindox, carbadox, nifursol. If such substances are authorised in a non-EU country, particularly in livestock production for the EU market, it should consider analytical and/or other control strategies to offer equivalent guarantees to those of EU legislation, which bans their use. If an exporting country authorises the use of certain steroid hormones or beta-agonists for growth promotion or the use of stilbenes, thyrostats or estradiol, their residues control plan can only be approved if there is a 'split system' in place, which guarantees that animals (or their products) for export to the EU have not been treated at any time during their rearing.

In respect of the Group B substances, the NRCP should contain those substances which are likely to be used in the livestock production system. The choice of substances tested should be justified with a documented risk-based approach.

Tools for such risk-based approach could include:

- information on available prescription medicine for animals;
- data collection system of frequently used veterinary medicine products;
- information on available relevant medicine from other sources (e.g. non-prescription, internet and other illegal imports);
- residue control results from previous year(s);
- recommendations from scientific and administrational bodies (WHO/FAO, EU-RLs[56] and FVO);[57]
- residue control results from countries with comparable production conditions;
- RASFFs[58] (especially for import control).

In addition to the calculated minimum number of samples in each subgroup, the remaining number of required samples should be allocated according to the experience and background information of the country.

Methods for misuse of substances for growth promotion or prevention of illness is not static, but should be expected to change. Thus, strategies for sampling should be laid down by specialist with knowledge of the possibilities for misuse as well as symptoms and effects of such not prescribed use.

56 EU Reference Laboratories – ec.europa.eu/food/food/controls/reference_laboratories/index_en.htm.

57 EU Commission's Food and Veterinary Office – ec.europa.eu/food/fvo/index_en.cfm.

58 Rapid Alert System for Food and Feed – ec.europa.eu/food/food/rapidalert/index_en.htm.

2.2.2.3. Sampling methods

Sampling for control of residues of veterinary drugs and certain other compounds in animals and animal products must follow the prescriptions laid down in CD 98/179.

Guidelines equivalent to these should be readily available for the relevant personnel (*i.e.* inspectors and sampling officers) in the local language.

Statistics on sampling should be reviewed by persons responsible at a central level in order to verify that samples adhere to the aims and prescriptions for the NRCP. Results from such reviews could be communicated to representatives of institutions participating in the NRCP (e.g. competent authority, national reference laboratory, regional laboratories and sampling institutions).

2.2.2.4. Analytical methodology

Analysis of samples analysed to fulfil the NRCP shall be carried out exclusively by laboratories approved for official residue control by the competent authority. These laboratories must have an accreditation system in place and must prove their competence by regular and successful participation in adequate proficiency testing schemes.

Only those analytical techniques, for which it can be demonstrated in a documented traceable manner that they are validated and have a false compliant rate of < 5% (ß-error)[59] at the level of interest, shall be used for screening purposes in conformity with Directive 96/23. In the case of a suspected non-compliant result, this result shall be confirmed by a confirmatory method.

Confirmatory methods for organic residues or contaminants shall provide information on the chemical structure of the analyte. Consequently, methods based only on chromatographic analysis without the use of spectrometric detection are not suitable on their own for use as confirmatory methods. However, if a single technique lacks sufficient specificity, the desired specificity could be achieved by analytical procedures consisting of suitable combinations of clean-up, chromatographic separation(s) and spectrometric detection.

For Group A substances, all positive findings must be confirmed using the reference method criteria laid down in accordance with measures implemented by the Commission – *i.e.* for residues of veterinary medicines in accordance to Commission Decision 2002/657 (CD 2002/657).

There is no requirement for specific method procedures ('standard methods', 'reference methods'). Instead, criteria for performance of analytical methods and interpretation of results are laid down in CD 2002/657. For some areas (e.g. pesticides) where other specific rules have been laid down in Community legislation, CD 2002/657 does not apply.

Documents from the EU Reference Laboratories (EU-RLs) give some guidelines for implementation of CD 2002/657 and for validation of screening methods.

59 *I.e.* the rate of false negative results must be below 5% for samples having a concentration at the level of interest.

Even when a fully validated 'reference method' is transferred and implemented, the laboratory must carry out their own validation. However, some of the gained experience from the initial validation may be transferred to the new laboratory provided that this information is available and documented during the initial validation (examples of issues that can be transferred in some cases: stability of reagents, standard solutions and sample extracts; some tests of robustness). The guidelines from the EU-RLs give further guidance on validation of transferred screening methods.

2.2.2.5. Reporting

CD 98/179 lists the information that must, at least, be present in the sampling report and the information that must be available to the analytical staff. This information should be extended as needed with other information necessary to identify the sample and its origin as well as all information necessary for the proper follow-up procedure. The information must be stored and not accessible to unauthorised persons.

- ***Sampling report***

A report shall be produced and signed after each sampling procedure. The inspector collects at least the following data in the sampling report:

- address of the competent authorities;
- name of the inspector or identification code;
- official code number of the sample;
- sampling date;
- name and address of the owner or the person having charge of the animals or the animal products;
- name and address of the animal's farm of origin (when sampling on farm);
- registration number of the establishment-slaughterhouse number;
- animal or product identification;
- animal species;
- sample matrix;
- medication within the last four weeks before sampling (when sampling on farm);
- substance or substance groups for examination;
- particular remarks.

All information relevant for the interpretation of the analytical results must be collected. This can include sex, age and weight of the animal tested.

Copies of the report are to be foreseen depending on the sampling procedure. The sampling report and its copies shall be signed at least by the inspector; in case of on-farm sampling, the farmer or his deputy may be invited to sign the original sampling report.

The original of the sampling report remains at the competent authority, which has to guarantee that unauthorised persons cannot access this original report.

If necessary, the farmer or the owner of the establishment may be informed of the sampling undertaken.

- ***Sampling report to the laboratory***

The laboratory report established by the competent authorities must contain at least the following information:

- address of the competent authorities;
- name of inspector or identification code;
- sampling date;
- official code number of the sample;
- animal species;
- sample matrix;
- substances or substance groups for examination;
- particular remarks.

This report is handed over to the routine laboratory together with the samples.

Guidelines available to the sampling officer should specify what information should be collected and how it should be transferred to relevant institutions.

The specific origin of the samples must not be known to the analytical staff until the analytical report has been completed and signed, but provided that proper procedures for restriction of access to the detailed sample information can be established, the full information on the sample could be available to the institution doing the actual analysis.

- **Follow-up**

Every case of non-compliant analytical results must be assessed by follow-up investigations. Procedures that enable immediate information exchange about non-compliant cases between laboratories, central administration, regional/local inspectors should be in place.

Measures vary depending on whether the finding indicates illegal treatment or non-compliance with residue levels of authorised substances.

In any case, the cause of the finding must be clarified through investigations:

- concerning the business operator or animal keeper;
- concerning the veterinary practitioner;
- concerning other parties, for instance, suppliers of feed.

At the same time, recall of contaminated product must be ensured.

Kind and scope of controls must be documented. A system of traceability for animals and products, as well as samples, must be in place.

Investigations concerning the business operator or animal keeper should include, at least:

- the identity of the animal(s) in question;
- tracing the animals to farm level in case the samples are collected at slaughterhouse;
- a review of the use of veterinary drugs, including a check of the drugs present on the farm, with regard to the kind of drugs used; the amount of drugs used; the origin of drugs used;
- in case of authorised veterinary drugs:
  - adherence to the rules of defined waiting periods must be assessed;
  - receipts proving sales of drugs, and other relevant documentation;
  - prescriptions by the veterinarian;
  - prescriptions of feeding drugs;
  - a review of the animal keeper's documentation if relevant.

Investigations concerning veterinary practitioners:

- checks of the documents to be kept according to national legislation;
- purchase and use of drugs containing substances listed in Table 2 of Regulation (EU) No. 37/2010;
- signs of use of drugs which are not allowed in animals intended for food production.

If follow-up investigations at the producer, farm or the veterinary practitioner produce signs that substances which may be used as veterinary drugs have been used, and have been bought from other farms or establishments, these farms and establishments must be inspected also.

When the laboratory has reported a residue finding, the competent food control or veterinary office shall take follow-up samples. The approach and amount of sampling should depend on the kind of residue found.

This follow-up sampling should be aimed to identifying the sources of contamination, possibly by taking additional samples of feed, drinking water, or other potential sources.

In case of confirmed non-compliant animals/products, measures must be taken to assure that the non-compliant animal/product does not reach the market.

The following actions – depending on circumstance and national legislation – could be taken in non-compliant cases:

- confiscating the feeding stuff or products;
- recall and destruction of the products;
- slaughtering the animals; products from such animals are prohibited for use in human consumption;
- determine restrictions for the circulation of products from the particular farm/establishment;
- determine the measures, which must be carried out for reducing of exceeded MRLs;

- oblige owner to improve self-control system in the establishment and ensure mandatory sampling on residues;
- tightened control of particular farm/establishment;
- ensure official control for every lot intended for export.

2.2.3. Setting up a national residue control plan (NRCP)

The following section describes the necessary actions to fulfil EU requirements for a national residue control plan.

An approved residue monitoring plan is one of the prerequisites for export to the EU. EU animal and public health conditions must also be satisfied.[60]

The plan will only have to cover those animal species from which products will be exported to EU, and those establishments that are involved in producing these foods.

In order to export food of animal origin to the EU, one of two prerequisites must be fulfilled:

- either all producers of such products must fulfil the requirements for residue control;
- or a strict scheme for registration and control of export approved establishments must be in place ('split system').

2.2.3.1. Initial residue control plan

The initial national residue control plan from a non-EU country must give details on the structure and legal background of the systems involved in the residue control.

The initial plan must include (where applicable) information on:

- The competent authority/authorities responsible for residue controls in all commodities included in the residue monitoring plan:
  - contact details (name and address of the central competent authority);
  - structure of the competent authority e.g. the levels involved (central, regional, local) and the personnel resources allocated for residues controls;
  - role of the central competent authority e.g. drawing up the residue monitoring plan, co-ordinating and supervising residue control activities at different levels (central, regional, etc.), collection of data (e.g. results of monitoring), evaluation of data (e.g. was sampling carried out in accordance with the plan), application of corrective measures if required, submission of annual data to the Commission etc.
- The residue monitoring plan (and results from the previous year):
  - existing (groups of) commodities which can currently be exported to EU and plans for expanding or restricting this list, which commodities that are included in the plan, and for which commodities results from the previous year's residue monitoring have been provided;

60 Further guidance on this subject can be found at ec.europa.eu/food/international/trade/index_en.htm.

- information on the legal basis of the residue monitoring plan;
- information on whether the plan is based on Council Directive No.96/23/EC or on an equivalent standard (e.g. *Codex Alimentarius*). If an equivalent standard has been used, this should be described;
- information on how the planned number of samples have been derived, in particular whether a 'split system' for animal production is in place;
- indicate whether all groups of residues are included in the plan for each of the relevant commodities (as listed in Annex I to Council Directive No. 96/23/EC). If not, explain on what basis substance groups have been excluded from the plan;
- the list of substances to be detected, the matrices to be tested, and the screening and confirmatory methods used, the analytical limits of detection and action levels / national tolerances (to determine non-compliant results) should be clearly laid out in the plan;
- indicate whether there are any national tolerances or Maximum Residue Limits/Levels (MRLs) which do not correspond with EU MRLs;
- for residues of substances which are unauthorised or illegal in the non-EU country, indicate what action limits are applied and the rationale for setting these. When those limits exist, information on whether they are consistent with EU minimum required performance limits (MRPLs) where applicable;
- information on which type of services/personnel are involved in official sampling, and whether sampling is carried out only by officials or if third parties are involved;
- description of the sampling strategies used;
- explain any discrepancies in the number of samples planned versus the number of samples analysed;
- briefly describe the measures taken – administrative, penal, professional and procedural (reinforcement of monitoring on the farms concerned) – for the non-compliant results detected during the implementation of previous year's plan.

- Laboratory network:
  - name(s) and address(es) of all laboratories involved in official residue testing;
  - information on the level of competence of the National Reference Laboratory (if one has been established in the country), as well as the routine laboratories, particularly as regards the implementation of quality assurance in accordance with ISO 17025:2005, including the identity of the accrediting body (if applicable);
  - information on the performance of the laboratories regarding their participation in relevant proficiency testing schemes.

- Authorisation and use of pharmacologically active and other substances in food producing animals:
  - information on whether:
    - stilbenes or thyrostats;
    - hormones and ß-agonists;
    - substances which are included in Table 2 of the Annex to Commission Regulation (EU) 37/2010 (e.g. chloramphenicol, nitrofurans and nitroimidazoles);
    - which are expressly prohibited from in-feed administration to food producing animals in the EU (e.g. carbadox, olaquindox, nifursol etc.);
    - antibiotics for the treatment of certain diseases in honey bees;
    - dyes such as malachite green and crystal violet;

    are authorised for use in food producing animals at any stage of production. Depending on the substance, additional information must be given.

Templates and further details for reporting such information is available on the Internet.[61] A copy has been included in Annex 2.

2.2.3.2. Subsequent (annual) residue control plans

- ***Description of the regulatory systems***

Non-EU countries are not required to send a detailed description of their regulatory systems every year. Only relevant updates or changes to the system need to be communicated to the European Commission. For non-EU countries with a well-established regulatory system, details of which were sent with the initial plan, subsequent communication with the European Commission would normally include:

- the (prospective) residue control plan;
- results of the previous year's plan, details of its implementation *i.e.* numbers of samples taken compared to the number planned and the measures taken for non-compliant ('positive') results. This is evidence of how the plan was implemented and an indicator of the competent authorities' performance.

- ***The (prospective) residue control plan***

The NRCP must provide information on which kind of samples that are planned for each of the categories of animal species (as listed in Annex 1) which are currently being exported to the EU (or which the country wishes to export to EU).

The plan must describe which substance groups are covered in the analytical scope, and for each substance give detailed information on the number of samples analysed and provide information on the performance of the methods used, including method

61 ec.europa.eu/food/food/chemicalsafety/residues/docs/table_1_information_required_for_tc_residue_control_programmes_20032012_en.pdf.

principle for both screening and confirmatory methods, detection capability (CCβ)[62] for screening methods, decision limit (CCα)[63] for confirmatory methods as well as the level of action.[64]

The number of samples within each species group and substance group are laid down in Directive No. 96/23 as a function of the size of the annual production for each species group. These sampling requirements are summarised in Table 1.

Table 1: Summary of sampling requirements by commodity/species

Species	Commodity	Frequency
Bovine	Meat	0.4 % of animal slaughtered the previous year
Bovine, ovine, caprine	Milk	One per 15,000 tonnes of annual production – minimum 300 samples
Porcine	Meat	0.05 % of the animals slaughtered the previous year
Caprine, ovine	Meat	0.05 % of the animals slaughtered the previous year older than 3 months
Equine	Meat	No frequency or minimum of samples established
Poultry	Meat	One per 200 tonnes of annual production (deadweight)
	Eggs	One per 1,000 tonnes of annual production for human consumption – minimum 200 samples
Rabbit	Meat	10 per 300 tonnes of annual production (deadweight) for the first 3,000 tonnes + 1 sample for every 300 tonnes thereafter
Farmed and wild game	Meat	At least 100 samples
Farmed fin fish	Meat	One per 100 tonnes of annual production (deadweight)
Bees	Honey	10 per 300 tonnes of annual production for human consumption for the first 3,000 tonnes + 1 sample for every 300 tonnes thereafter

Source: ec.europa.eu/food/food/chemicalsafety/residues/docs/requirements_non_eu.pdf

62 If the concentration level in the sample is at CCβ, then the probability that the analytical result will be at or above CCα is 95 % (for β = 5 %). CD 2002/657: detection capability (CCβ) means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β. In the case of substances for which no permitted limit has been established, the detection capability is the lowest concentration at which a method is able to detect truly contaminated samples with a statistical certainty of 1 – β. In the case of substances with an established permitted limit, this means that the detection capability is the concentration at which the method is able to detect permitted limit concentrations with a statistical certainty of 1 – β.

63 CD 2002/657: decision limit (CCα) means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant.

64 Level of action: concentration above which a result is deemed non-compliant.

Templates for reporting such information are available.[65] The minimum numbers of samples under EU rules are automatically updated, when the production data are entered. Details of the analytes, materials to be tested, screening and confirmatory analytical methods etc., can be entered.

When reporting the plan, care must be exercised in distinguishing between number of samples and number of analysis.

As an example, if the plan has indicated that 12 samples of horses are to be analysed for substances in subgroup B1 (stilbenes) and 12 samples of horses are to be analysed for substances in subgroup B2 (antithyroids), a total of 24 samples must be taken – even when each sample is analysed for both subgroups B1 and B2.

This must be reflected in reporting the plan as well as the results.

The templates presented on the Web[66] for reporting the plan or the results are not well suited to report such information.

One possible solution for the example above (when only samples of 12 horses are planned to be taken) could be to report in the template that 12 horses are planned to be analysed for substances in group B1 and to add a note, stating that the 12 samples of horses reported for subgroup B1 also will be analysed for substances in subgroup B2 (and including information on the substances analysed for). These 12 horses **should then not** be reported in the template under subgroup B2. Alternatively could be reported that 6 horses were to be analysed for substances in group B1, and 6 horses were to be analysed for substances in group B2 (supplementary information could be added in notes).

2.2.4. Special rules for certain commodities

In addition to the general rules described above, special rules exists for export of horses (and products of horses), casings and honey.

These rules are described in "Imports of animals and food of animal origin from non-EU countries. Manual on residue requirements for non-EU countries exporting to the EU".[67]

65 ec.europa.eu/food/food/chemicalsafety/residues/plantemplate.xls.

66 Plan: ec.europa.eu/food/food/chemicalsafety/residues/plantemplate.xls
Results: http://ec.europa.eu/food/food/chemicalsafety/residues/resultstemplate.xls.

67 ec.europa.eu/food/food/chemicalsafety/residues/docs/requirements_non_eu.pdf.

2.2.5. Reporting results from the annual monitoring programme

Results from the annual monitoring programme must be reported to the EU Commission on an annual basis. Templates for reporting such information are available.[68] When reporting the results, care must be exercised in distinguishing between number of samples and number of analysis, see remarks above (Subsequent (annual) residue control plans).

2.2.6. Systems and procedures for approval and registration for VMPs

In the EU, a company can market a veterinary medicinal product only after a marketing authorization has been issued by an EU member state or by the EU Commission through EMA.[69] Before the EU member state or the EU Commission issues a marketing authorization, the company must submit a marketing authorization application, called the 'dossier'. The dossier includes data from studies showing the product's quality, safety, and efficacy.

The legislation on residues of veterinary medicinal products used in food producing animals provides for a scientific evaluation before respective products are authorised. The objectives of marketing authorisation are to ensure that the product is safe for the consumer of food derived from treated animals, the animal itself, those handling the product, and the environment. If necessary, maximum residue limits (MRLs) are established and in some cases the use of substances is prohibited.

For an animal drug, the EMA Committee for Medicinal Products for Veterinary Use (CVMP) is responsible for the scientific evaluation. In the CVMP, experts from all EU Member States are seated. The Rapporteur, or lead reviewer on the dossier, prepares an overview of the committee's scientific evaluation, called the CVMP Assessment Report.

The CVMP Assessment Report:

- summarizes the data submitted by the company on the product's quality, safety, and efficacy;
- explains the assessment done by the CVMP to support the committee's recommendation to the EU Commission to issue a marketing authorization;
- is the basis for the European Public Assessment Report (EPAR) published on EMA's Web site.

68 ec.europa.eu/food/food/chemicalsafety/residues/resultstemplate.xls.

69 European Medicines Agency – http://www.ema.europa.eu/ema/index.jsp.

Le lien ne fonctionne pas

2.3. PESTICIDE RESIDUES

Pesticides cover a wide range of very different chemical substances. Pesticides are toxic compounds that are deliberately spread in nature, precisely because of their toxic properties. Pesticides are therefore different from the other chemicals used in the modern society. Since the toxicity of pesticides is not necessarily specific to the organisms it must fight, contaminants or residues in food may cause harm humans.

Pesticides are mainly used to prevent pests in the production of fruit, vegetables and cereals. Additionally, small quantities are used in the production of meat, to fight insects in stables and on animals, and – with less relevance to food – also wood preservatives. There exist different types of pesticides, depending on the pests to be controlled; insecticides, herbicides, fungicides and plant growth regulators. Around thousand active substances are produced and used worldwide. As a consequence of the use of pesticides, it is possible to identify residues in a large number of food products.

The description in the present chapter of controls with pesticide residues in foods will be described with reference mainly to EU legislations.

2.3.1. Legislation concerning residue control

In many countries, there is national legislation regulation on which pesticides are authorized. Many countries also have national legislation on the maximum amounts of pesticide residues in different food commodities. Such upper limits are also referred to as Maximum Residue Levels (MRLs) or tolerances (in the United States).

In countries with no national legislation, the MRLs set by the *Codex* system are often used. MRLs are normally set for raw agricultural commodities (RAC), for example, banana with peel, lettuce, and apples.

The *Codex Alimentarius* Commission (CAC) is an international body that aims to protect the health of consumers, ensure fair trade practices in the food trade, and promote coordination of all food standards work undertaken by international governmental and nongovernmental organizations. CAC also set MRLs, which are indicative and not statutory. The *Codex* MRLs are to be used as guidance on acceptable levels when there is no other legislation in place; for example in countries without their own national MRLs or they can be used if national MRLs have not been set for a particular compound.

MRLs set by *Codex* are evaluated and negotiated through a stepwise procedure. Initially, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) considers recognized use patterns of good agricultural practice (GAP) and evaluates the fate of residues, animal and plant metabolism data, and analytical methodology as well as residue data from supervised trials conducted according to GAP. Based on these data, MRLs are proposed for individual pesticides. Toxicologists evaluate the toxicological data related to the pesticides and propose acceptable daily intakes (ADI) and acute reference doses (ARfD). The toxicological data originate from animal studies and include both studies on the short-term and long-term effects. The ADI is a measure of the amount of specific substance (in this case, a pesticide) in foods and drinks that can be consumed over a lifetime without any appreciable health risk. ADIs are expressed as milligram/kilogram body weight/day. The ARfD of a substance (here pesticide) is an estimate of the amount of the substance in food or drinks, normally expressed on a body weight basis, that can be ingested during a period of 24 hours or less without appreciable health risks to the consumer on the basis of all known facts at the time of the evaluation. ARfD apply only to pesticides that cause acute effects, e.g. phosphorus pesticides that are cholinesterase inhibitors.

The *Codex* Committee on Pesticide Residues (CCPR) considers at their annual meetings the MRLs proposed by the JMPR. CCPR is an intergovernmental meeting with the prime objective to reach agreement on proposed MRLs. The MRLs are discussed in an eight-step procedure and after the final step, the CCPR recommends MRLs to CAC for adoption as *Codex* MRLs. To protect the health of the consumers, the intake calculated using the proposed MRLs is compared with the ADI or the ARfD and if the calculated intake exceeds one of these two values the MRL cannot be accepted.

Often, when national MRLs are set, an evaluation is performed on a national level, that in many ways are similar to the evaluation performed by JMPR. Some countries also set their own ADIs or ARfDs. As part of the evaluation of pesticides within the European Union (EU), ADIs and ARfDs are set on the EU level which then applies in all Member States. These values can differ from the values set by *Codex*.

Food and feed imported into the community should comply with EU or equivalent requirements for food safety in the EU (see 2.1. Legislation concerning residue controls).

The present chpater is widely based on legislation and practices required for or implemented by EU Member States.

The Member States within the EU set harmonized EU MRLs for pesticides. All harmonized legislation can be found on the Web site of the EU Commission.[70] In April 2005, new legislation (Regulation (EC) 396/2005) entered into force in which only harmonized EU MRLs can be set and all national legislation are turned into EU legislation.

Attention should be paid to the EU Commission Regulation 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin as some compounds has dual use and can be used both as pesticide and as a veterinary drug.

EU countries must monitor food for the presence of pesticide residues and draw up respective residue monitoring plans. How these plans need to be designed and implemented is outlined in the following legislation:

- Regulation (EC) No. 178/2002 – general principles and requirements of food law;
- Regulation (EC) No. 396/2005 and amendments – Pesticides MRLs in/on food and feed of plant and animal origin and Commission implementing rules;
- Regulations amending Annexes II and III to Regulation (EC) No. 396/2005 – amendments from 2008 to 2011;[71]
- Commission Regulation (EC) No. 178/2006 – food and feed to which pesticide MRLs apply;
- Commission Regulation (EU) No. 600/2010 – additions and modification of examples of related varieties or other products to which the same MRL applies;
- Directive 2002/63/EC – establishing Community methods of sampling for the official control of pesticide residues in and on products of plant and animal origin and repealing Directive 79/700/EEC;
- EU multi-annual control programmes, Commission Implementing Regulation (EU) 788/2012 of 31 August 2012 concerning a coordinated multiannual control programme of the Union for 2013, 2014 and 2015 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin.

Codex,[72] EU[73] and many countries publish their MRLs on their Web site, e.g. United States,[74] Australia,[75] Japan,[76] and South Africa.[77] In New Zealand,[78] and the United States,[79] authorities have compiled information about legislation and MRLs

70 ec.europa.eu/sanco_pesticides/public/index.cfm. Le lien ne fonctionne pas
71 ec.europa.eu/food/plant/plant_protection_products/legislation/max_residue_levels_en.htm.
72 www.codexalimentarius.net/pestres/data/index.html?lang=en. Le lien ne fonctionne pas
73 ec.europa.eu/sanco_pesticides/public/index.cfm. Le lien ne fonctionne pas
74 www.ecfr.gov/cgi-bin/text-idx?c=ecfr&sid=bd32aab1f2263d189c2ea7ae45c321e9&tpl=/ecfrbrowse/Title40/40cfr180_main_02.tpl.
75 www.apvma.gov.au/residues/standard.php#tables. Le lien ne fonctionne pas
76 www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/MRLs-p.
77 www.doh.gov.za/healthtopics.php?t=Food%20Control&c=Legislation. Le lien ne fonctionne pas
78 www.foodsafety.govt.nz/industry/sectors/plant-products/pesticide-mrl/worldwide.htm.
79 www.fas.usda.gov/htp/MRL.asp. Le lien ne fonctionne pas

worldwide. Other countries do not have their own legislation and MRLs published on Websites but the information can be gathered by contacting the relevant authorities. For countries that have published MRLs on Websites, be aware that addresses changes and the most recent legislation is not yet published.

2.3.2. Planning and implementing residue control

The task of setting up and implementing the national residue control should be assigned to a central public department or body. This institution should draw up the plan and coordinate the activities of central and regional departments involved in the implementation of the plan, including inspections, sampling, analysis, reporting and follow-up activities.

The following sections describe the elements necessary for the implementation of the residue plan.

2.3.2.1. Sampling strategy

The residue control plan should be aimed at controlling food on the market (domestically produced as well as imported) to check compliance with statutory limits, e.g. MRLs or monitor the intake of pesticides. Normally, the two purposes are combined.

Consequently, the sampling plan should include samples that are allocated according to the consumption pattern of commodities in the country and samples that are allocated according to the frequency of findings e.g. for the last five years. In addition, a maximum and minimum of samples should be set on each included commodity, e.g. 100 and 10.

Likewise, the relative allocation of the samples between domestically produced commodities and imported commodities should be based on supplies on the market and expected residues as known from examinations from previous years.

The examinations cover food commodities from domestic production, from other Member States and from non-EU countries. The samples are taken randomly. Additionally, check sampling can be performed in cases where high levels of pesticide residues may be expected. Such examinations will be instigated by violations of regulations, e.g. MRLs, and they normally cover a single pesticide in one commodity from a specific area or country.

To cover all the different commodities consumed in the country, a rolling programme can be implemented. An example of this is the EU coordinated multi-annual control programmes, (Commission Implementing Regulation (EU) No. 788/2012[80]), which the Member States should fulfil in addition to their national control programme. Thirty to forty foodstuffs constitute the major components of the diet in the Union. Since pesticide uses show significant changes over a period of three years, pesticides should be monitored in those foodstuffs over a series of three-year cycles to allow consumer exposure and the application of Union legislation to be assessed.

80 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:235:0008:0027:EN:PDF.

The rolling programme is:

Year 1: Beans with pod (fresh or frozen), carrots, cucumbers, oranges or mandarins, pears, potatoes, rice, spinach (fresh or frozen) and wheat flour.

Year 2: Aubergines, bananas, cauliflower or broccoli, table grapes, orange juice, peas without pod (fresh or frozen), peppers (sweet), wheat and virgin olive oil (oil processing factor = 5, taking into account an olive oil production standard yield of 20 % of the olive harvest).

Year 3: Apples, head cabbage, leek, lettuce, peaches including nectarines and similar hybrids; rye or oats, strawberries, tomatoes and wine (red or white) made from grapes.

2.3.2.2. Pesticides to monitor

In principle, all pesticides used worldwide should be monitored (around 1000). However, this is probably not done by any laboratory due to state of art in the analysis. For domestically produced foods, the pesticides approved in the country should be analysed for and if suspicion on use of not approved or illegal pesticides, these compounds should be added to the analytical scope.

For the imported food, it is even more difficult to decide which pesticides to be monitored as the pesticides use in the exporting countries is most likely not know. Consequently, global information on pesticide residue findings can be examined e.g. via reports (e.g. EU reports),[81] scientific articles on monitoring or databases like Pesticides-Online.[82]

EU multi-annual control programmes[83] includes a list in Annex 1 of approximately 200 pesticides that all Member states must analyse for in the coordinated programme. However, some Member States analyse up to 800 pesticides.

2.3.2.3. Sampling methods

Sampling for control of pesticide residues must follow the prescriptions laid down in Commission Directive 2002/63/EC or CAC/GL 33 – recommended method of sampling for the determination of pesticide residues for compliance with MRLs.

Guidelines equivalent to these should be readily available for the relevant personnel (*i.e.* inspectors and sampling officers) in the local language.

In many cases, there will be a need to obtain samples over a 12-month period to take account of seasonal variations.

Statistics on sampling should be reviewed by persons responsible at a central level in order to verify that samples adhere to the aims and prescriptions for the NRCP. Results from such reviews could be communicated to representatives of institutions participating in the NRCP (e.g. competent authority, national reference laboratory, regional laboratories and sampling institutions).

81 www.efsa.europa.eu/en/efsajournal/pub/2430.htm.

82 www.pesticides-online.com.

83 ec.europa.eu/food/plant/plant_protection_products/max_residue_levels/eu_multi-annual_control_programme_en.htm.

2.3.2.4. Sampling point

Samples should be representative of the supply chain. It could be retail outlets (supermarkets, local shops, market stalls, and farm shops), wholesale outlets, points of entry (Border Inspection Point e.g. ports and airports) and manufacturers (processing industries).

2.4. ORGANIC CONTAMINANTS

Environmental contaminants are chemicals that accidentally or deliberately enter the environment and are often a result of human activities. They are undesirable, harmful substances, which can be found at trace level in foodstuffs. They are not present in food due to a deliberate action, but as they are present in the environment

in which the food is grown, harvested, transported, stored, packaged, processed, and consumed. They may end up as food contaminates which can be a thread to consumer safety.

Environmental contaminants are in this chapter regarded as persistent organic pollutants (POPs), which is defined as "chemical substances that persist in the environment, bio-accumulate through the food chain, and pose a risk of causing adverse effects to human health and the environment". Stockholm Convention on Persistent Organic Pollutants is an international environmental treaty, signed in 2001, which aims to eliminate or restrict the production and use of persistent organic pollutants, including a number of organochlorine pesticides such as DDT, aldrin, chlordane and heptachlor, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-o-dioxins ('dioxins') and polychlorinated dibenzofurans ('furans'). Most of the POPs can be found in food items, as a common characteristic for the compounds is fat solubility, which allow them to accumulate through the food chain and biomagnified in higher species. Therefore, the most important human food intake sources for the POPs are fatty foods including fish, meat, eggs and dairy products.

Dioxins and furans are the common names for a group of chemicals that are formed during combustion processes such as waste incineration, power generation, metal production, and fuel burning. These compounds are found in small amounts in the air, water and soil. As a result of their chemical persistence and presence in the environment, they also enter the food chain. Human exposure to dioxins and furans is mainly through the diet.

Polychlorinated Biphenyls (PCBs) are man-made chemicals that are banned from manufacture in many countries. They are very persistent and can be transported over long distances. As a result, they are found throughout the environment. Humans are still exposed to small amounts of PCBs, primarily through foods.

Organochlorine pesticides are a number of pesticides which has previously been used as pesticides, but today banned in many countries due to the persistency and toxicology. They are generally fat soluble and can accumulate in the food chain, human exposure is therefore mainly through foods. However, if still used, there is the possibility of direct exposure.

Polycyclic aromatic hydrocarbons (PAHs) are one of the most widespread organic pollutants. In addition to their presence in fossil fuels they are also formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco, and incense. PAHs are lipophilic and are therefore present in fatty foods as a consequence of environmental contamination or they can be found, for example, on the surface of smoked food due to the smoking process.

Considering the huge variety of chemical agents and the complexity of conceivable contamination pathways, operators must conduct a precise evaluation of chemical risks of the product, evaluate the risk of environmental contaminants in the feed, considering the production processes, machinery used, technical agents used, etc., to determine the possible origin and probability of contamination, and to take appropriate action as needed to reduce or prevent such risks. As an example, fruit and vegetables can be exposed to contamination from hydrocarbons from

the exhaust during transport or different types of oils from machinery used either in the production or during transport and precautions should be taken in order to avoid contamination of the food.

In EU general principles and requirements of food law, Article 14, the requirements for food safety are the following: *"food shall not be placed on the market if it is unsafe and food shall be deemed to be unsafe if it is considered to be injurious to health or unfit for human consumption"*.

In determining whether any food is unsafe, regard shall be had:

- to the normal conditions of use of the food by the consumer and at each stage of production, processing and distribution;
- to the information provided to the consumer, including information on the label, or other information generally available to the consumer concerning the avoidance of specific adverse health effects from a particular food or category of foods.

In determining whether any food is injurious to health, regard shall be had:

- not only to the probable immediate and/or short-term and/or long-term effects of that food on the health of a person consuming it, but also on subsequent generations;
- to the probable cumulative toxic effects;
- to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers.

Authorities and food producers must therefore take measures to ensure the foods, both by monitoring and control of the products during the production chain, but also by on-going protection of feed, food production, manufacturing, packaging and transport to ensure there is no risk of contamination of the final product. In determining whether any food is unfit for human consumption, regard shall be had to whether the food is unacceptable for human consumption according to its intended use, for reasons of contamination, whether by extraneous matter or otherwise, or through putrefaction, deterioration or decay.

Authorities as well as food manufacturers and companies dealing with foods should pay attention to the general food laws description: when any unsafe food is part of a batch, lot or consignment of food of the same class or description, then it shall be presumed that all the food in that batch, lot or consignment is also unsafe and therefore not be placed on the marked. Food that complies with specific provisions for food safety shall be deemed to be safe insofar as the aspects covered by the specific provisions are concerned. Conformity of a food with specific provisions applicable to that food shall not bar the competent authorities from taking appropriate measures to impose restrictions on it being placed on the market or to require its withdrawal from the market where there are reasons to suspect that, despite such conformity, the food is unsafe. Where there are no specific provisions, food shall be deemed to be safe when it conforms to the specific provisions of national food law of the Member State in whose territory the food is marketed and the food shall be in compliment with that.

Food contaminants are substances that may be present in certain foodstuffs due to environmental contamination, cultivation practices or production processes.

If present above certain levels, these substances can pose a threat to human health. EU rules ensure that food placed on the market is safe to eat and does not contain contaminants at levels which could threaten human health:

- Maximum levels are set for the contaminants of greatest concern to EU consumers, either due to their toxicity or their potential prevalence in the food chain. These include aflatoxins, heavy metals (such as lead and mercury), dioxins and nitrates.
- The levels are set on the basis of scientific advice provided by the European Food Safety Authority (EFSA). Member State authorities are responsible for sampling food products, to ensure that they comply with the legislation.
- For imported foodstuffs, the country of origin is responsible for compliance with EU legislation, and this is controlled at EU borders and on the market.

The EU promotes best practice among all those involved in the production, storage and delivery of food to ensure that contaminant levels are kept to a minimum.

A number of countries and international organisations have set MRL or maximum values for the presence of environmental contaminants in foods. For pesticides, the most important for international trade are the values set in the *Codex Alimentarius*[84] and the European Union MRL values.[85] The MRL value to be taken into consideration is always the value applied on the market of destination.

When discussing environmental contaminants in food, attention should be drawn to the sources of the contaminants into the foods and in that perspective, one of the major factors is the presence of environmental contaminants in feed. The overall objectives for official control of feeding stuffs are:

- to avoid that feed causes problems with food safety;
- to keep food and feed producers to their obligations to ensure human and animal health and the environment in regard to their products;
- to create good conditions for fair trade with feed.

The authorities should inspect food business operators as well as performing direct analytical control of the foods from the companies. The purposes of the analytical chemical control are to monitor, control and survey the levels of environmental contaminants in the different food items, including:

- control imposed under EU rules or *Codex Alimentarius* rules;
- EU recommendations to conduct surveys for selected chemical contaminants in various food categories;
- control of products with EU import restrictions. These are, for example, aflatoxins, heavy metals, melamine, pesticide residues or dioxins as regulated, for example, in Commission regulation No. 258/2010 imposing special conditions on the imports of guar gum originating in or consigned from India due to contamination risks by pentachlorophenol and dioxins and similar regulations.

84 www.codexalimentarius.net/pestres/data/index.html?lang=en. Le lien ne fonctionne pas

85 ec.europa.eu/sanco_pesticides/public/index.cfm. Le lien ne fonctionne pas

To obtain maximum chemical food safety and avoid environmental contaminants in foods, several factors should be included in the food system. The official authorities should inspect the food business operators and perform the official control. Input to both the official authorities and the food business operators should be information about food contaminants and arising problems in foods as for example a rapid alert system described in a later chapter. The food business operators (FBOs) should perform self-assessment including required documentation from sub suppliers concerning the absence of environmental contaminants. The FBOs should furthermore use best practices in the entire food production line.

Figure 4 - Factors that contribute to increased chemical food safety

2.4.1. Legislation concerning environmental contaminants and residue controls

The European Union (EU) has strictly regulated controls on the use of veterinary drugs and pesticides and guidelines for controlling of residues and contaminants. These are found, for example, in Council Directive No. 96/23/EC for animal products and their products with detailed procedures for EU Member States to set up national monitoring plans, including details on sampling procedures. Furthermore, the EU Commission implemented Regulation No. 788/2012 concerning a coordinated multiannual control programme for 2013, 2014 and 2015 to ensure compliance with maximum residue levels of pesticides, including the organochlorine pesticides and to assess the consumer exposure to these compounds in and on food of plant and animal origin, which all Member States are obliged to follow.

Commission Regulation 1881/2006 setting maximum levels for certain contaminants in foodstuffs and Commission Regulation No. 835/2011 amending Regulation (EC) No. 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs, are setting maximum levels for polycyclic aromatic hydrocarbons (PAHs), more specific for benzo(a)pyrene and for the sum of benzo(a)pyrene, benz(a) anthracene, benzo(b)fluoranthene and chrysene. Maximum levels for dioxin are included in Commission Regulation No 1881/2006 and Commission Regulation No. 420/2011 amending Regulation No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs for both the sum of dioxins and the sum of dioxins and dioxin-like PCBs. Furthermore, in Commission Regulation No. 1259/2011 amending Regulation (EC) No. 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs, maximum levels are set for the indicator PCBs as the sum of PCB-28, 52, 101, 138, 153 and 180. In addition to these maximum levels, Commission recommendation of 23 August 2011 on the reduction of the presence of dioxins, furans and PCBs in feed and food, introduces

action levels in order to stimulate a pro-active approach to reduce the presence of dioxins and dioxin-like PCBs in food.

2.4.2. Legislation concerning sampling and performance of analytical method

In Commission Regulation No. 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs, the legislation for the sampling and official control are described for the compounds mentioned. Similar, Commission Regulation 252/2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs.

2.4.3. Planning and implementing residue control

Planning and implementing official chemical control in food and feed shall be carried out by the national competent authority. Some aspects of the control, e.g. chemical analyses, can be outsourced to private analytical laboratories but the final quality control and responsibility lies with the competent authority.

A food control system must be developed and implemented in a transparent manner. The confidence of consumers in the safety and quality of the food supply depends on their perception of the integrity and effectiveness of food control operations and activities (FAO/WHO).

The number of chemical compounds belonging to the group of organic environmental contaminants is large. National and international legislation for control and sampling are established for a small number of compounds but for the majority of compounds, the analytical control must be carried out based on the current available scientific knowledge.

The overall structure for planning of chemical analytical control can be described by four steps (modified from US FDA 2011). In Figure 5, the four steps are shown:

1. understand the potential hazard;
2. identify critical points for control;
3. develop a control strategy;
4. publishing of control results.

Figure 5 - Developing and planning of chemical control

1. Understand the potential hazard

The starting point for all activity regarding chemical control in food and feed is to understand the potential hazard on human health posed by a specific chemical compound or group of compounds with similar properties. Information of toxicology, sources of the environmental contamination, potential species related and process-related hazard is important issues to consider. Accumulation in certain tissues of the animal and possible changes in concentration due to processing is relevant to take into account as well (e.g. extraction of fish oil will increase the content of fat soluble compounds on fresh weight basis). When all aspects have been considered, it must be determined whether the potential hazard is significant or not. The evaluation can be used for prioritising the control between different potential hazards.

2. Identify criticals points for control

From a food safety point of view, it is essential to look at the entire production process when conducting analytical control. Even if maximum limits have been set for the food item to be eaten and therefore samples for control purposes have to been taken accordingly, it can be of value to take additional samples earlier in the production chain. For aquaculture fish, the feed is often more relevant to monitor than the fish itself because the feed in many cases is the only source of the contaminants. The feed can be manufactured and sold to several farms and thus, it is much more efficient from an economic as well as a food safety point of view to carry out control or monitoring on the feed.

3. Develop a control strategy

Every action of chemical control must be carried out on the basis of some kind of tolerance levels. It can be maximum limits from international or national legislation or *ad hoc* tolerance levels. *Ad hoc* tolerance levels can be set from evaluation of TDI (tolerable daily intake) and food consumption figures or other scientific advice.

Sampling procedures and frequency of testing must be specified. Corrective actions must be established and they can be adjusted in relation to the purpose of the programme: whether it is official control or monitoring and survey. Emergency procedures can be established for dealing with particular hazards (e.g. recall of products). A part of the control strategy can be a check of the food business operator's self-assessment results.

4. Publishing of control results

Publishing of control results will increase the public awareness of potential food hazards for food business operators and maintaining consumer confidence in the food system. The origin of the control samples can be made anonymous depending on the actual situation.

2.5. INORGANIC CONTAMINANTS

The amount of metals in food and feed depends upon the natural content and the conditions under which food and feed are produced and processed. Some metals have nutritional functions and are essential to the health. But others such as lead, cadmium and mercury have no nutritional relevance and can cause serious illnesses (Table 2).

Table 2: Elements commonly monitored in food (Capar and Szefer, 2011)

Element	Primary purpose
Aluminium (Al)	Toxicity
Arsenic (As)	Toxicity
Bore (B)	Nutrition
Cadmium (Cd)	Toxicity
Calcium (Ca)	Nutrition
Chromium (Cr)	Nutrition/toxicity
Copper (Cu)	Nutrition
Fluorine	Nutrition/toxicity
Iodine (I)	Nutrition/toxicity
Iron (Fe)	Nutrition
Lead	Toxicity
Magnesium (Mg)	Nutrition
Manganese (Mn)	Nutrition
Mercury (Hg)	Toxicity
Molybdenum (Mo)	Nutrition
Nickel (Ni)	Toxicity
Phosphorus (P)	Nutrition

Element	Primary purpose
Potassium (K)	Nutrition
Selenium (Se)	Nutrition/toxicity
Sodium (Na)	Nutrition
Tin (Sn)	Toxicity
Zinc (Zn)	Nutrition

2.5.1. Legislation concerning residue controls

2.5.1.1. Food

To reduce the risk to human health associated with a high heavy metal content in food and feed, maximum allowed limits in several commodities have been laid down in the European legislation.

In certain foods maximum levels for the heavy metals, cadmium, lead and mercury and inorganic tin (Table 2) have been established by Commission Regulation (EC) No. 1881/2006.[86] The methods of sampling and analysis for the official control of the maximum levels of these metals are described in Commission Regulation (EC) No. 333/2007. Surveillance for residues of chemical elements in foods of animal origin is also specified in Council Directive 96/23/EC (subcategory B3c).

Table 3: Examples of maximum levels (ML) in certain foods established in Commission Regulation (EC) No. 1881/2006

Foodstuffs	Maximum levels (mg/kg wet weight)
Lead	
Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.10
Muscle meat of fish	0.30
Bivalve molluscs	1.5
Food supplements	3.0
Cadmium	
Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.050
Muscle meat of the following fish: bullet tuna (*Auxis* species)	0.20
Bivalve molluscs	1.0
Bran, germ, wheat and rice	0.20

86 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:02006R1881-20100701:EN:NOT.

Foodstuffs	Maximum levels (mg/kg wet weight)
Mercury	
Food supplements	0.10
Muscle meat of the following fish: • eel (*Anguilla* species) • mullet (*Mullus* species) • redfish (*Sebastes marinus*, *S. mentella*, *S. viviparus*) • shark (all species) • swordfish (*Xiphias gladius*) • tuna (*Thunnus* species, *Euthynnus* species, *Katsuwonus pelamis*)	1.0
Tin (inorganic)	
Canned foods other than beverages	200
Canned beverages, including fruit juices and vegetable juices	100
Canned baby foods and processed cereal-based foods for infants and young children, excluding dried and powdered products	50

2.5.1.2. Feed

Directive 2002/32/EC[87] contains maximum limits for heavy metals including arsenic, lead, mercury and cadmium in certain feed materials, feed additives and feeding stuffs (Table 4). It prohibits the dilution of contaminated feed materials.

Table 4: Examples of maximum levels (ML) in certain feeds established in EU Directive 2002/32/EC

Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feeding stuff with a moisture content of 12 %
Lead	
Complete feed	5
Complementary feed with the exception of:	10
• mineral feed	15
Cadmium	
Feed materials of vegetable origin	1
Mercury	
Feed materials with the exception of:	0.1
• fish, other aquatic animals and products derived thereof	0.5
• calcium carbonate; calcium and magnesium carbonate	0.3

87 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32002L0032:EN:NOT.

Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feeding stuff with a moisture content of 12 %
Compound feed with the exception of:	0.1
• mineral feed	0.2
• compound feed for fish	0.2
• compound feed for dogs, cats and fur animals	0.3
Arsenic	
Complete feed with the exception of:	2
• complete feed for fish and fur animals	10*
• complete feed for pet animals containing fish, other aquatic animals and products derived thereof and/or seaweed meal and feed materials derived from seaweed	10*

* Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 mg/kg.

2.5.2. Legislation concerning sampling

In the EU, the sampling of foodstuff for the official control of the levels of lead, cadmium, mercury and inorganic tin shall follow Commission Regulation (EC) No. 333/2007.[88] Careful sampling can be time consuming since it is important that the analysed samples are representative of the original bulk product. All procedures used for acquisition, reduction and preservation of the sample may affect the reliability of the analytical result. For the sampling of foodstuffs intended for metal analysis, it is important to pay special attention to avoid contamination and analyte loss during handling and transport to the laboratory.

2.5.2.1. General sampling requirements

The general provisions include sampling by authorised personnel and separate sampling of each lot or sublot which is to be examined. A quantity of sample material (incremental sample) shall be taken at various places distributed throughout the lot or sublot. An aggregate sample shall be made up by combining the incremental samples. Samples for enforcement, defence and referee purposes shall be taken from the homogenised aggregate sample. Each sample shall be placed in a clean, inert container. All necessary precautions shall be taken to avoid any changes affecting the levels of contaminants and analytical determination or in other ways make the samples unrepresentative. Each sample taken for official use shall be sealed and labelled at the place of sampling. A sample record shall be kept permitting each lot or sublot to be identified unambiguously (lot number, date and place of sampling) together with any additional information likely to be of assistance to the analyst. Any departure from the sampling procedure shall be noted in the sample record (Commission Regulation [EC] No. 333/2007).

88 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32007R0333:EN:NOT.

2.5.2.2. Sampling methods

Large lots shall be divided into sublots on condition that the sublot may be separated physically. For products traded in bulk consignments (e.g. cereals), Table 5 shall apply. For other products, Table 6 shall apply.

Table 5: Subdivision of lots into sublots products trated in bulk consignments

Lot weight (ton)	Weight or number of sublots
≥ 1,500	500 tonnes
> 300 and < 1,500	3 sublots
≥ 100 and ≤ 300	100 tonnes
< 10	—

Table 6: Subdivision of lots into sublots for other products

Lot weight (ton)	Weight or number of sublots
≥ 15	15-30 tonnes
< 15	—

Table 7: Minimum number of incremental samples to be taken from the ***lot or sublot***

Weight or volume of lot/sublot (in kg or litre)	Minimum number of incremental samples to be taken
< 100	3
≥ 50 and ≤ 500	5
> 500	10

Table 8: Number of packages or units (incremental samples) which shall be taken to form the aggregate sample if the lot or sublot consists of individual packages or units

Number of packages or units to be taken	Number of packages or units to be taken
≥ 25	At least one package or unit
26-100	About 5 %, at least two packages or units
> 100	About 5 %, at maximum 10 packages or units

The minimum number of incremental samples to be taken from the lot or sublot to form the aggregate sample is specified in Table 7 (bulk) and Table 8 (individual packages or units). Figure 6 illustrates sampling of 1200 tonnes bulk product. Since the weight of the lot is not always an exact multiple of the weight of the sublots,

the weight of the sublot may exceed the mentioned weight by a maximum of 20 %. If it is a bulk **liquid** product, the lot or sublot shall be thoroughly mixed. Then, a homogeneous distribution of contaminants is assumed within a given lot or sublot and it is therefore sufficient to take three incremental samples from a lot or sublot to form the aggregate sample.

The weight of an incremental sample shall be at least 100 grams or 100 millilitres, resulting in an aggregate sample of at least about 1 kg or 1 litre except where it is not possible e.g. when the sample consists of 1 package or unit.

When received in the laboratory, the complete aggregate sample shall be finely ground and thoroughly mixed using a process that has been demonstrated to achieve complete homogenization (Commission Regulation [EC] No. 333/2007).

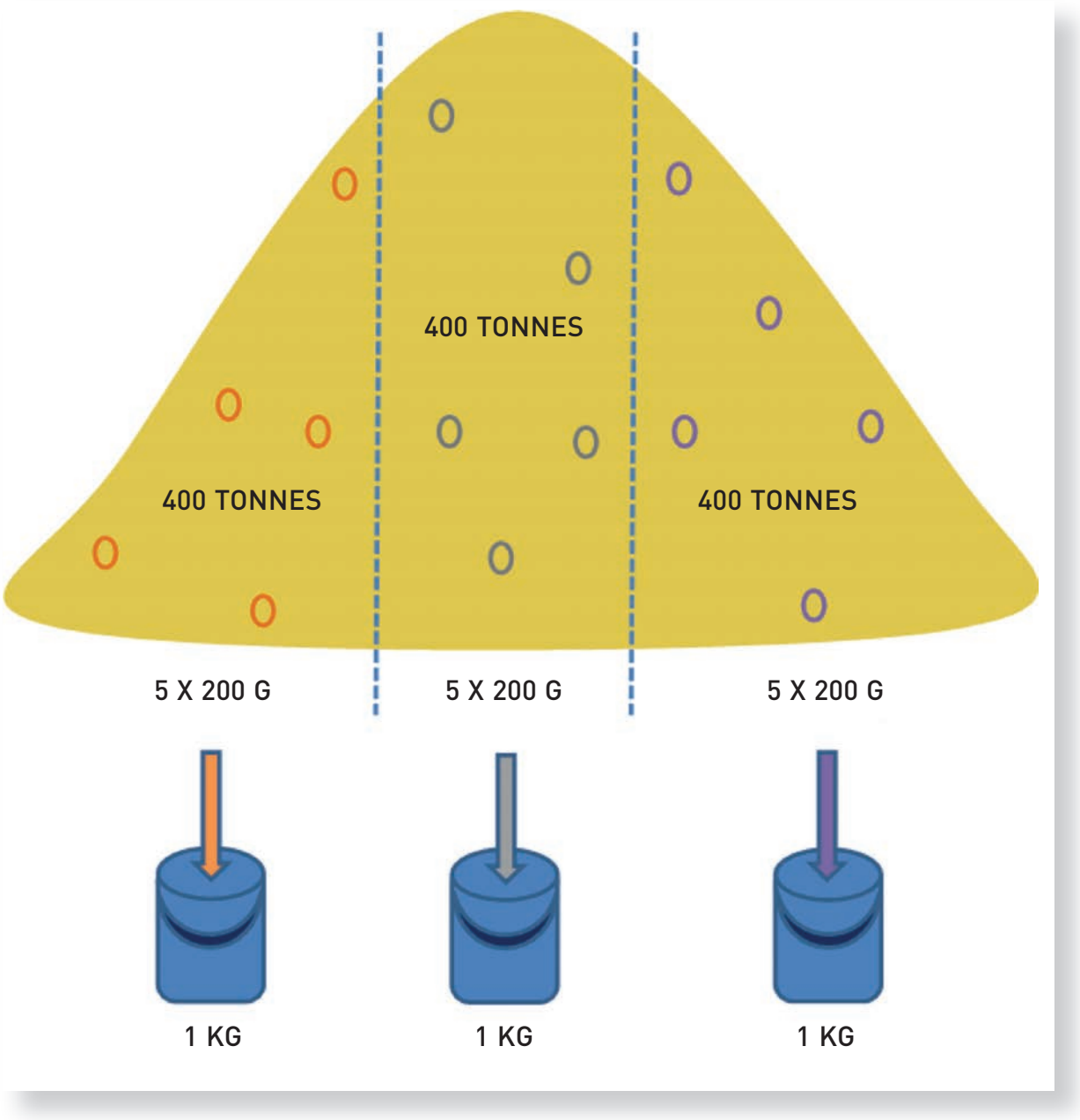


Figure 6 - Sampling of 1200 tonnes bulk product (e.g. cereal) divided into 3 sublots of 400 tonnes. Each sublot is sampled 5 times by 200 g increment sample to form 1 aggregate sample which is taken for analysis

2.5.2.3. Analytical methods for official control

For determination of e.g. lead in wine, a specific analytical method for the official control should be applied (Commission Regulation [EC] No. 2676/90). However, if no specific methods are prescribed by the EU, any validated method can be applied if the selected method meets specific performance criteria on detection and quantification limit, precision, recovery and specificity (Table 9). CEN, ISO and AOAC have published several official methods for the determination of heavy metals in feed and food. These standards provide both general and specific instruction for trace element analysis (Table 10).

Table 9: Performance criteria for methods of analysis for lead, cadmium, mercury and inorganic tin (Commission Regulation [EC] No. 333/2007 amended by Commission Regulation [EU] No. 836/2011[89] of 19 August 2011)

Parameter	Criterion		
Applicability	Foods specified in Regulation (EC) No. 1881/2006		
Specificity	Free from matrix or spectral interferences		
Repeatability (RSD_r)	$HORRAT_r$ less than 2		
Reproductibility (RSD_R)	$HORRAT_R$ less than 2		
Recovery	If an extraction step is applied in the analytical method, the analytical result shall be corrected for recovery. In this case, the level of recovery must be reported. In case no extraction step is applied in the analytical method (e.g. in case of metals), the result may be reported uncorrected for recovery if evidence is provided by ideally making use of suitable certified reference material that the certified concentration allowing for the measurement uncertainty is achieved (*i.e.* high accuracy of the measurement), and thus that the method is not biased. In case the result is reported uncorrected for recovery, this shall be mentioned.		
	Inorganic tin	**Lead, cadmium, mercury**	
		ML < 0,100 mg/kg	ML ≥ 0.100 mg/kg
LOD	≤ 5 mg/kg	≤ one fifth of the ML	≤ one tenth of the ML
LOQ	≤ 10 mg/kg	≤ two fifths of the ML	≤ one fifth of the ML

Le lien ne fonctionne pas

89 eur-lex.europa.eu/Notice.do?val=583712:cs&lang=en&list=583712:cs,&pos=1&page=1&nbl=1&pgs=10&hwords=.

Table 10: Examples of CEN, ISO and AOAC standards

CEN – EN 13804:2002 Foodstuffs – Determination of trace elements – Performance criteria, general considerations and sample preparation
CEN/TS 15506:2007 Foodstuffs – Determination of trace elements – Determination of tin in fruit and vegetables preserved in cans by flame atomic absorption spectrometry (AAS)
CEN – 16278:2012 Animal feeding stuffs – Determination of inorganic arsenic by hydride generation atomic absorption spectrometry (HG-ASS) after microwave extraction and separation by solid phase extraction (SPE)
AOAC – 990.04 Mercury (Methyl) in seafood by liquid chromatography-atomic absorption spectroscopy (LC-AAS)
ISO/TS 6733:2006 Milk and milk products – Determination of lead content – Graphite furnace atomic absorption spectrometric method

2.5.3. Planning and implementing residue control

In live animals and animal products, metals are regulated by Council Directive 96/23/EC (chemical elements, subcategory B3c). The directive establishes the frequencies and level of sampling and the groups of substances to be controlled for each food commodity. Surveillance should be aimed particularly at controlling and monitoring the metal contamination. The EU Member States should draft a national residue monitoring plan. The control plan is aimed at monitoring of environmental contaminants and at surveying and revealing the reasons for residue hazards in foods of animal origin. Sampling must be unforeseen, unexpected and effected at no fixed time and on no particular day of the week. Normally targeted sampling (selecting products with known or suspected contamination) should be applied.

Commission Decision 97/747/EC provides further rules for certain animal products: milk, eggs, honey, rabbits and game meat. Commission Decision 98/179/EC lays down detailed rules for official sampling procedures and official treatment of samples until they reach the laboratory responsible for analysis.

The number of samples needed for control of compliance with Commission Regulation (EC) No. 1881/2006 has not been specified. It is only described in general terms that *foodstuffs shall not be placed on the market if they contain a contaminant level exceeding the maximum level (ML).*

2.6. ANNEXES

A.1. EU legislation

Commission Decision 97/747/EC fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products (*OJEC*, No. L 303 of 6 November 1997, p. 12).

Commission Decision 98/179/EC laying down detailed rules on official sampling for the monitoring certain substances and residues thereof in live animals and animal products (*OJEC*, No. L 65 of 5 March 1998, p. 31).

Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (*OJEC*, No. L 221 of 17 August 2002, p. 8).

Commission Decision 2005/34/EC laying down harmonised standards for the testing for certain residues in products of animal origin imported from third countries (notified under document number C(2004) 4992) (Text with EEA relevance) (*OJEU*, No. L 16 of 20 January 2005, p. 61).

Commission Directive 2002/63/EC establishing Community methods of sampling for the official control of pesticide residues in and on products of plant and animal origin and repealing Directive 79/700/EEC (*OJEC*, No. L 187of 16 July 2002, p. 30).

Commission Implementing Regulation (EU) No. 788/2012 concerning a coordinated multiannual control programme of the Union for 2013, 2014 and 2015 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin (*OJEU*, No. L 235 of 1 September 2012, p. 8).

Commission Recommendation 2011/516/EU on the reduction of the presence of dioxins, furans and PCBs in feed and food (*OJEU*, No. L 218 of 24 August 2011, p.23).

Commission Regulation (EEC) No. 2676/90 determining Community methods for the analysis of wines (*OJEC*, No. L 272 of 3 October 1990, p. 1), eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31990R2676:EN:NOT.

Commission Regulation (EC) No. 178/2006 amending Regulation (EC) No. 396/2005 of the European Parliament and of the Council to establish Annex I listing the food and feed products to which maximum levels for pesticide residues apply (*OJEU*, No. L 29 of 2 February 2006, p. 3).

Commission Regulation (EC) No. 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs (*OJEU*, No. L 70 of 9 March 2006, p. 12).

Commission Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs (*OJEU*, No. L 364 of 20 December 2006, p. 5), consolidated version: 1 September 2012, eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20120901:EN:PDF.

Commission Regulation (EC) No. 1883/2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs (*OJEU*, No. L 364 of 20 December 2006, p. 32).

Commission Regulation (EC) No. 333/2007 laying down the methods of sampling and analysis for the official control of levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs (*OJEU*, No. L 88 of 29 March 2007, p. 29).

Commission Regulation (EC) No. 124/2009 setting maximum levels for the presence of coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed (*OJEU*, No. L 40 of 11 February 2009, p. 7).

Commission Regulation (EU) No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (*OJEU*, No. L 15 of 20 January 2010, p. 1).

Commission Regulation (EU) No. 258/2010 imposing special conditions on the imports of guar gum originating in or consigned from India due to contamination risks by pentachlorophenol and dioxins, and repealing Decision 2008/352/EC (*OJEU*, No. L 80 of 26 March 2010, p. 28).

Commission Regulation (EU) No. 600/2010 amending Annex I to Regulation (EC) No. 396/2005 of the European Parliament and of the Council as regards additions and modification of the examples of related varieties or other products to which the same MRL applies (*OJEU*, No. L 174 of 9 July 2010, p. 18).

Commission Regulation (EU) No. 1259/2011 amending Regulation (EC) No. 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs (*OJEU*, No. L 320 of 3 December 2011, p. 18).

Commission Regulation (EU) No. 252/2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No. 1883/2006 (*OJEU*, No. L 84 of 23 March 2012, p. 1)

Commission Regulation (EU) No. 277/2012 amending Annexes I and II to Directive No. 2002/32/EC of the European Parliament and of the Council as regards maximum levels and action thresholds for dioxins and polychlorinated biphenyls (*OJEU*, No. L 91 of 29 March 2012, p. 1).

Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC, and decision 89/187/EEC and 91/664/EEC (*OJEC*, No. L 125 of 23 May 1996, p. 10).

Council Directive 002/32/EC on undesirable substances in animal feed (*OJEC*, No. L 140 of 30 May 2002, p. 10), consolidated version: 6 September 2012, eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20120906:EN:PDF.

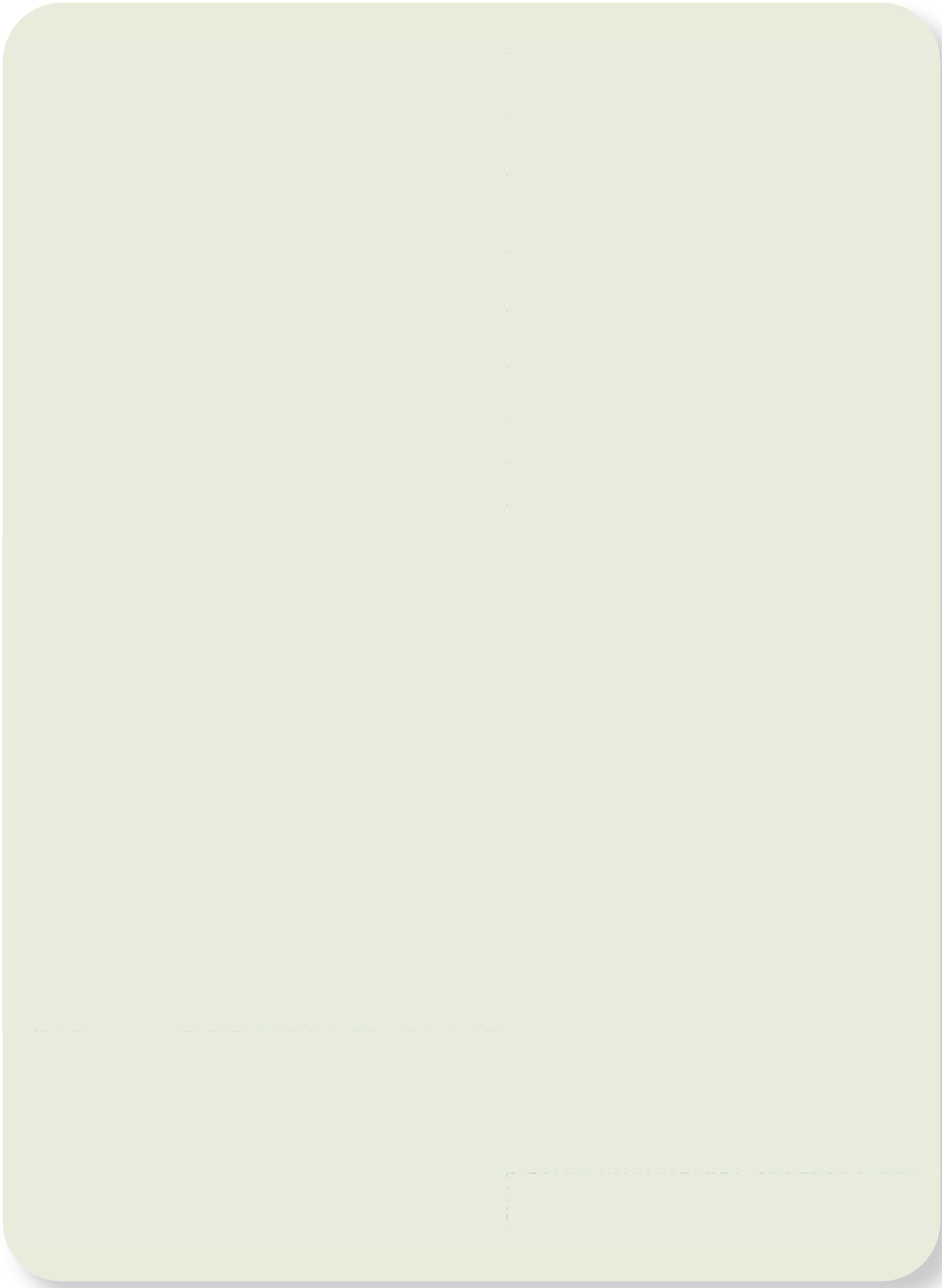
Regulation (EC) No. 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (*OJEC*, No. L 31 of 1 February 2002, p. 1).

Regulation (EC) No. 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules (*OJEU*, No. L 165 of 30 April 2004, p. 1).

Regulation (EC) No. 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC (*OJEU*, No. L 70 of 16 March 2005, p. 1) (as amended).

Regulation (EC) No. 470/2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No. 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No. 726/2004 of the European Parliament and of the Council (*OJEU*, No. L1 52 of 16 June 2009, p. 11)

A.2. Substances or Group of substances to be monitored (veterinary drugs)



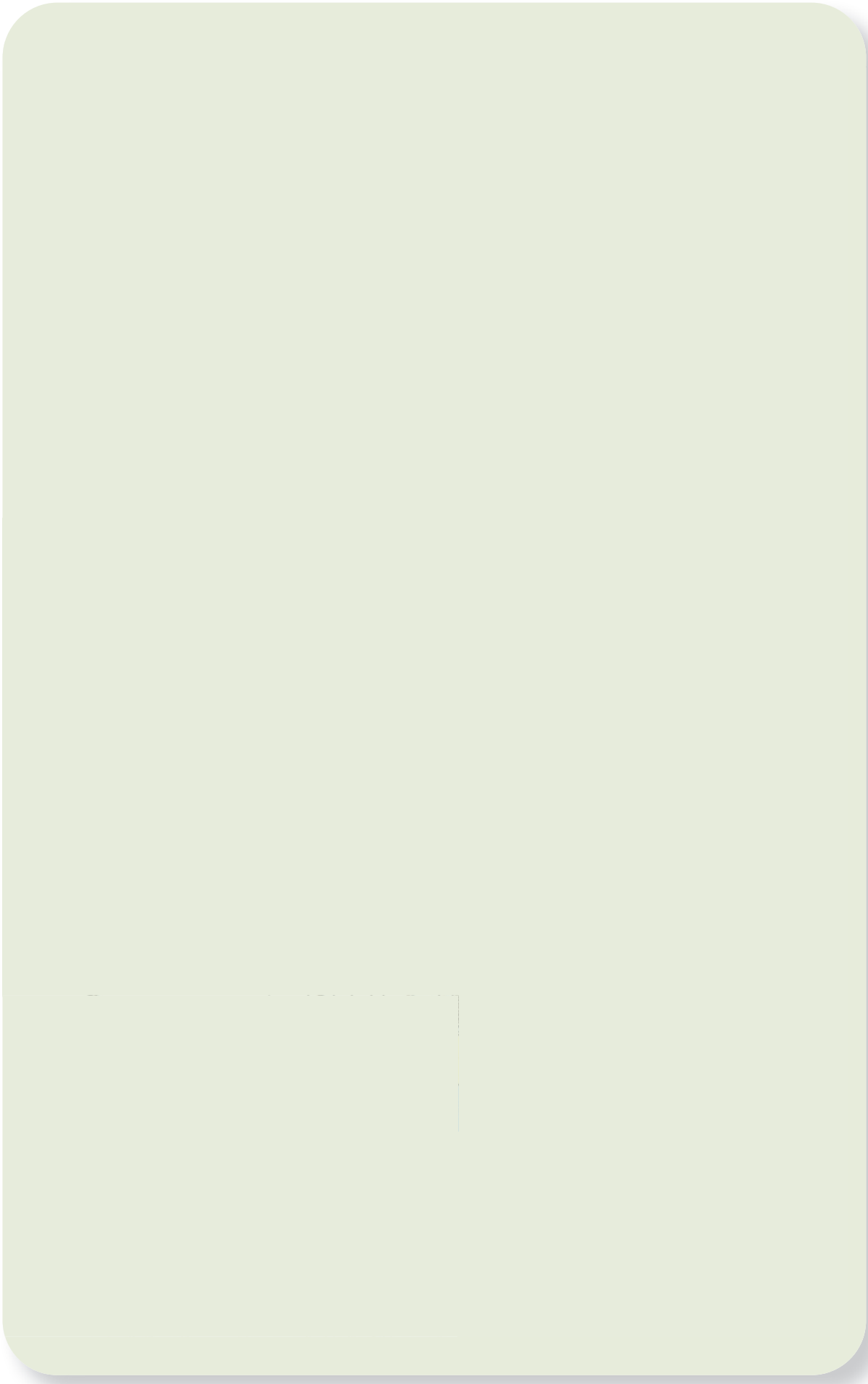
Source: ec.europa.eu/food/food/chemicalsafety/residues/docs/table_2_101106_en.pdf

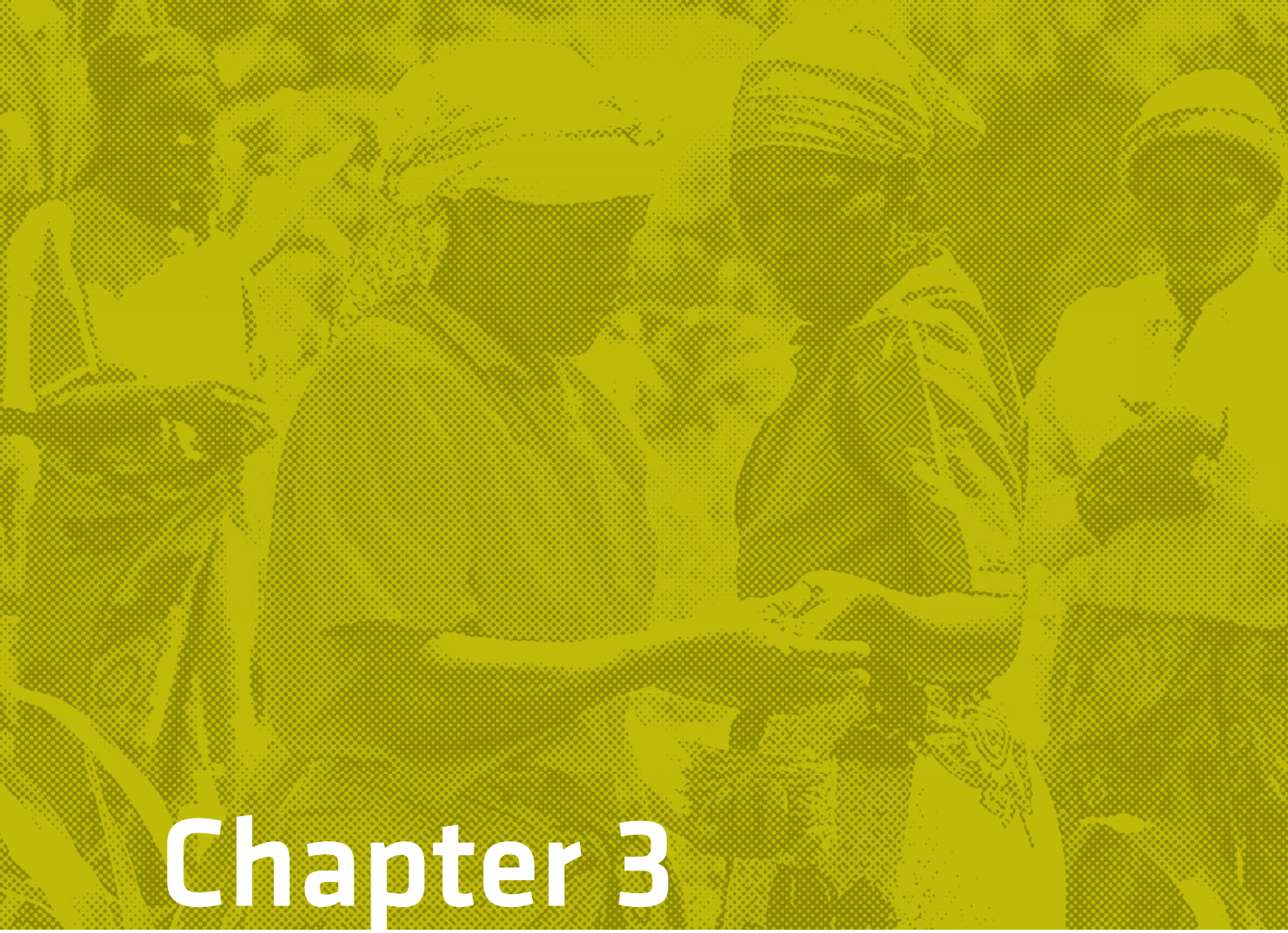
A.3. Information required for third country residue control programmes (veterinary drugs)

Source: ec.europa.eu/food/food/chemicalsafety/residues/docs/table_1_information_required_for_tc_residue_control_programmes_20032012_en.pdf

A.4. Example of a completed specimen plan for aquaculture products (veterinary drugs)

Source: http://ec.europa.eu/food/food/chemicalsafety/residues/plan_template_specimen_en.pdf





Chapter 3

Programming official controls of residues in plant products

3.1. PROGRAMMING OFFICIAL CONTROLS

3.1.1. Place of risk-based official controls

The performance of 'official controls' is one of the key activities **devolved to the risk manager** (*i.e.* the competent authorities) to ensure that food (**imported or exported**) put on the market is safe. One of the sectors to be controlled is crop production, in particular to identify the presence of pesticide residues or other chemical or biological contaminants.

In general, the object of an official control is to check that all food sold or consumed in a State complies with the (local or international) standards laid down by statute or under regulations with respect to various residues and contaminants referred to in that legislation or regulations. With regard to exports, it is the **standards applicable to the destination market** that apply to the exporting producer.

Compliance with these standards should contribute to the availability of food of satisfactory sanitary quality and thus enable consumers to obtain healthy food and remain in good health. **Specific testing for pesticide residues** is aimed at checking for:

1. the absence of "prohibited" pesticides (not authorised under the legislation and regulations in the destination market);
2. in the case of authorised products, to check whether their presence, if any, is in concentrations that are within the ceiling set in the legislation or regulations (Maximum Residue Limits, or MRLs, for pesticides).

The same applies for other chemical compounds covered by legislation and regulations on maximum limits (MLs), such as certain **mycotoxins** (aflatoxins, ochratoxins, fumonisins etc.) and **certain heavy metals** (above all Cd, Pb and Hg) for which tests must be conducted to ensure that the statutory maximum limits have not been exceeded.

The **programming of controls must be risk based** so that the resources earmarked for this activity can be allocated as judiciously as possible. A risk-based approach is also required to justify any action under international legislation and regulations, such as the Agreement on the Application of Sanitary and Phytosanitary Measures (the '**SPS Agreement**') adopted by the WTO.

Drawing up a programme of risk-based controls should ideally be undertaken in conjunction with **two types of stakeholders** (Figure 1):

- risk assessors (scientists, academics, etc.);
- managers (officials from the Ministries of Agriculture, Public Health, etc.).

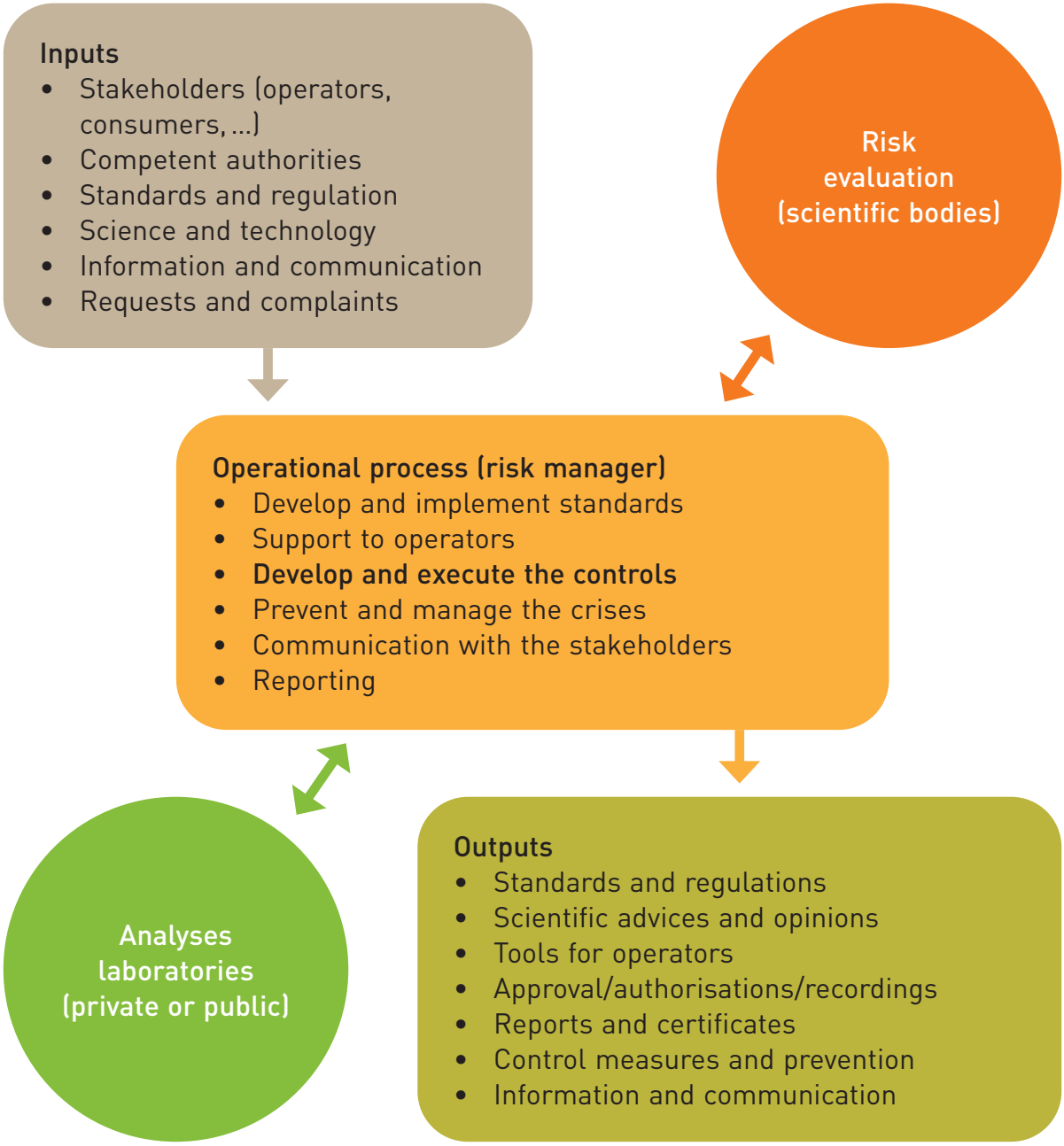


Figure 1 - Place of official controls as regards activities devolved to the bodies responsible for managing food safety

3.1.2. Key definitions

3.1.2.1. Control

Depending on the context, this term should be understood, in its broad sense, as covering physical checks, identity checks, documentary checks, etc., but also inspection, observation, verification etc., or, in its narrow sense, as sampling followed by analysis.

There are therefore **two key types of control activity**:

1. **Inspections** (of establishments, visits to operators), including checks, retesting, investigations following complaints etc. These activities may or may not be programmed.
2. **Sampling** (followed by analyses of the samples taken): on-site sampling may or may not be based on a sampling plan.

3.1.2.2. Official control

Any form of **control performed by the Competent Authority** of each country to verify compliance with feed and food law, animal health and animal welfare rules (e.g. control as defined in Regulation [EC] No. 882/2004, Article 2). By extension, any form of check performed by the competent authority of a third country to verify compliance with feed and food law, as well as any other sanitary or phytosanitary legislation, in particular in relation to international trade and the protection of local consumers (e.g. official controls performed in Europe on products imported from third countries, such as analyses of pesticide residues or antibiotics, verification of phytosanitary certificates at the frontiers or checks for regulated pests in plants).

3.1.2.3. Programming in controls

All activities aimed at making control plans available to the services concerned. These activities include the collection of basic information, risk prioritisation, control plan preparation, planning of controls and their performance, reporting, analysis of the findings and monitoring.

The programming of controls forms part of an ongoing process consisting of four stages, as set out in Figure 2.

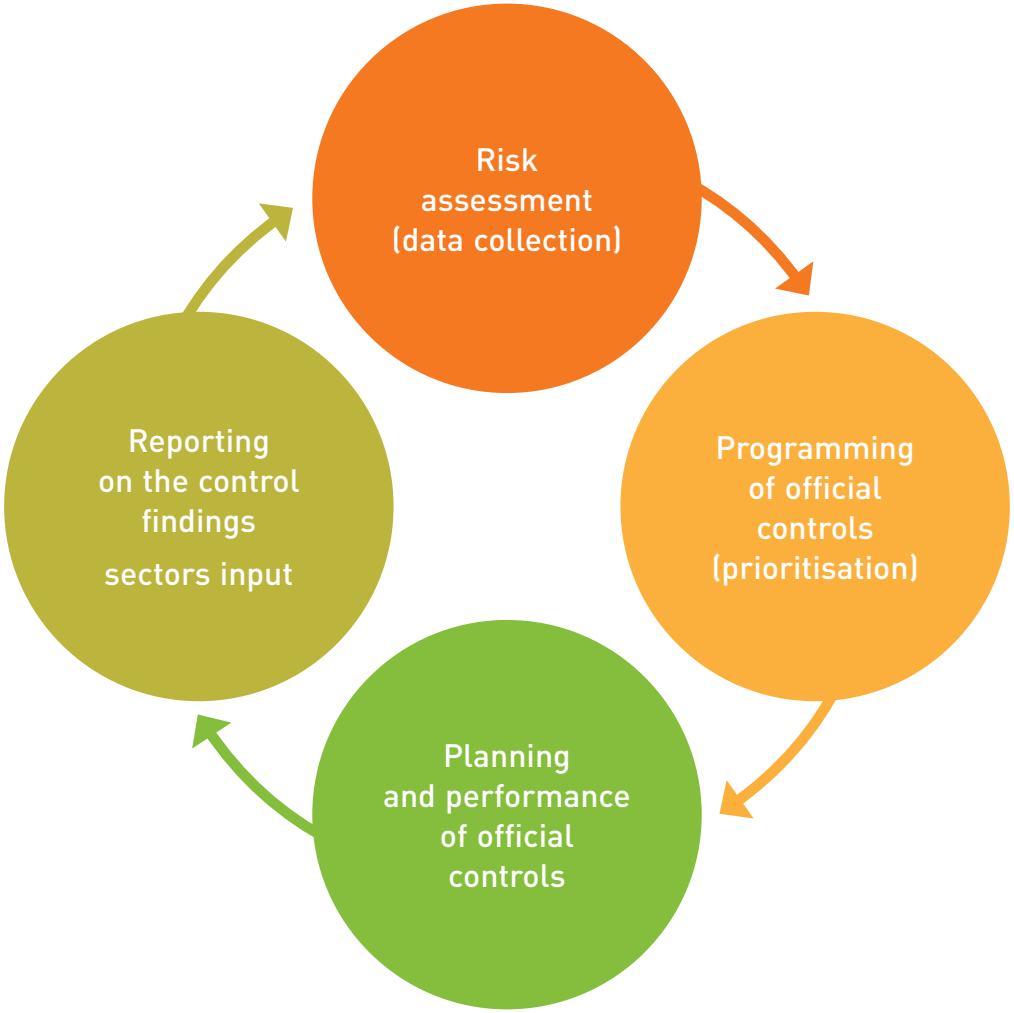


Figure 2 - The ongoing controls programming process showing the place of risk assessment and risk prioritisation

3.1.2.4. Monitoring plan and control plan (MPCP)

1. A **monitoring plan** is a campaign of analyses of animals, plants or foodstuffs with the primary objective of assessing the prevalence of a contaminant in a defined population and therefore the exposure of domestic consumers to this hazard. **The sample is representative and samples are taken at random** among an identified population or sub-population.
2. A **control plan** is a campaign of analyses of animals, plants or foodstuffs with the primary objective of identifying anomalies, non-conformities or even fraud. The **sampling is targeted** and samples are taken on the basis of pre-set criteria.

3.1.2.5. Pluri-annual control plan

A control plan to be conducted on the basis of a pluri-annual cycle (generally three to five years) in order to apportion the different types of products to be controlled over time, based on a revolving programme. At the end of a cycle, all the products covered by the overall programming have been assessed and a new cycle covering all the products to be analysed can be started. This approach is particularly relevant

in the case of pesticide residue given the large number of 'pesticide-commodity' pairs to be analysed. Spreading this over a number of years makes it possible to cover all the 'commodity-pesticide' pairs more effectively, which may be of particular importance.

3.1.2.6. Phytopharmaceutical product residue, Maximum Residue Limits (MRLs) and residue to be controlled

1. **A phytopharmaceutical product residue** is defined as one or more substances present in or on plants or products of plant origin, comestible products of animal origin, or elsewhere in the environment, and constituting the residue from the use of a plant protection product, including its metabolites and products deriving from its degradation or from its reaction. A definition must be proposed for residues in food produced, the soil and water, taking account of both the levels found and their toxicological and environmental significance.
2. **The Maximum Residue Limit (MRL)** is fixed on the basis of the residue content present in the foodstuff that constitutes good practice (dose applied, waiting period etc.) and after assessment of the risks. The maximum residue limit is the regulatory ceiling for the concentration of residues which if exceeded means that the marketing of a food product is no longer authorised, be it food for human consumption or animal feed. For the EU, maximum residue limits are set in Regulation (EC) No. 396/2005.
3. **The residue to be controlled** is formed by all the toxicologically significant compounds that are sufficiently abundant and easily identifiable, as set out in the definition of maximum residue limits. For example, for triadimefon, the residue to be controlled is the total triadimefon and triadimenol, a pertinent metabolite in food products.

3.1.2.7. Multi-residue and multi-analyte methods

1. **Multi-residue methods** (MRM) Instrument-based methods for residue detection that are generally based on high performance liquid chromatography (HPLC) coupled with mass spectrometry, making it possible to analyse in tandem numerous residues in a single chromatographic pass. These multi-residue methods apply not only to pesticide and medicinal products (veterinary) but also to other contaminants, such as mycotoxins.
2. **Multi-analytes methods**. These are instrument-based methods for detecting several different chemical compounds (analytes) in a single operation. Metal trace elements (MTEs), such as lead or cadmium, can be detected simultaneously in a given matrix using techniques such as inductively-coupled plasma mass-spectrometry (ICP-MS) and inductively-coupled plasma-atomic/optic emission spectroscopy (ICP-OES/ICP-AES). Multi-residue methods are a specific form of multi-analyte method.

3.1.2.8. Batch

Quantity of foodstuff delivered at one time and with characteristics presumed to be uniform, such as the origin (the land parcel, for example, or the same harvesting date), producer, variety, packager, packing type, brand, shipper etc.

3.1.2.9. Population

All batches for a product or category of products to which the control relates.

3.1.3. Legislation and regulations relating to official residue controls of plant products

Every nation must establish a **food control infrastructure** capable of ensuring maximum consumer protection and promoting fair practices in the food trade. There are large numbers of national legislation and regulations on official pesticide residue controls. In Europe, however, these are often a transposition into domestic law of common legislation and regulations established at European level.

In Europe, all issues relating to statutory limits on pesticide residues in food and feed are governed by **Regulation (EC) No. 396/2005**.This regulation also contains provisions on official controls of food of plant and animal origin for residue of pesticides used on plants. In addition, Regulation (EC) No. 396/2005 provides a **legal definition of pesticide residues** and sets the maximum limits applicable to such residues (MRLs). A European MRL database has been put in place and is regularly updated. In addition, all matters relating to official controls for ensuring legislative compliance are covered by Regulation (EC) No. 882/2004.

Furthermore, **Regulation (EC) No. 669/2009** establishes rules governing the **increased level of official controls** to be made on food and feed introduced from certain third countries or when **repeated violations of EU food law** have been observed (e.g. when pesticide residues frequently exceed the MRLs for a given product). The food products, the country of origin, **the frequency of the controls to be performed** at the point of entry into EU countries and the hazards (including certain pesticides) are also set out in Annex I to this Regulation. (As a result, concentrations of dimethoate above the MRL in French beans imported into Europe led to increased controls on import, posing a threat for the economy of the exporting country.)

At European level, there is also a coordinated control programme for pesticide residues in food of plant origin in which all Member States are required to cooperate. As part of this whole monitoring exercise, each of the 29 reporting countries conduct two control programmes: a domestic programme, developed by each country, and a programme coordinated by the EU (Regulation [EU] No.2015/595) that requires all national bodies to conduct harmonised control activities. Finally, the European Commission has also drawn up a regulation on increased official controls for certain food not of animal origin as regards combinations of hazardous commodities on which particular attention should be focused given the frequency of non-compliance observed in the past (Regulation [EC] 669/2009). The list of commodities and related hazards is regularly updated.

Globally, United Nations consumer protection guidelines have served as the foundation stone for national policies and legislation and have led to the **adoption of the *Codex Alimentarius*** standards.

The **Joint Meeting on Pesticide Residues** (JMPR) is a body administered jointly by the FAO and WHO with the purpose of harmonizing the requirements and risk assessment as regards pesticide residues. The work of the JMPR forms

the fundamental basis for the ***Codex*** **MRLs** for food and agricultural commodities circulating in international trade. The MRLs set by the *Codex* can be applied in the absence of domestic legislation on residues.

3.2. CONTROL OF PESTICIDE RESIDUES IN PLANT PRODUCTS

3.2.1. Role of the authorities

The **planning** and **implementation of official controls** of pesticide residues in food and feed are conducted by the competent national authority. This 'authority' varies from one country to another. Certain aspect of this control, for example residue analyses, may be entrusted to private-sector analysis laboratories (preferably accredited in accordance with the ISO/CEI 17025 standards and licensed by the competent authority), but quality control and ultimate responsibility for such analyses always lies with the competent authority.

A **transparent** food control system must be developed and implemented (e.g. the list of controls conducted and the control findings must be published in an annual report or put on the Internet). The confidence of consumers in the safety and quality of food available depends on their **perception of the integrity and effectiveness** of food control operations and activities.

3.2.2. Specific requirements for plant product pesticide residue controls

Since the objective of the official controls programme is, firstly, **to detect non-compliances** (e.g. where the statutory maximum limits have been exceeded), the control plan to be drawn up must include **targeted sampling** and the collection of samples based on predetermined criteria, such as a predefined prevalence level to be controlled (PLC) and a given level of confidence. In fixing these parameters allowance must be made for the level of risk for the consumer presented by 'commodity-hazard' pair.

The **number of pesticide residues to be controlled is very high** (different pesticides in different foods). Not only must all the pesticides authorised under domestic legislation be taken into account but careful attention must also be paid to many pesticides not authorised in the country and/or on the crop concerned so that checks can be made to detect **fraudulent use** of certain plant protection products. It is estimated overall that, given the number of authorisations that have been granted for various crops, **the number of pesticides to be found in our food is likely to be in excess of one thousand compounds**. Finally, account must also be taken of the fact that several different residues may exist for any one pesticide, all of which must be analysed, where appropriate (see the definition of residue to be controlled).

For example, under the 2012 European coordinated programme relating to 12 foods, a total 205 different pesticides were covered by the programming. Several hundred samples were taken per commodity (from 362 to 1,327) and between 19 and 90 pesticides were detected in the various commodities concerned.

The number of 'commodities' (foods) to be controlled is also very high given that plant health products are likely to be used on all plant crops and, in certain cases, also post-harvest (to preserve plant products).

Furthermore, **work must be undertaken to identify the relevant pesticide residues** and the various commodities (products) likely to be contaminated and therefore to present a risk to food safety.

Consequently, a specific strategy must be adopted to be able to conduct such control programmes successfully.

3.2.3. Potential approaches (strategic choices)

Firstly, it is possible to adopt a **pluri-annual-type programme extending over 3 to 5 years** so that the products (or groups of products) to be analysed can be apportioned over time. At the end of the cycle, an overall assessment can be made before a new cycle is programmed.

In addition, since the number of '**commodity-pesticide**' pairs can be in the thousands, it is also essential to favour certain approaches that make it possible to group both the pesticides to be detected and the number of matrices (commodities) to be analysed. To achieve this, **pesticide profiles can be established for each product** or, better still, for a certain number of comparable commodities that will be grouped together into **a plant product category**.

3.2.3.1. Determining 'product categories' (groups of commodities)

A **product category** can be defined as a group of products (commodities) with common characteristics and likely to be contaminated by the same pesticides. **Several levels of aggregation are possible** and a level must be sought that makes it possible, firstly, to allocate a coherent pesticide profile (corresponding to a single testing method, for example) and, secondly, to limit the total number of populations so that the data can be processed easily.

For this, pre-existing systems can be adopted, such as the FoodEx system developed by the European Food Safety Authority (EFSA) or the commodity categories established in Annex I of Regulation (EC) No. 396/2005. Then principal objective of FoodEx is to facilitate assessment of the exposure of food to potentially hazardous chemical products by establishing a precise correspondence of chemical product contamination data sets and food consumption data. FoodEx is a hierarchical system based on **20 principal food categories** that are then divided into subgroups up to a maximum of 4 levels. Recourse to this form of categorisation is particularly useful for comparing residue data against consumption data for the population. However, to compare the findings against the legislative guidelines on MRLs, it is better to adopt the categorisation used in Regulation (EC) No. 396/2005, Annex I of which sets out the various categories to which the MRLs relate (e.g. citrus fruits, pome fruits, brassicas etc.) and the list of the principal commodities relating thereto (e.g. for citrus fruit: grapefruit, oranges, lemons, limes, mandarins and 'others') (see Annex 1).

3.2.3.2. Determination of 'pesticide profiles'

Pesticide residues to be identified in a population are listed in the 'profiles' corresponding to the product groups to be analysed. Several criteria can be used to select the pesticides to be included in a profile. These include: use (in large quantities) on the crop(s) in question; detection that has occurred in similar programmes or during previous controls; or any other useful information.

Pesticide residue tests are generally **conducted using multi-residue methods** and, for certain substances, specific methods (mono-analyte).

In the case of multi-residue methods, **hundreds of different compounds may be detected during a single analysis** (e.g. in Belgium, a laboratory proposes the detection of 550 residues by combining two multi-residue methods applied to a single sample), making it possible, where they are suitably tailored to the products or groups of products concerned, to include a relatively large number of pesticides into the profile, including pesticides not authorised or which may be used fraudulently.

However, on the grounds of feasibility, it is important to be able to produce profiles with a reasonable number of compounds to be detected, the focus being placed instead on the relevance of the compounds and the scale of the risks linked to the pesticides selected in the crop(s) concerned. Even if multi-residue methods offer a very broad range of possibilities, in practice **laboratories are required to validate the methods** and a reasonable number of pesticides in a profile is the rule to make this possible in practice (the reason why several methods may be combined so that the scope of the analysis is extended, *i.e.* the number of compounds identified on the sample.

3.2.3.3. Determination of the 'severity scores' for a profile

To **prioritise the risks**, classification based on various '**Commodity-hazard**' pairs is generally established depending on the **severity of the risk and the probability** of being exposed to it through the commodity consumed.

A heavily consumed commodity with a serious risk will be considered to be 'at risk', in contrast to a commodity with low consumption and containing a low severity hazard.

For the 'pesticide residue' approach, **the hazards are grouped into a profile**. To prioritise the 'commodity-hazard' pairs, it is therefore essential to be able to **allocate a severity score to the entire profile** (of the pesticide group) that will be associated with a particular commodity or category of products. To ensure that the risk is not underestimated, a score must be allocated to the profile that reflects the **severity of the most hazardous pesticide in the profile** to ensure that it is not treated as a hazard with a probability of presence that is insufficient. As a precaution, **the pesticide score presenting the highest level of severity is therefore allocated to the entire profile**. This means that if just one pesticide in the profile has a high score, the entire profile will be allocated a high score even if that pesticide is not truly representative of the profile and the probability of encountering it is low. For this reason, it is essential to establish profiles on the basis of pesticides that can be shown to be relevant.

Since multi-residue methods are likely to involve a very high number of pesticides but with various degrees of relevance based on the information available on the frequency of detection in the commodity, **it must be ensured that there is no confusion between the profiles of relevant pesticides (or major pesticides)** for a given commodity and all the pesticides that could be detected using one or other multi-residue method applied by the laboratory but that encompasses both major and **minor** pesticides.

It must be possible for major pesticides to be **analysed by the method** that will be employed and that they are all taken into account in determining the profile severity score, the minor pesticides being disregarded in allocating a severity score to the profile.

Finally, since the hazards profile approach may also be used for other contaminants that can be analysed using multi-analyte methods (e.g. mycotoxins and heavy metals), determining the '**profile severity score**' must be appropriate to these different hazards and take into account all the relevant toxicological properties.

3.3. METHODOLOGY FOR DRAWING UP AN OFFICIAL CONTROLS PROGRAMME

The methodology for risk-based pesticide residue controls programming is fundamentally identical to that adopted for other types of contaminants. The theoretical bases can be found in Maudoux *et al.* (2006).

This methodology consists of the following **four stages**:

1. **definition of the field** covered and the strategy to be adopted;
2. **collection of** the necessary **information**;
3. **prioritisation** and **determination of the number of samples to be set**;
4. **planning** of the controls.

3.3.1. Definition of the field covered and the strategy to be adopted

This means defining exactly what will be covered by the controls programme, e.g. by responding to the following questions:

- Does it relate essentially to primary production or is the focus rather on processed products? And to which products exactly?
- Which of the productions are considered to be a priority based on the risks they present and their economic and/or dietary importance for the country?
- Is the control limited to productions for the local market or should products destined for export also be included?
- What is the situation as regards imported products?
- Etc.

The strategy to be adopted **will also encompass the type of programme selected**: annual or pluri-annual. If the pluri-annual formula is chosen, what will the timespan of its cycle: 3, 4 or 5 years? How will products be apportioned across the cycle timespan?

Choices will likewise have to be made as regards the **purpose of the controls**:

- **control plan** to ensure legislative compliance (detection of a minimum percentage of non-compliance);
- **increased control plan** for certain more problematic products (problems with exports or imports, for example);
- **monitoring plan aimed at obtaining data for use** in determining the prevalence of residues or estimating the exposure level of consumers through their diet. In the latter case, the programme could be profiled to make it more compatible with a study along the lines of a "Total Diet Study" aimed at estimating the exposure of consumers to pesticide residues and, where there is exposure, at establishing sampling that is less targeted (more random) but extends to more foods that are consumed.

Finally, **resources must be made available that meet the real needs**, and the feasibility of the controls planned must be examined (accredited laboratories available, sustainable cost, logistical aspects, etc.).

Ideally, this type of work will be undertaken by drawing on different types of expertise pooled through, say, a working party or project that is programmed and piloted by the competent authority.

The strategy to be followed means that **a risk assessment must be conducted before the process is launched**, and then followed by the programming *per se* and the planning of the controls.

The **last stage is the reporting** and analysis of the findings before information can be extracted to serve as guidance for the next cycle to be programmed.

It is therefore an ongoing process, as already indicated above and can be seen from Figure 2.

3.3.2. Collection of the necessary information

The information to be collected is of various orders, such as data on products, pesticides and production sectors, as well as on the analytical capabilities of the laboratories, the sampling methods applicable, etc.

3.3.2.1. Data on the food products to be controlled

A series of sources must be consulted to collect a series of data:

- sectors of production and the organisation of those sectors (existing professional organisations);
- data on the trading and transport of agricultural products and foods;
- data on imports and exports (volumes);

- data on primary agricultural production (plant sector and animal sector, including products from fishing and hunting etc.);
- data on processing and the production of foods by the agro-industrial sector (nature, volumes);
- findings of national or regional food surveys (consumption);
- data on the use of plant protection products (local production, imports, sales etc.).

The sources to be consulted are multiple (to be adapted to what is available in the country concerned): customs and excise, Ministry of Trade, sectoral publications, national statistical services and the FAO Statistical Databases of the United Nations (FAOSTAT).[90] The existence of "Sectoral self-checking guides" may facilitate access to such data.

The anticipated outcome is a **data set on production** (type of food, populations to be controlled), the number of establishments, the state and organisation of the operators. The staff (quantities, number of batches, etc.) for each population to be controlled, classified by category, are known or estimated.

There must therefore **be tables listing all the plant products** obtained at country level or imported by (or exported to) third countries, with indications of the tonnage and number of batches. Such tables should also be drawn up for processed products (national production, exports and imports).

3.3.2.2. Data relating to pesticides

Given the large number of pesticides likely to leave residues in plants, **various sources of information must be cross-referenced** to make it possible to identify the most relevant products.

- ***Data on the quantities of pesticides used***

Relatively crude sources of information exist, such as data relating to the quantities of pesticides imported or manufactured at national level. Such data must then be analysed to try to estimate the quantities used for each crop. To do this, information is needed on local practices (which pesticides are used on a given crop, at what dosage and at what frequency?). Ideally, there should be a complete table on the use of plant health products at national level, crop by crop, year by year. Such data must be cross-checked against the overall data available, such as the quantities imported or manufactured annually. It will be possible to extract from this table data on the intrinsic risks linked to the nature of the commodity to be analysed. This should make it possible to assess the inherent risk of a commodity based on the probability that it will contain residues as a result of the treatments performed based on common practice.

90 faostat3.fao.org/faostat-gateway/go/to/download/Q/*/F.

- ***Frequency*** of detection and establishment of profiles per crop

In addition, all sampling plans must also take into account notifications for purposes such as the European Rapid Alert System for Food and Feed (RASFF) and other sources that make it possible to obtain information on breaches of MRLs. The inherent risk in a commodity must also take into consideration the quantities consumed by the population and greater priority must be given to the most commonly consumed commodities.

Information is published on the nature of pesticides that are most likely to be present in various agricultural products. For example, for Belgium, Delcour *et al.* (2015) proposes a list of 10 key pesticides for certain fruit and vegetable crops, such as apples, strawberries, grapes, carrots and lettuce.

The **method used** for this classification can be summarised as follows:

- For each product, the ten principal pesticide residues are selected on the basis of the residue monitoring data base established under the monitoring programmes put in place in Belgium from 2005 to 2009.
- For this selection, the five-year monitoring data for each product are analysed separately to identify (by order of importance) the active ingredients that:
  1. breach the MRL;
  2. were not authorised when the sample was taken;
  3. were detected in the greatest number of samples;
  4. were detected on several consecutive occasions with rising residue levels.
- Then, a qualitative classification of the active ingredients is undertaken. Consequently, the active ingredients with recent and/or a high number of MRL infringements are classed first.
- This classification is then combined with the appearance of illegal residues.
- The final classification is undertaken by including the percentage of samples with detectable residues over several consecutive years.
- This means that the active ingredients chosen present the highest risk of being (illegally) present in high levels in the commodities concerned.

Once this research has been performed, a list of the principal pesticides likely to be found in the various commodities (or groups of commodities) concerned is drawn up so that profiles can be established for major pesticides for the product or product category. This type of work must be regularly updated.

- ***Quantification of hazard severity***

Quantification of the severity of pesticide residues may be undertaken with a view to protecting consumers exposed through their diet. There are **two toxicological baseline values** corresponding to **acute risks** (Acute Reference Dose – ARfD –, expressed in mg/kg pc) and **chronic risks** (Acceptable Daily Intake – ADI–, expressed in mg/kg pc/day) respectively.

Since long-term exposure is the most important threat to the health of consumers (in general, there are indeed residues present in their food, even if in low quantities,

at every meal and throughout their lives), **it is proposed that pesticides be allocated a severity score of 'S', based on the Acceptable Daily Intake.**

An example of the scores allocated (1 < S < 4) on the basis of the Acceptable Daily Intake is given in table 1.

Table 1: Grading of the severity (S) of pesticide residues based on the ADI

ADI (mg/kg pc/day)	Severity score (S)
< 0.001	4
0.001 ≤ ADI ≤ 0.01	3
0.01 ≤ ADI ≤ 0.1	2
0.1 < ADI	1
ADI not necessary	1

It is then possible to drill down this grading to also take into account the Acute Reference Dose value where the acute toxicity is relatively high (ARfD < 0.1 mg/kg pc) **as compared with the chronic toxicity**.

Consequently, for pesticides with an **Acute Reference Dose value of less than 0.1 mg/kg pc**, it is proposed that **the severity score be increased by one unit** where the initial score based on the Acceptable Daily Intake is 1, 2 or 3. This means that a pesticide with an Acceptable Daily Intake value of 0.05 mg/kg pc/day will have a score of 2. The same pesticide but with an Acute Reference Dose value of 0.05 mg/kg pc will be allocated a score of 3 (2 + 1).

For other hazards, such as mycotoxins and heavy metals, it is sometimes necessary to take into consideration, more specifically, the **carcinogenicity potency of the compound** (as a basic principle, a pesticide with proven carcinogenic properties is not authorised and, consequently, this property is not taken into account in setting the Acceptable Daily Intake). In view of this, the maximum severity score (S = 4) must be allocated to substances classed in category 1 by the WHO International Agency for Research on Cancer (IARC) (Sufficient proof of being *carcinogenic to humans*) and a S score = 3 will be reserved for substances classed in category 2A (Probably carcinogenic to humans) or even 2B (Possibly carcinogenic to humans).[91]

Once the major pesticides that must form part of the profile for each commodity have been identified, the hazard severity level for the profile must be established since this will be essential for risk prioritisation during the controls programming stage. As specified above in point 3.2.3., the precautionary approach means that it is the score for the most hazardous pesticide that is allocated to the profile, while making sure, however, that the particular pesticide is in fact relevant and justified in the profile based on the criteria set out in the previous paragraph (pesticide data). These criteria are based in particular on the frequency of detection in previous years.

91 Group 2A has 81 agents, while 294 substances are classed in Group 2B by the IARC. For information, the IARC classification has 5 groups (1, 2A, 2B, 3 and 4).

3.3.2.3. Other data

It is important to have other data on the way in which the different sectors involved in crop production are organised and put into practice, at their particular level, pesticide residue management measures. It can therefore be of advantage to have feedback on the results of self-checking by companies and even the results of sectoral product sampling and analysis campaigns for the various links in the chain.

Other miscellaneous information, such as the **level of consumption of foods** by the local population, the nature and impact on the residue content of the various stages involved in the processing of raw materials to turn them into foods, as well as the effect of culinary preparations.

Data relating to the **organisation of the distribution network** and **points of sale** to consumers are another category of data that is useful for planning controls.

Finally, information must be obtained on the analytical capabilities of laboratories (methods used and level of validation, nature of the pesticides analysed and analytical matrices (commodities), number of samples analysed by time unit, costs etc.).

3.3.3. Prioritisation and determination of the number of samples to be set

3.3.3.1. Preparation of the 'Product groups – Hazards to be controlled' matrix

Preparation of this matrix involves:

a. firstly, **putting together, in a table, all the commodities to be controlled**, as set out in stage 3.3.2., and, for each of them, **listing the pesticides** deemed to be relevant based on the procedure also set out in section 3.3.2.;

b. secondly, **focusing on the different commodities** that can be seen as being comparable in terms of risk. They must be regrouped as explained above (3.2.3.). For example, all forms of cabbage must be grouped into the "*brassicas*" category; all types of citrus fruit must be placed in the "*citrus fruits*" category, etc.[92]

For each category, profiles must be drawn up for major pesticides, *i.e.* **the list of relevant pesticides that must be included** in the **Multi-Residue Method** (**MRM**) for analysis. For example, the multi-residue method for citrus fruits will be 'MRM-citrus fruits' and the multi-residue method for brassicas will be 'MRM-brassica'.

Each 'product category-pesticide for analysis profile' or 'product category-MRM-...' pair is considered to be a '**commodity-hazard**' **pair for which the number of samples to be analysed must be determined** based on risk.

If analyses applying specific methods must planned (since it not feasible technically to include them in the MRM), these must also be mentioned but will be the object of a new 'commodity-hazard' pair for which the number of samples to be analysed must be calculated separately. An example is given in table 2.

92 In the case of the programming of controls specifically targeting compliance with MRL legislation, these categories must be compatible with those set out in Regulation (EC) No. 396/2005 (see Annex 1).

Table 2: Example of a 'product category-hazards to control' matrix (each line corresponds to a product-hazard pair requiring the risks to be prioritised)

Product category	Pesticides profile = hazards to be controlled
Citrus fruits	Pesticides covered by the 'MRM-citrus fruits'
Brassicas	Pesticides covered by the 'MRM-brassica'
	Bromides (specific method)
	Dithiocarbamates (specific method: all the dithiocarbamates are analysed using the same method given that they generate the same residue, CS_2)
Cereals	Pesticides covered by the 'MRM-cereals'
	Glyphosate (specific method)
	Chlormequat (specific method)

3.3.3.2. Risk prioritisation and calculation of the number of batches to be sampled

Risk prioritisation is performed by **calculating the number of batches to be sampled based on a method that takes into account the prevalence of the hazard and its severity**. The number of samples to be analysed is determined with the aim of detecting a minimum percentage of non-conformities with a certain degree of reliability.

The method for calculating the number of batches to be sampled and analysed falls into **4 stages**, which are set out below.

- ***Stage 1: determining the prevalence level to be controlled (PLC) for the different 'product population-pesticide profile' pairs***

After having fixed N, the total number of batches within the population, the prevalence level to be controlled (PLC) is established. The higher the severity (S) of the hazard (harmful effects anticipated), the lower the prevalence level to be controlled. The values for the prevalence level to be controlled given in Table 3 are used for the 4 classes of severity, as defined for the pesticide profiles in point 3.2.2.

Table 3: Prevalence level to be controlled for a given product population (commodities) based on the severity class established for the corresponding pesticides profile

Profile hazard class	Prevalence level to be controlled (PLC)
1	10 %
2	5 %
3	2,5 %
4	1,0 %

- *Stage 2: Calculation of the confidence index*

Three parameters are needed to calculate the confidence index (CI):

- The first is the hazard severity score (S) defined above.
- The second is the prevalence (P), which reflects the extent to which hazard is present in the population to be controlled. It also consists of 4 classes, defined as follows:
  1. little hazard detected and MRL not exceeded (score 1);
  2. hazard sometimes detected that is above the MRL or present at a regular frequency while not exceeding the MRL (score 2);
  3. MRL regularly exceeded or frequently detected with the MRL sometimes being exceeded (score 3);
  4. frequent detection accompanied by the MRL regularly being exceeded (score 4).
- The third parameter is linked to the contribution (C) made by the hazard (in the matrix considered) to the exposure of consumers Here too, there are 4 categories and, consequently, 4 scores:
  1. exposure to pesticides analysed through the matrix concerned is only marginal (score 1);
  2. exposure to the pesticides analysed through the matrix concerned is average (score 2);
  3. exposure to the pesticides analysed through the matrix concerned is substantial (score 3);
  4. exposure to the pesticides through the matrix concerned is very substantial since the matrix is widely consumed or equates to a high level of exposure (score 4).

Calculation of the confidence index is then undertaken applying the following formula:

$$IC = G + (P \times C)$$

The confidence index values may range from 2 to 20 depending on the value of the scores of each of the parameters involved. The higher the confidence index value, the higher the risk linked to the 'products-pesticides' pair.

- ***Stage 3: Allocation of the 'alpha' confidence level to determine the number of batches to be controlled***

The 'alpha' confidence level is allocated as follows:

- 90 % if the total score (CI) is between 2 and 6;
- 95 % if the total score (CI) is between 7 and 12;
- 99 % if the total score (CI) is between 13 and 20.

- ***Stage 4: Calculation of the number of samples to be taken***

This last stage involves calculating the number *n* (number of samples to be analysed within the population) using the modified Cannon & Roe formula:

$$n = [1 - (1 - \text{alpha})^{1/NPC}] * [N - (NPC - 1)/2]$$

3.3.4. Practical example of determination of the number of batches to be sampled

To provide a concrete example of the method for calculation number *n*, we use a **fictitious example of the production of fruit and vegetable at the level of a given country** and perform all the stages required to ultimately obtain the number *n*. We restrict ourselves to two categories of fruits and vegetables, notably pome fruits and leafy vegetables. With regard to pome fruits, we presume that a large part of national production is exported; for leafy vegetables, the production and consumption are essentially local so that we ignore any imports or exports. It is therefore assumed that the entire national production is consumed by the local population (10 million inhabitants).

3.3.4.1. Data collected

- ***Volume of production and number of batches produced annually***

Table 4 sets out the figures for production at national level (e.g. FAO data or national statistics). A batch is taken to be a volume of production obtained under the same conditions and with the same characteristics and therefore equates to the approximate quantity of products obtained from a representative parcel of land (1 ha for fruit trees and 10 ares for leafy vegetables).

Table 4: Production figures for leafy vegetables and pome fruits for the (fictitious) country considered

Product category	Products-commodity	Annual production volume (tonnes)	Batch size (tonnes)	Number of batches
Leafy vegetables	Butterhead lettuce	62,000	2.5	15,500
	Curly endive	16,000	2.5	6,400
	Iceberg lettuce	3,000		1,200
	Lamb's lettuce	2,100	2.5	840
	Lollos (green and red)	2,000	2.5	800
	Arugula	1,800	2.5	720
	Oak-leaf salad	1,200	2.5	480
	Other	6,500	2.5	2,600
	Total			**28,540**
Pome fruits	Apples	11,000	10	11,800
	Pears	4,000	10	4,700
	Other	3,000	10	3,200
	Total			**19,700**

3.3.4.2. Pesticide data

The Delcour *et al.* (2014) study is used as the basis for identifying relevant pesticides that must be included in the pesticides profile to be established for the two categories chosen as examples. The list of pesticides concerned is given in table 5.

Table 5: List of relevant pesticides for the two product categories selected

List of relevant pesticides for lettuces (Leafy vegetables category)	List of relevant pesticides for apples (Pome fruits category)
mepronil	daminozide
azoxystrobin	phosalone
mandipropamid	primicarb
pymetrozine	dithianon
iprodione	boscalid
propamocarb	pyraclostrobin
boscalid	cyprodynil
dimethomorph	flufenoxuron
deltamethrin	triadimefon
tolclofos	carbendazim

(Source: Delcour *et al.*, 2014)

Based on this first list, a draft '**profile for residues to be tested for**' is established **by incorporating all the other pesticides seen as relevant** based on their use in the crops considered, the import data and retail sales figures, previous detections etc., and taking into account the definition of the relevant residue. We consider that all ten key pesticides identified by the Delcour *et al.*, (2014) study are the core pesticides for the profile for the leafy vegetables and pome fruits category and that they can all be analysed based on the Multi-Residue Method applicable to these two profiles.

3.3.4.3. Steps in the calculation of n

To calculate the *n*, or number of samples to be controlled, based on risk, the five stages described in point 3.3.2 (Risk prioritisation and calculation of the number of batches to be sampled) must be followed. Figure 3 shows a diagram of the different components involved in calculating the number of samples to be analysed.

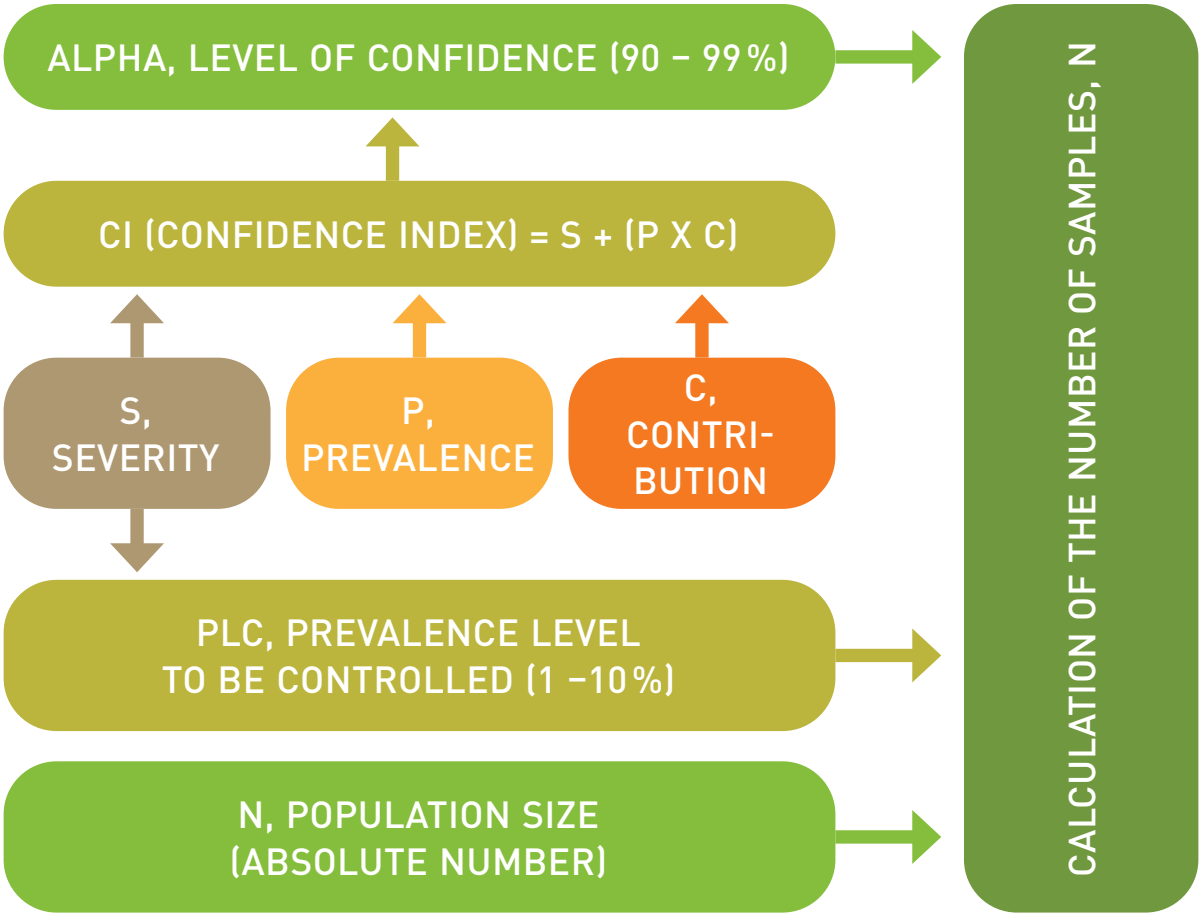


Figure 3 - Diagram showing the different parameters involved in calculating the number of samples n to be taken to conduct a risk-based control

- ***Stage 1: determining the prevalence level to be controlled for the different 'Product categories-pesticide profile' pairs***

For this stage, a **severity score** must be allocated to the pesticide **profile** established.

If we take our first draft profile as set out in table 5, we first need to **examine the Acceptable Daily Intake values** for each pesticide (identify the lowest Acceptable Daily Intake value since the greatest risk is chronic exposure), and **then** the Acute Reference Dose values for those pesticides (identify the lowest Acute Reference Dose value) to be able to establish **the most 'sensitive' product**, *i.e.* that which will determine the **severity score to be allocated to the profile as a whole**.

Tables 6 and 7 set out all the values that will be analysed to establish the severity score for the two profiles.

Table 6: Details for calculating the severity score of the profile for the leafy vegetables pesticides to be controlled

List of relevant pesticides for lettuces (leafy vegetables category)	DAI (mg/kg pc/day)	ARfD (mg/kg pc)	Severity score (S)
mepronil	No information	No information	4
azoxystrobin	0.2	Not applicable	1
mandipropamid	0.15	Not applicable	1
pymetrozine	0.03	0.1	2
iprodione	0.06	Not applicable	2
propamocarb	0.029	1	1
boscalid	0.04	0.03	3 (2 + 1)
dimethomorph	0.05	0.6	2
deltamethrin	0.01	0.01	3 (2 + 1)
tolclofos	0.064	Not applicable	2

(Source: EU Pesticides Database available at ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.detail&language=EN&selectedID=1270)

Since **mepronil** is the pesticide deemed to be the most relevant for residue analysis, it will be allocated the highest severity score by default since it is a **prohibited pesticide for which there is no toxicological documentation (no Acceptable Daily Intake value = the lowest value since the risk is high)**. Consequently, the entire profit is characterised by the **maximum severity score of 4**.

Table 7: Details for calculation of the severity score for the 'pome fruits' profile

List of relevant pesticides for apples (pome fruits category)	DAI (mg/kg pc/day)	ARfD (mg/kg pc)	Severity score (S)
daminozide	0.45	Not applicable	1
phosalone	**0.01**	**0.1**	**2**
primicarb	0.035	0.1	2
dithianon	**0.01**	**0.12**	**2**

List of relevant pesticides for apples (pome fruits category)	DAI (mg/kg pc/day)	ARfD (mg/kg pc)	Severity score (S)
boscalid	0.04	Not applicable	2
pyraclostrobin	0.03	0.03	3 (2 + 1)
cyprodynil	0.03	Not applicable	2
flufenoxuron	**0.01**	**Not applicable**	**2**
triadimefon	0.03	0.08	3 (2 + 1)
carbendazim	0.02	0.02	3 (2 + 1)

(Source: EU Pesticides Database available at ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.detail&language=EN&selectedID=1270)

The **lowest Acceptable Daily Intake value** (0.01 mg/kg pc/day) is found for three of the pesticides in the profile and this corresponds **to a score of 2**. As the Acute Reference Dose value is either not applicable or not less than 0.1 mg/kg pc/day, **the severity score of 2 remains unchanged**. The score of 2 will therefore be allocated to the entire profile (this would be the case even if a score of 3 had been allocated to other active substances).

The prevalence level to be controlled (PLC) is then determined based on the severity of the hazard. This stage is summarised in Table 8.

Table 8: Prevalence level to be controlled for the two categories of products considered

Pesticides profile to be controlled	Severity score (S)	PLC
'Leafy vegetables' profile	4	1 %
'Pome fruits' profile	2	5 %

- ***Stage 2: Calculation of the confidence index***

This stage is the most complex (numerous parameters to be taken into consideration) and greatly dependent on the quality of the information collected, and in particular that relating to the prevalence of pesticide residues (frequency of detection) and consumption of the various commodities concerned.

Table 9 sets out all the elements necessary for calculating the Confidence Index (CI).

- The ***gravity score*** (S) remains that determined previously for the entire profile.
- The ***prevalence*** (P) is estimated based on the frequency of detection. As the number of pesticides included in the profiles is known to have high detection frequencies or MRLs that are exceeded, it is proposed that the highest score (P = 4) be allocated for both leafy vegetables and pome fruits.
- The ***parameter for contribution*** (C) of the commodity to pesticide exposure will be set at the maximum value of 4 for leafy vegetables since the product

that is the most relevant for the profile is characteristic of leafy vegetables and there are few other product categories that contain this residue. In addition, leafy vegetables are consumed in large amounts in the country considered. Consequently, leafy vegetables are one of the key vectors for exposure to the pesticides characteristic of that profile and the value for C will be the maximum (C = 4). In contrast, for pome fruits, the pesticides representative of the profile are present in numerous other commodities (stone fruits, exotic fruits, bush berries, numerous vegetables) and pome fruits are just one of many sources of consumer exposure. In addition, pome fruits produced in the country concerned are largely exported to third countries and the proportion consumed locally is relatively low based on the consumption data that it has been possible to collect. Consequently, the C value for pome fruits is set at 2.

The various score values make it possible to calculate the Confidence Index (CI), as set out in Table 9. The confidence index value may be seen as the outcome of the risk assessment: the higher the CI value, the greater the risk.

Table 9: Allocation of scores for severity (S), prevalence (P) and contribution (C), and calculation of the confidence index (CI = S + [P*C])

'Product category-pesticides profile' pair	G (Severity)	P (Prevalence)	C (Contribution)	CI (Confidence index)
Leafy vegetables	4	4	4	20
Pome fruits	2	4	2	10

- ***Stage 3: Allocation of the alpha confidence level to determine the number of batches to be controlled***

For leafy vegetables, given that the confidence index is 20, the alpha confidence level should be 99% whereas it will only be 95% for pome fruits for which the confidence index value is 10.

- ***Stage 4: Calculation of n (the number of samples to be taken***

The last stage is conducted by calculating the number *n* using the modified formula produced by Cannon & Roe and presented earlier. It is suggested that the WinEpi software available over the Internet[93] be used for the calculations. Table 10 sets out the input data to be entered into the system and the result obtained (value of *n*).

93 www.winepi.net/uk/index.htm.

Table 10: Calculation of the number *n* (samples to be taken) using the WinEpi software (the text in italics corresponds to the title of the parameter to be input into the system once the 'detection of disease' functionality has been chosen)

Product category	Size of the population N	Alpha confidence level *(Confidence level, %)*	Prevalence level to be controlled *(Expected minimum prevalence, %)*	Number of samples to be taken *(needed sample size)*
Leafy vegetables	28,540	99 %	1 %	456
Pome fruits	19,700	95 %	5 %	58

3.3.4.4. Analysis of the findings

The statistical approach used is based on a prevalence level to be controlled with a given level of confidence. This method is based on risk (probability of a harmful impact) since, to determine the prevalence level to be controlled and the confidence level, it includes, firstly, parameters associated with the hazard severity and, secondly, parameters associated with:

1. the occurrence of the hazard in the matrix considered;
2. the contribution of the matrix in question to consumer exposure to that contaminant.

The number of analyses is calculated so that if the real prevalence is under the prevalence level to be controlled, all the samples taken will provide a "negative" result. The confidence level indicates the degree of certainty that the real prevalence will in fact be below the prevalence level to be controlled, provided all the samples give a "negative" result.

The two examples set out below relate to relatively large production volumes each containing some tens of thousands of batches. However, it is clear that in the case of pome fruits, the number *n* of batches to be sampled is proportionately lower (0.30 % of the total number of batches) than in the case of leafy vegetables (1.60 % of the total number of batches). This shows that the calculation method takes into consideration a certain number of risk factors linked to the hazard severity (S) and the potential exposure of the consumer (P + C).

This is a method particularly well suited to the needs of risk managers, who may need to establish a controls programme for a large number of product categories and a variety of hazards.

3.3.4.5. Applicability of the method to other hazards

The method can also be adopted where multi-analyte techniques are used **to detect mycotoxins and metal trace elements** in food productions.

In this case, however, **a severity score assessment would need to be undertaken in advance** given the different toxicological properties of these contaminants and the **carcinogenic potential or other relevant toxicological properties** associated

with these hazards. The different mycotoxins or metal trace elements would, in fact, need to be grouped within the relevant hazard profiles and a severity score would need to be established based on the most toxic component in the profile. The different "commodities – hazards profiles" pairs would be listed and the number *n* would be calculated by applying the same procedure as that described above for pesticides

3.3.5. Planning of controls

The planning of controls is **the intermediate stage between the programming and implementation of controls**. It makes it possible to provide precise and concrete information on the nature, number and sites for the samples to be taken over a given time line. We deal here solely with controls that involve samples being taken to analyse residues in a laboratory, while acknowledging that other forms of control are possible (inspection, documentary controls etc.).

Since the control programme may be pluri-annual, the first task to be undertaken is to **apportion the categories of products to be controlled over time (3 to 5 years)**, ensuring that the apportionment is as uniform as possible in terms of workload and breakdown of the categories that are most at risk. Consequently, since it is known that fruits and vegetables form a series of categories with many high-risk products, it is logical to ensure that the various categories are apportioned over time while ensuring that a reasonable proportion of the most sensitive products are covered each year.

With regard to products, the number of samples established must then be **apportioned** by category. Unless otherwise indicated (no products more at risk than others within the category), the apportioning can be made *pro rata*, based on the batches produced (or marketed), as determined for each commodity during the stage in which the size of the total population for the category was calculated (see table 4).

The **sites and sample collection time line** must then be **determined**. The choice of sampling sites may depend on the objective that the risk managers have set. If the objective is to check compliance with residue legislation (which is generally the primary objective of a control programme), the samples will be taken at the site that is closest to the production, that is to say at the level of the production companies themselves. However, for logistical reasons, it is often preferable to take samples at a more centralised site, such as wholesale markets and auctions for example, always providing, of course, that traceability can be established and, in the event of non-compliance, it is possible to identify the producer.

As to the **sample collection time line**, this will of course depend on the cultivation methods used for the commodity. In the case of seasonal production, the sampling must primarily be focused on this period, with particular attention being paid to early fruit and vegetables since these are the most likely to contain residues.

The result of this activity must be translated into a plan that contains all the practical information required for the inspectors to complete their task.

3.4. CONCLUSIONS

Risk-based controls programming may follow the general methodology described for contaminants and other hazards present in the food chain. Given the considerable number of "commodities – hazards" pairs involved in the area of pesticide residues in all products intended as food, the **methodology must be adapted** to be able to control all the parameters that come into play.

It is therefore proposed that the **programming be achieved by first working on the aggregated data as a whole** in order to establish a reasonable number of 'products-hazards' pairs.

To this end, categories of products must be considered and, for each category, a profile of relevant pesticides drawn up (major pesticides). Since risk assessment will be undertaken at this level of aggregation, it is important that **the parameters that characterise the risk (prevalence and severity) are based on the most critical representatives** in the profile so that the risk is not diminished by too much weight being given to less problematic pesticides present in the profile.

Using this methodology, **it is possible for risk managers** (who will ideally perform their task in conjunction with risk assessors to determine the hazard severity scores) **to draw up a concrete control plan** (the number of samples to be collected is determined for each food) **that is based on risk**.

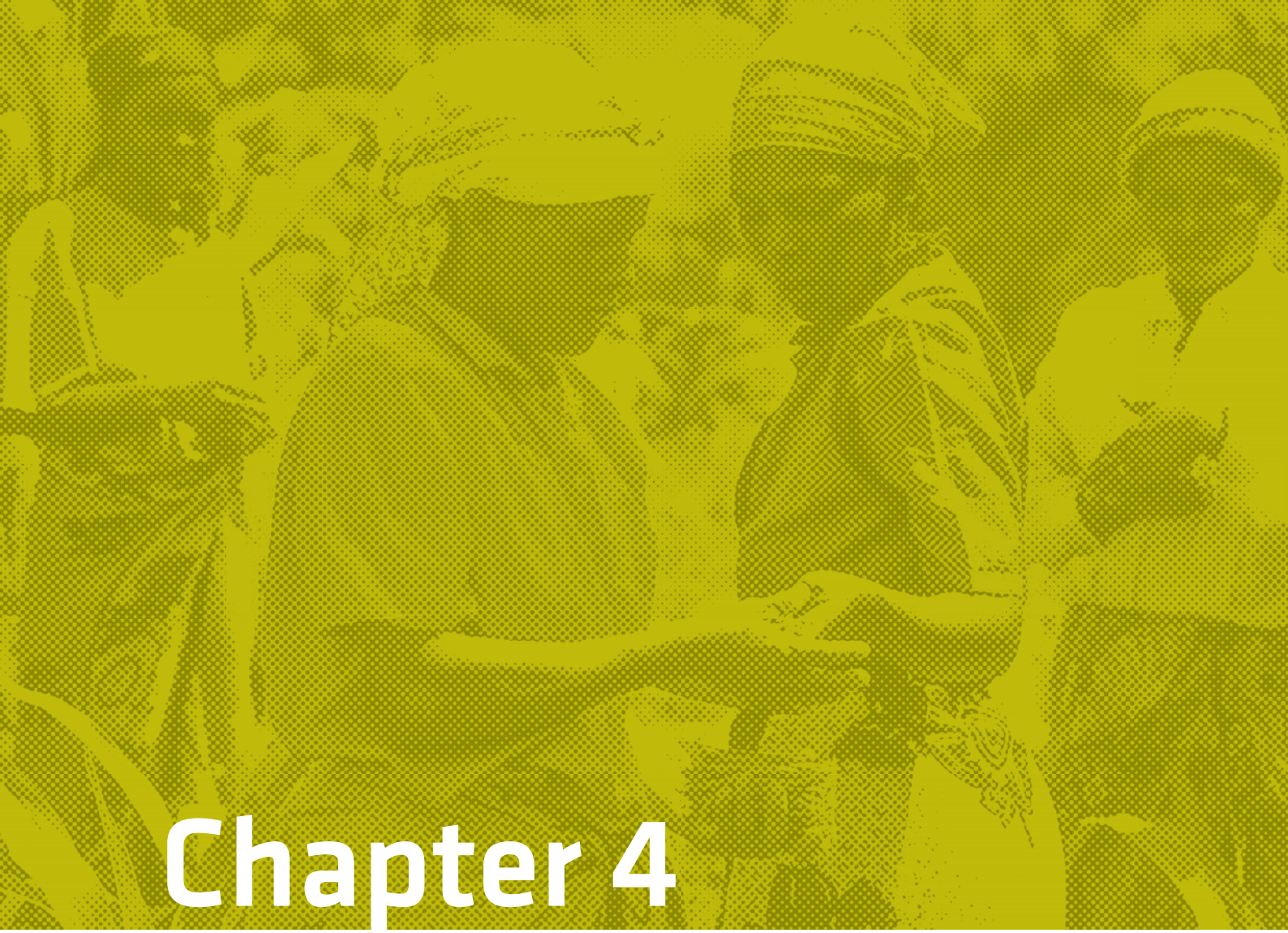
The approach described is applicable to all residues and contaminants present in agricultural products and food that must be examined to determine their compliance with the statutory provisions and that may be analysed using a multi-analyte-type method, as is the case for pesticides but also, increasingly, for mycotoxins and metal trace elements.

3.5. ANNEX

A.1. Products-commodities categories and groups in accordance with Regulation (EC) No. 396/205

Code number	Product categories	Groups to which MRLs apply
0100000	FRESH OR FROZEN FRUIT; NUTS	
0110000		Citrus fruits
0120000		Nuts (shelled or otherwise)
0130000		Pome fruits
0140000		Stone fruits
0150000		Berries and small fruit
0160000		Sundry fruits
0200000	FRESH OR FROZEN VEGETABLES	
0210000		Root vegetables and tuber vegetables
0220000		Bulb vegetables
0230000		Vegetables – fruits
0240000		Brassicas
0250000		Leafy vegetables and mixed herbs
0260000		Legume vegetables (fresh)
0270000		Stem vegetables
0280000		Mushrooms
0290000		Algae
0300000	DRIED VEGETABLES	
0400000	GRAINS AND OLEAGINOUS FRUITS	
0401000		Oilseeds
0402000		Oleaginous fruits
0500000	CEREALS	
0600000	TEA, COFFEE, INFUSIONS AND COCOA	
0610000		Tea
0620000		Coffee beans
0630000		Infusions
0640000		Cocoa
0650000		Carob
0700000	HOPS	
0800000	SPICES	

0810000		Seeds
0820000		Fruits and berries
0830000		Bark
0840000		Roots or rhizomes
0850000		Buds
0860000		Flower stigma
0870000		Aril
0900000	SUGAR CROPS	
1000000	PRODUCTS OF ANIMAL ORIGIN-LAND ANIMALS	



Chapter 4

Laboratory organisation

4.1. INTRODUCTION

4.1.1. Context

Laboratory Organisation is a key aspect of any effective laboratory. For testing laboratories the internationally accepted structure for laboratory organisation is provided in ISO/IEC 17025 and in the documented Principles of Good Laboratory Practice, also known as GLP. Box 1 below provides links to freely downloadable documents regarding GLP.

Compliance with GLP is necessary when a laboratory is carrying out testing work in support of the preparation of a dossier for submission to Regulatory Authorities for registration or market approval. Examples of such testing work are all novel food ingredients, veterinary medicines, agrichemicals such as herbicides and pesticides. Indeed GLP is required for all pre-clinical safety studies of chemical and other products for human and animal use. Laboratories may find themselves sub-contracted to do specific tests as part of such a safety study for another organisation, or they may have full responsibly for the study.

Laboratories carrying out on-going testing are not required to be GLP compliant, but nonetheless have to have a management system in place that demonstrates in a transparent way that they are technically competent and provide consistently technically valid test results. To demonstrate this they have to have a management system (formerly called a quality system) in place.

The management system which most food control and food safety laboratories implement is specified in the **ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories.**[94] Supporting application documents for this standard are available to download for free from the websites of ILAC[95] (International Laboratory Accreditation Cooperation) and EA[96] (European co-operation for Accreditation) and from many national accreditation bodies.

94 A laboratory has to purchase this from a standards body that is a member of ISO or from ISO itself. See: www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=39883.

95 www.ilac.org/guidanceseries.html. Le lien ne fonctionne pas

96 www.european-accreditation.org/publications.

Most GLP monitoring agencies expect laboratories to have a management system to the requirements of ISO/IEC 17025 in place (though not necessarily either certified or accredited). However specific requirements exist under GLP and particularly for *in vivo* studies (testing with live animals or other biota).

The fundamental difference between full compliance with the requirements of ISO/IEC 17025 and GLP lies in the following:

- Compliance with ISO/IEC 17025 serves to demonstrate technical competence, impartiality and performance capability;
- GLP is a legal requirement for laboratories that are conducting regulatory studies and embodies a set of principles that provide a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived.

GLP studies usually evaluate the properties of a test item to either determine its characteristics or to evaluate the effect that it has upon a test system, such as for example the potential of a new agrochemical active ingredient to persist in soil.

DOCUMENTS AVAILABLE TO DOWNLOAD FREE FROM THE OECD WEB SITE

www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/oecdseriesonprinciplesofgoodlaboratorypracticeglpandcompliancemonitoring.htm#GLP_consensus_documents.

No. 1: OECD Principles on Good Laboratory Practice (also available to download as an Annex to the EU Directive 2004/10/EC for Good Laboratory Practice)

See: http://ec.europa.eu/enterprise/sectors/chemicals/documents/specific-chemicals/laboratory-practice/index_en.htm

GLP CONSENSUS DOCUMENTS

No. 4: Quality Assurance and GLP (revised 1999);

No. 5: Compliance of Laboratory Suppliers with GLP Principles (revised 1999);

No. 6: The Application of the GLP Principles to Field Studies (revised 1999);

No. 7: The Application of the GLP Principles to Short Term Studies (revised 1999);

No. 8: The Role and Responsibilities of the Study Director in GLP Studies (revised 1999);

No. 10: The Application of the Principles of GLP to Computerised Systems (1995);

No. 13: The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies.

GUIDANCE DOCUMENTS FOR COMPLIANCE MONITORING AUTHORITIES

No. 2: Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice (also available as an Annex to the EU Directive 2004/9/EC for the Monitoring of Good Laboratory Practice, see Chapter 1);

No. 3: Revised Guidance for the Conduct of Laboratory Inspections and Study Audit;

No. 9: Guidance for the Preparation of GLP Inspection Reports.

ADVISORY DOCUMENTS OF THE WORKING GROUP ON GLP

No. 11: The Role and Responsibility of the Sponsor in the Application of the Principles of GLP;

No. 12: Requesting and Carrying Out Inspections and Study Audits in Another Country;

No. 14: The Application of the Principles of GLP to in vitro Studies;

No. 15: Establishment and Control of Archives that Operate in Compliance with the Principles of GLP.

POSITION PAPERS

- The Use of Laboratory Accreditation with reference to GLP Compliance Monitoring (1994);
- 'Outsourcing' of Inspection Functions by GLP Compliance Monitoring Authorities (2006).

ISO/IEC 17025 is a standard for laboratory competence that accreditation bodies use and which is concerned with the technical competence of a laboratory. Accreditation by a national accreditation body serves to demonstrate impartiality and performance capability. Tests conducted to ISO/IEC 17025 are usually performed to evaluate a specific property of a sample, for example traces of residues or contaminants such as pesticides, mycotoxins etc. in a food product or raw material.

Both GLP and ISO/IEC 17025 require documented management systems and verification that activities are conducted in a controlled and consistent manner. The GLP Principles also describe specific roles and responsibilities that do not directly correlate to (but are compatible with) those within ISO/IEC 17025. Consequently, whilst compliance with ISO/IEC 17025 will have addressed many of the elements required for GLP compliance, it is unlikely that a quality system designed to meet the requirements of compliance with ISO/IEC 17025 will satisfy all the requirements of GLP. None the less applying the requirements of ISO/IEC 17025 is a useful way to start establishing a laboratory organisation, and if for actual or potential GLP compliance, doing this while being attentive to the Principles of GLP will also tend to be beneficial.

4.1.2. Basic laboratory organisational requirements

While ISO/IEC 17025 and GLP can be applied in a wide variety of laboratories and testing situations some basic requirements are necessary. The laboratory may be a public or private entity, an established business or corporation, or an identifiable division or in-house activity of a business or corporation, which meets the applicable legal requirements of the governmental jurisdiction in which it conducts business. Legal responsibility aids in addressing issues of liability/accountability, uniqueness, and independence of operation.

There must be a clearly defined laboratory unit with own resources and budget. This does not prevent it from being a part of a larger organisation but for its testing activities it must be clearly separate and free from influences that might affect or even invalidate its tests. If it is part of an organisation that carries out other laboratory activities such as teaching or research, the testing laboratory must control and be responsible for its own resources including staff and equipment to meet the requirements of ISO/IEC 17025 and GLP. Personnel (staff) must clearly be answerable to the testing laboratory while engaged in testing work. The equipment and facilities used for the purposes of ISO/IEC 17025 and GLP testing must be completely under the control of the testing laboratory (this will be discussed further

in Section 4). Equipment may not be used for other purposes (training or research) except under the supervision of the testing laboratory staff; all such use must not affect its operation for testing purposes and must be documented.

EXTRACT FROM OECD PRINCIPLES OF GOOD LABORATORY PRACTICE (MONOGRAPH NO.1) SECTION ON DEFINITIONS

- Test facility means the persons, premises and operational unit(s) that are necessary for conducting the non-clinical health and environmental safety study. For multi-site studies, those which are conducted at more than one site, the test facility comprises the site at which the Study Director is located and all individual test sites, which individually or collectively can be considered to be test facilities.
- Test site means the location(s) at which a phase(s) of a study is conducted.

Testing laboratories/ facilities can have many different forms:

- they may be in permanent facilities, and may include specified out of door areas (for example test plots or animals for agricultural testing, or veterinary testing or for sources of special reagents as well as test systems);
- a testing laboratory might also be a mobile unit which is located close to the test items (for example, a field laboratory where a crop or a plantation is growing, or where a herd might be grazing etc.);
- testing might also be carried out in the field with no actual premises. To meet the requirements of ISO/IEC 17025 and GLP there are strict requirements to ensure that the circumstances (for example the environmental conditions) are fully recorded.

EXTRACT FROM THE SCOPE OF ISO/IEC 17025

1.1. This International Standard specifies the general requirements for the competence to carry out tests and/or calibrations, including sampling. It covers testing and calibration performed using standard methods, non-standard methods, and laboratory-developed methods.

1.2. This International Standard is applicable to all organizations performing tests and/or calibrations. These include, for example, first-, second- and third-party laboratories, and laboratories where testing and/or calibration forms part of inspection and product certification.

This International Standard is applicable to all laboratories regardless of the number of personnel or the extent of the scope of testing and/or calibration activities. When a laboratory does not undertake one or more of the activities covered by this International Standard, such as sampling and the design/development of new methods, the requirements of those clauses do not apply.

Where a laboratory is part of a larger organisation, the organisational arrangements should be such that departments having conflicting interests, such as production, commercial marketing or financing do not adversely influence the laboratory's compliance with the requirements of ISO/IEC 17025 or with the Principles of GLP.

If the laboratory wishes to be recognised as a third-party laboratory, it should be able to demonstrate that it is impartial and free from any undue commercial, financial and other pressures which might influence technical judgement (this includes the laboratories personnel). The third-party testing or calibration laboratory should not engage in any activities that may endanger the trust in its independence of judgement and integrity in relation to its testing or calibration activities.

4.1.3. Outline of the process of putting in place a laboratory organisation

Assuming that the appropriate personnel (Section 3) and facilities (Section 4) are available, the nature and scope of testing have been identified, and that appropriate equipment (Section 5) either is in place or has been identified, the following steps must be implemented:

- suitable management and staff structure put in place;
- the appropriate documentation and procedures prepared and implemented;
- particular attention must be paid to the implementation of key administrative procedures such as:
  - setting up test item registration procedures (tender /contract review),
  - setting up approved vendors,
  - requirements for Suppliers (GLP),
  - setting up a subcontractor register,
  - setting up the investigation procedures for complaints, non-conformances, corrective and preventive actions and improvement,
  - setting up record systems, filing and archiving systems,
  - setting up an internal auditing team (ISO/IEC 17025),
  - requirements for periodic management review of the management system.

Before the laboratory has fully implemented its management system to either ISO/IEC 17025 or the Principles of GLP it must also attend to:

- metrology and management of the traceability chain;
- methods for validation;
- putting in place a quality assurance programme.

4.1.4. How to get started

The laboratory management must first of all define its objectives:

- What kind of testing it wishes to carry out and whether this requires GLP compliance or not?

- What will the scope of testing be, that is what will its clients want tested and for what purposes will the test results be used? This will enable the technical staff to decide what test procedures are suitable, what equipment they might need, and what qualifications and training the existing staff require or those to be recruited will require;
- The scope of testing will also indicate what physical facilities will be required.

Having defined its objectives the laboratory management should carefully study the requirements of the ISO/IEC 17025 standard and, if GLP compliance is contemplated, the Principles of GLP. This generally requires the attendance of and / or training courses if available. In the absence of conveniently available workshops and training courses management and key staff should set aside time to study and tease out the requirements together.

As a next stage (or after the study above) a 'Gaps Analysis' should be carried out.

4.1.5. 'Gaps Analysis'

This requires a sub-clause by sub-clause examination of the standard writing down:

- Firstly whether the standard applies to the proposed activities of the laboratory, and if it does whether or not the laboratory has the necessary requirements in place and how they are documented. This is normally addressed as a policy in the Laboratory Quality Manual or in a procedure or in some other (documented) way. Examples of such 'gap analyses' will be provided in the relevant sections below.
- Secondly, where an element is not in place a programme needs to be developed showing what needs to be done, who is to do it, any resources necessary to do it and a target date for its completion. Laboratories might find it useful to use project organisation computer programmes to keep their implementation programme under control. Such programmes may be downloadable from the Internet.

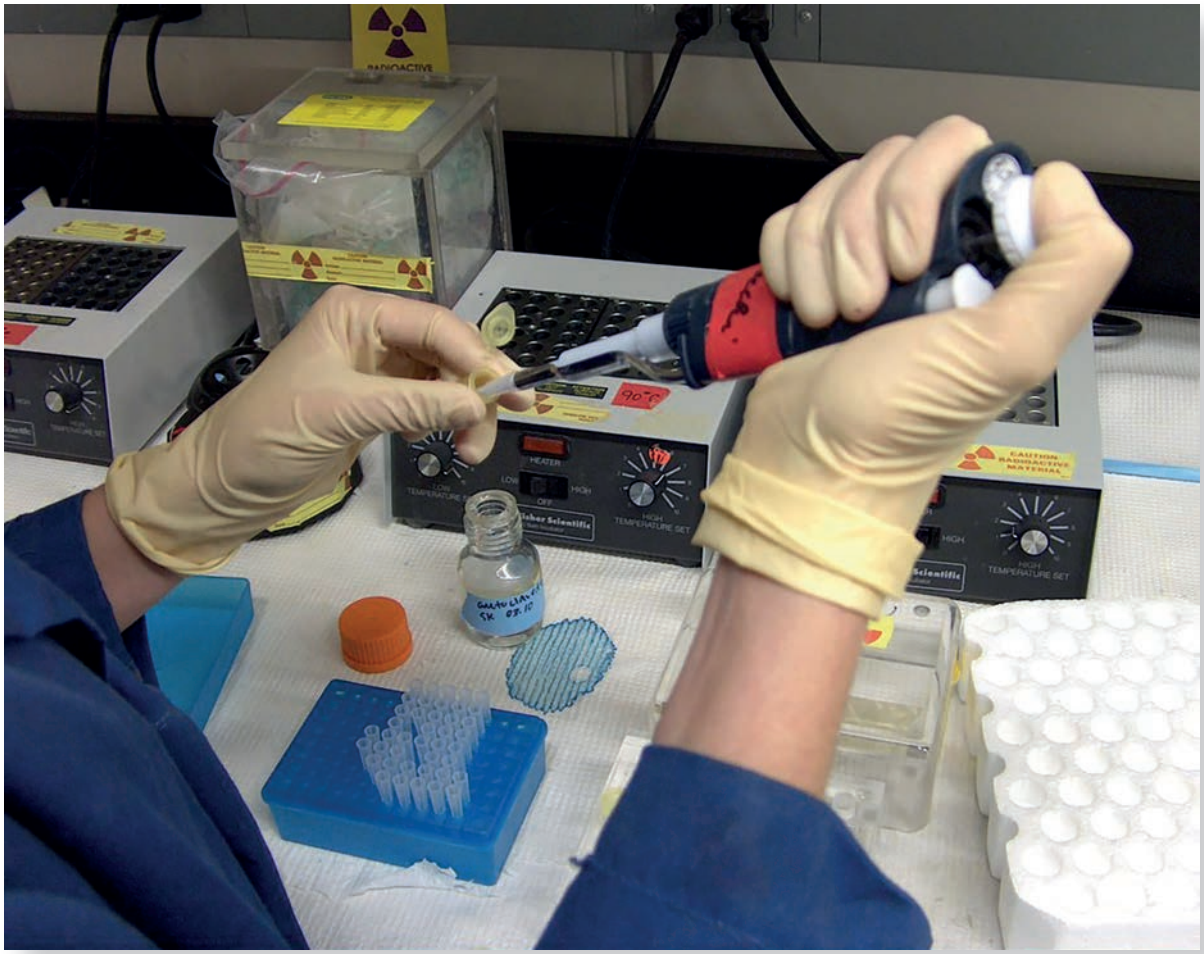
4.2. ESSENTIAL INFRASTRUCTURE

For a laboratory to operate a laboratory organisation to the requirements of GLP or ISO/IEC17025 certain important infrastructural elements should be in place:

- The laboratory should have ready access to equipment calibration services, with the calibration traceable to international measurement reference standards.
- An adequate and convenient source of supply of the required chemicals (reagents and high purity solvents), media, test kits, pure compressed gases (alternatively have their own gas generators), source of suitable laboratory animals (see Section 10) and other laboratory consumables. These should be available to the laboratory in timely manner, particularly materials etc. which have a short shelf-life.
- Convenient service backup for all essential laboratory equipment.
- General uninterrupted power supply with steady frequency and voltage, or special uninterruptible power supply units for equipment which require it.

These above should be seen in addition to access to suitable qualified personnel from which to recruit management, supervisors and staff and to suitable premises.

4.3. PERSONNEL



The most important element of the organisation of a laboratory is its personnel. Without suitable trained and-for certain tests-professionally qualified staff a laboratory cannot operate.

All staff should be carefully selected and have appropriate qualifications and experience for the job. In many cases staff is selected on the basis of their basic training and will be trained for the specific jobs they have to carry out. It will always be necessary to provide training in the requirements of the testing work to be carried out but also in the application of the management system including procedures of the specific laboratory. Some or all of this training may be carried out in-house and on the job. It is essential that records of all such training be retained.

A procedure should ensure that *Curriculum Vitae* (CVs) exist for all personnel in a standard approved format, and that these are kept up to date. These should be in the required languages (local and sometimes English for regulatory submissions) and should be carefully archived to ensure historical reconstruction.

A typical *Curriculum Vitae* includes:

- name and age of the person;
- education, including diplomas and qualifications awarded by recognised institutions;

- professional experience gained both within the laboratory (as recorded in the training records) and before joining it;
- relevant training courses completed (records of these are retained in the training records);
- if relevant:
  - any scientific publications;
  - membership of relevant associations;
  - languages spoken.

All staff should have a CV. Even if some personnel do not have extensive qualifications, they will have professional experience which should be listed in their CV. It is good practice to have the CV signed and dated by the person concerned, confirming the content.

The laboratory must have an updated organization chart indicating the place and the role of each staff member, including the responsible(s) of quality management, the archivist(s) etc.

4.4. PHYSICAL FACILITIES

4.4.1. Premises

The basic requirement for premises in which testing is carried out implies the test environment is such that technically valid testing can be carried out without any interference or contaminants that might invalidate test results. Most standard tests methods specify the environmental conditions which are necessary to ensure a valid test. The validation of the test may be carried out under certain environmental conditions and the validation report will specify the necessary range (of, say, temperature, atmospheric pressure, relative humidity etc.).

Both ISO/IEC 17025 and GLP require that test facilities be of appropriate size, construction and location to meet the requirements of testing or of the study and to minimise disturbances that would interfere with the validity of the tests or study. They should be designed to provide an adequate degree of separation between the various activities.

The purpose of these requirements is to ensure that testing or a study is not compromised because of inadequate facilities. This does not necessarily mean providing "state of the art" constructions, but carefully considering the objectives of the testing activities and how to achieve them. It is up to management to define what is adequate, which will depend on the kind of testing being performed.

Separation ensures that different functions or activities do not interfere with each other, or in the case of GLP affect the study. ***For example, it is important for agricultural testing laboratories testing for traces of contaminants such as pesticides or veterinary medicine to be separated (ideally in different unconnected buildings) from where the bulk pesticides or medicines are being tested or formulated.***

Minimising disturbance by separation can be achieved by:

- physical separation: this can be achieved by walls, doors or filters, or by the use of isolators. When designing new buildings or renovating existing buildings, separation should be built in;
- separation by organisation, for example by establishing defined work areas within a laboratory carrying out different activities in the same area at different times. This will allow for cleaning and preparation between operations or maintaining separation of staff.

The laboratory must be big enough to accommodate the number of working staff allowing them to carry out their own work without risk of interfering with the work of others. Each operator should have a workstation sufficiently large to be able to carry out the operation efficiently. There should be sufficient physical separation between the workstations to reduce the chance of cross contamination.

In general the design of the laboratory should ensure that no direct sunlight falls on the working areas and equipment. This may be achieved by arranging that service corridors etc. are situated along the outer sun facing walls and the laboratory (testing) areas in the central area with suitable partitioning to prevent direct sunlight from entering. This can also be of assistance when equipment or testing necessitates air-conditioned rooms maintained at an even temperature.

The laboratory should be built of materials that allow easy cleaning. Furthermore, test items or contaminants should not be allowed to accumulate in corners and cracks have to be avoided to prevent cross contamination. A proper ventilation system should be in place with filters that serve to protect personnel and prevent cross contamination.

Ideally there should be separate areas for:

- storage of test items under different conditions;
- storage of control items;
- handling of volatile materials;
- weighing operations;
- cleaning equipment;
- offices and refreshment rooms;
- changing rooms.

4.4.2. Animal testing facilities

Special attention is necessary for animal testing facilities. To minimise the effects of environmental variables on the animal, the facility should be designed and operated to control selected parameters, such as temperature, humidity and light. In addition, the facility should be organised in a way that prevents the animals from coming into contact with diseases or with a test item other than the one under investigation.

Requirements will be different depending on the nature and duration of the studies being performed in the facility. Risks of contamination can be reduced by a 'barrier' system, where all supplies, staff and services cross the barrier in a controlled way.

A typical animal house should have separations maintained by provision of areas for:

- different species;
- different studies;
- quarantine;
- changing rooms;
- receipt of materials;
- storage of materials;
- bedding and diet;
- test doses;
- cages;
- cleaning equipment;
- necropsy;
- waste disposal.

The building and rooms should provide sufficient space for animals and studies, allowing the operators to work efficiently. The environment control system should constantly maintain the temperature, humidity and airflow at the defined levels for the species concerned.

Design should allow easy and thorough cleaning of surfaces of walls, doors, floors and ceilings. There should be no gaps or ledges where dirt and dust can accumulate. Water should not accumulate on uneven floors *i.e.* floors should be smooth and even

and without crevices. Non-laminated posters, notices, etc. should not be attached to the wall or doors of cupboards etc. Easily cleaned laminated documents, notices, etc. may be hung on walls; other transient notices, etc. should be sleeved.

Whatever the capabilities or needs of the laboratory, sensible working procedures can reduce the damage from outside influences. Such procedures may include:

- minimising the number of staff allowed to enter the building;
- restricting entry into animal rooms;
- organising work flow so that clean and dirty materials are moved around the facility at different times of the day and ensuring that corridors are cleaned between these times;
- requiring staff to put on different clothing for different zones within the animal facility;
- ensuring that rooms are cleaned between studies.

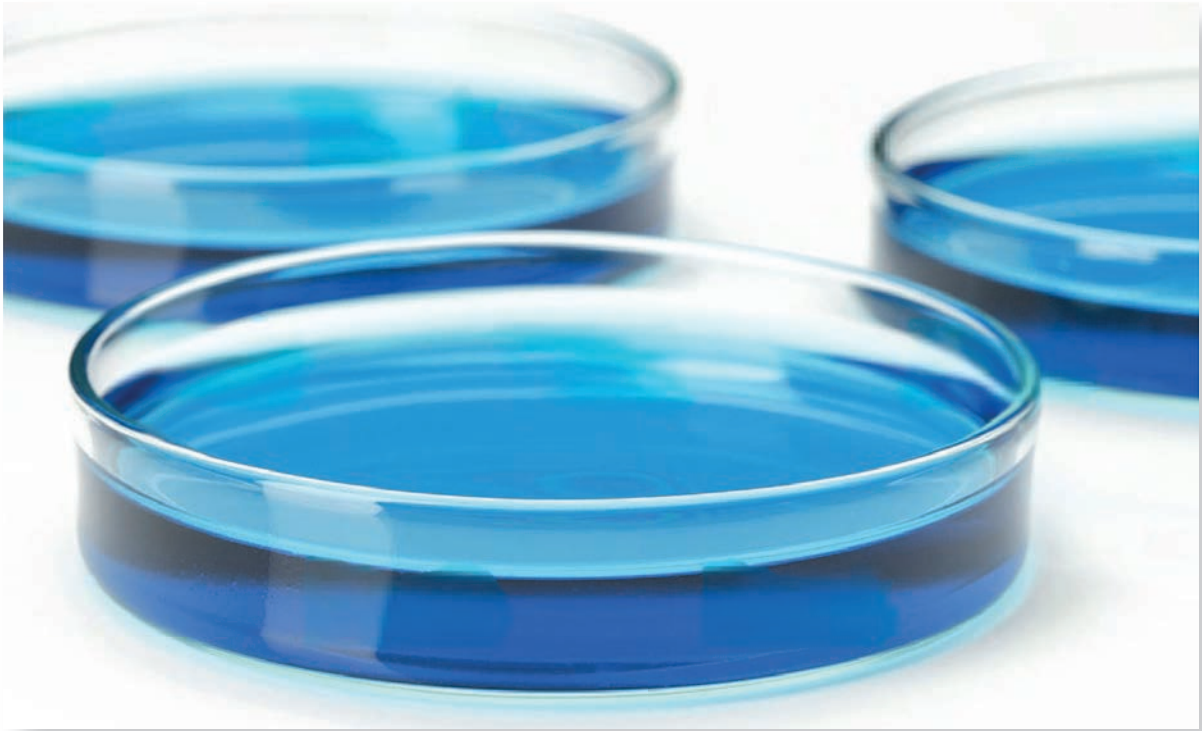
4.4.3. Microbiological testing laboratories

Microbiological testing laboratories require to a large extent the same conditions and procedures as animal test facilities. Only trained personnel should be permitted in microbiological laboratories. A separate area (preferably a separate room) should be used for opening samples. All associated rooms (sample storage, media storage, sample preparation, media preparation and sterilisation, plating and testing, area for incubators, and autoclaves/ ovens for sterilising waste, etc.) should ideally be connected with no passageways with general access between them.

Note

Laboratories are required to monitor, control and record environmental conditions. This includes monitoring biologic sterility, dust, electromagnetic disturbances, radiation, humidity, electrical supply, temperature, sound, vibration as relevant to the testing activities and the equipment in use.

4.5. EQUIPMENT



ISO/IEC 17025 requires that "*The laboratory shall be furnished with all items of sampling, measurement and test equipment required for the correct performance of the tests (including sampling, preparation of test items, processing and analysis of test and/or calibration data). In those cases where the laboratory needs to use equipment outside its permanent control, it shall ensure that the requirements of this International Standard are met*". GLP have the same implicit requirements.

Laboratories that are part of a research organisation or of an educational establishment should either have equipment which is exclusive for its purposes or only permit use by others under strict supervision. Laboratories are required to verify that such use has not affected the correct performance of the equipment.

Key requirements for equipment are:

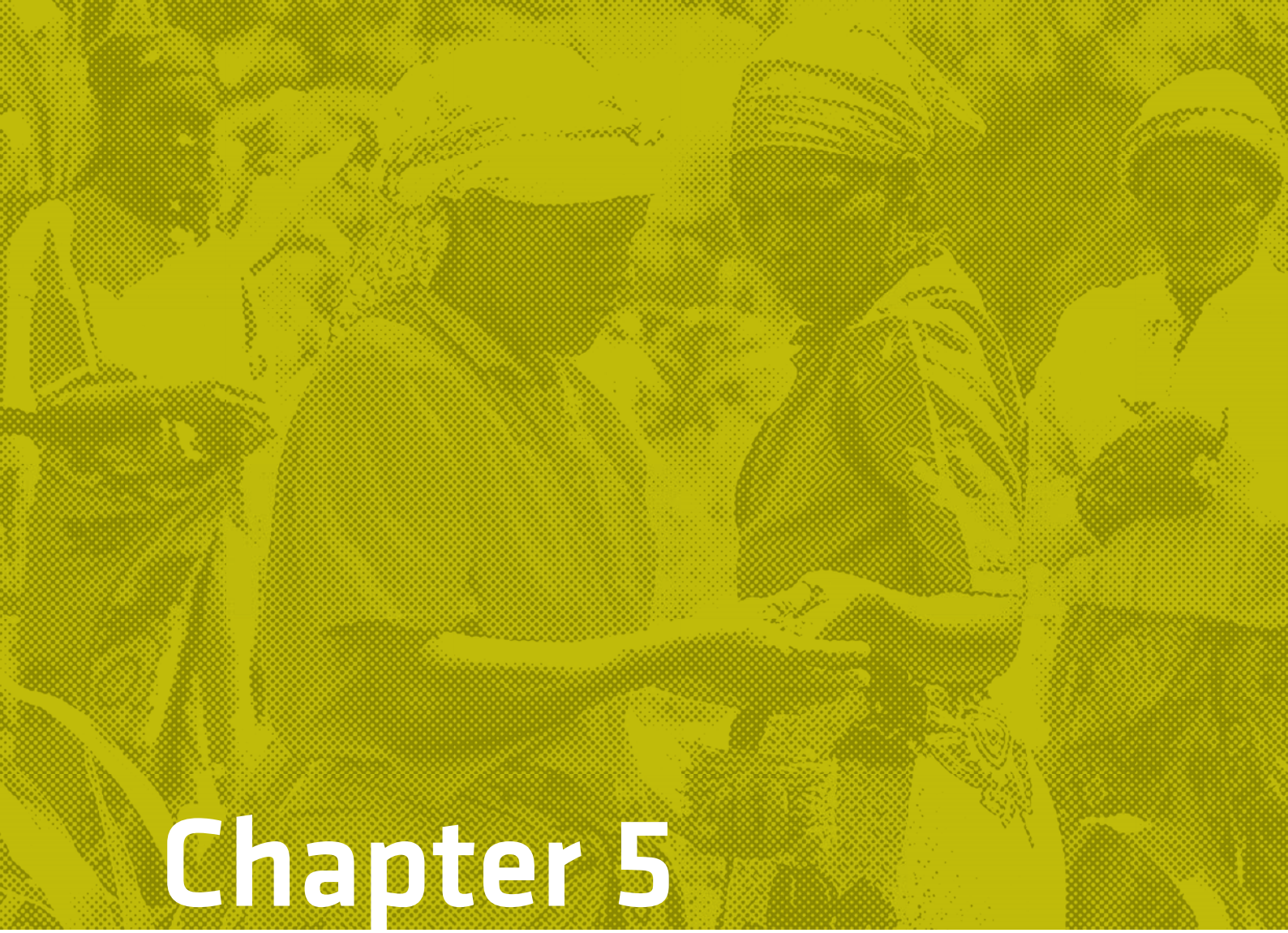
- equipment shall comply with specifications relevant to the tests. This is not "according to the manufacturers specifications", but relevant to the tests;
- before being placed in service, equipment shall be calibrated or checked to establish that it meets the laboratory's specification requirements. This process is sometimes called operational qualification;
- each item of equipment and its software used for testing and significant to the result shall, when practicable, be uniquely identified;
- records shall be maintained for each item of equipment and its software significant to the tests performed (see below). This includes, for example, version numbers of firmware and software. It also includes test results for qualification;

- the laboratory shall have procedures for planned calibration and maintenance;
- defective equipment shall be taken out of service. It shall be isolated and clearly marked to prevent its use until it has been repaired. Equipment found to be out of calibration (by for example and as indicated above daily calibration checks) shall not be used until it has been recalibrated;
- after repair it should be demonstrated that equipment performs correctly;
- the status of calibration should be indicated on each instrument;
- equipment shall be operated by authorised personnel;
- up-to-date instructions on the use and maintenance of equipment (including any relevant manuals provided by the manufacturer of the equipment) shall be readily available for use by the appropriate laboratory personnel. The instructions for use are generally written by the laboratory itself in simple language and easy to follow by the equipment operator and relevant to the testing work as carried out in the laboratory;
- procedures for the safeguard transport and use of equipment which might be used outside the laboratory premises (for example, in the field) should be prepared and available.

Records shall be maintained for each item of equipment and its software significant to the tests performed. The records shall include at least the following:

a. the identity of the item of equipment and its software;
b. the manufacturer's name, type identification, and serial number or other unique identification;
c. checks that equipment complies with the specification;
d. the current location, where appropriate;
e. the manufacturer's instructions (if available) or reference to their location;
f. dates, results, copies of reports and certificates of all calibrations, adjustments, acceptance criteria, and the due date of next calibration;
g. the maintenance plan, where appropriate, and maintenance carried out to date;
h. any damage, malfunction, modification or repair to the equipment.

Test equipment, including both hardware and software, shall be safeguarded from adjustments which would invalid



Chapter 5

Quality management of laboratories: accreditation and certification

5.1. INTRODUCTION

5.1.1. Context

The European Union pursues an integrated approach to food safety aiming to assure a high level of food safety, animal health, animal welfare and plant health through coherent farm-to-table measures and adequate monitoring. At the same time the effective functioning of the internal market of the EU is to be ensured.

The Directorate General for Health and Consumers (DG SANCO), as the main organ overseeing food safety at EU level, has as its mission to assure that effective control systems exist and to evaluate compliance with EU standards in the food safety and quality, animal health, animal welfare, animal nutrition and plant health sectors within the EU and third countries.

This mission also extends to the management of international relations with third countries and international organizations concerning food safety, animal health, animal welfare, animal nutrition and plant health. DG SANCO also manages relations with the **European Food Safety Authority** and ensures science-based risk management.

5.1.2. Legal framework of EU on the role of laboratories in the agro-food sector

In support of this extensive legislation is regularly updated to ensure that food production in all its aspects is carried out in such a way so as to ensure safe quality of food. The basic framework law for **food of animal origin** is Regulation (EC) No. 178/2002 which was issued in 2002. It lays down the general principles and requirements of food law, establishing EFSA and defining procedures in matters of food safety.[97] This regulation requires that official control laboratories and reference laboratories be designated by competent authorities that "may only designate laboratories that operate and are assessed and accredited[98] in accordance with the following European standards":

a. EN[99] ISO/IEC 17025 on "General requirements for the competence of testing and calibration laboratories";
b. EN 45002 on "General criteria for the assessment of testing laboratories";
c. EN 45003 on "Calibration and testing laboratory accreditation system. General requirements for operation and recognition".

97 *OJEC*, No. L 208 of 24 July1992, p. 9. Regulation as last amended by Regulation (EC) No. 806/2003 (Note: *OJEC* refers to the *Official Journal of the European Communities* until 2003, it is called later *OJEU*, *Official Journal of the European Union*; 'L' refers to the legislative series – the reference given is to where the referenced item is published).

98 See Section 5.2 of this chapter.

99 EN stands for European Norm or a European Standard adopted by CEN, the European Committee for Standardization. The designation EN in front of ISO means that that ISO standard has been adopted in Europe. See Section 5.2 of this chapter.

Taking into account criteria for different testing methods laid down in Community feed and food law. The accreditation and assessment of testing laboratories referred to above may relate to individual tests or groups of tests".

There is no requirement for third countries to have reference laboratories. However, Regulation (EC) No. 882/2004[100] requires laboratories that are engaged in verifying compliance with EU food standards to be accredited.

Such laboratories may be private laboratories that have been designated for the purpose of verifying compliance with EU food standards by the body in charge of official controls.

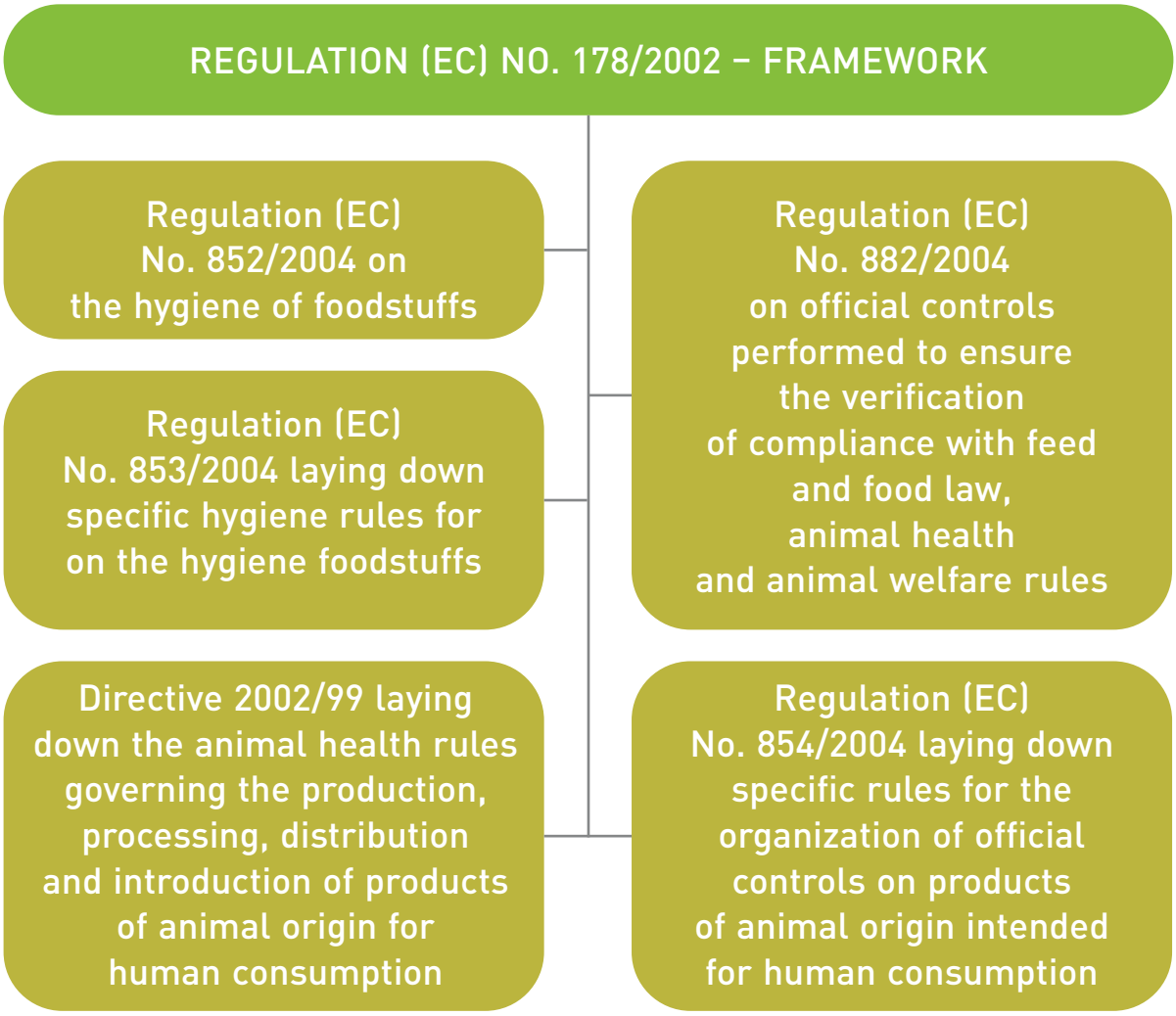


Figure 1 - EU legal framework on food safety

For **plant and plant products**, while there is no reference for laboratories conforming to any standards in Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community, the European and Mediterranean

100 Regulation (EC) No. 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules (*OJEU*, No. L 191 of 28 May 2004).

Plant Protection Organization[101] recommends that laboratories supporting its activities be accredited to ISO/IEC 17025.

In the case of **aquaculture products**, a control plan on heavy metals, contaminants, residues of pesticides and veterinary drugs must be in place to verify compliance with EU requirements.

For **all food products** Commission Decision 98/179/EC[102] laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products specifies in 1.2 of its Annex:

"The analysis of the samples shall be carried out exclusively by the laboratories approved for official residue control by the competent authority.

Participation in an internationally recognized external quality control assessment and accreditation scheme is required for authorized laboratories.

These laboratories must prove their competence by regularly and successfully participating in adequate proficiency testing schemes recognized or organized by the national or European community reference laboratories".

With regard to imports from third countries the EU does not demand accreditation for the laboratories approved by their competent authorities.

Registration of **novel foods or food ingredients** is laid down in Commission Recommendation 97/618/EC[103] of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No. 258/97[104] of the European Parliament and of the Council. The term 'novel' refers to any food or ingredient which was not on the market, in Europe in any event, before 1997.

Section 3.10 on allergenic potential specifies that studies to test the allergenic potential of novel foods should be compliant with principles of good clinical practice and good laboratory practice. Moreover, Chapter XI on nutritional information on novel foods requires that studies should be compliant with principles of good laboratory practice, in particular regarding the numbers involved in study groups.

Directive 2004/9/EC[105] lays down the obligation of the Member States to designate the **authorities responsible for GLP inspections** in their territory. This Directive also specifies reporting and internal market requirements (that is for ensuring mutual acceptance of data). The Directive requires that the OECD Revised Guides for Compliance Monitoring Procedures for GLP and the OECD Guidance for the Conduct of Test Facility Inspections and Study Audits must be followed during laboratory inspections and study audits.

---

101 European and Mediterranean Plant Protection Organization (EPPO) is an intergovernmental organization responsible for European cooperation in plant protection in the European and Mediterranean region.

102 *OJEC*, No. L 221/8 of 17 August 2002.

103 *OJEC*, No. L 253/1 of 19 September 1975.

104 *OJEC*, No. L 43/1 of 14 February 1997.

105 *OJEU*, No. L 50/28 of 28 February 2004.

Directive 2004/10/EC[106] requires Member States to take all measures necessary to ensure that laboratories carrying out safety studies on chemical products comply with the **OECD Principles of Good Laboratory Practice**.

5.1.3. Implications of EU legislation for laboratories in developing countries wishing to export to the EU

To export products of animals including game (animal includes milk and honey), fishery, aquaculture and plant origin, the exporter must certify that the products were produced in accordance with the relevant provisions of Regulations (EC) No. 178/2002, (EC) No. 852/2004[107], (EC) No. 853/2004[108] and (EC) No. 854/2004.[109]

Where such certification requires any testing to support it, the testing must be carried out in a laboratory accredited to ISO/IEC 17025 by an accreditation body with mutual recognition agreement with ILAC or one of its Regional Cooperation Bodies for the relevant tests.

If a developing country wishes to get approval for exporting into any member state of the EU Novel Food or Novel Food ingredients which require any allergenic or nutritional studies, these studies need to be carried out by laboratories compliant with the Good Laboratory Practice regulations.

106 *OJEU*, No. L 50/48 of 28 February 2004.
107 *OJEU*, No. L 226/3 of 25 June 2004.
108 *OJEU*, No. L 226/22 of 25 June 2004.
109 *OJEU*, No. L 226/83 of 25 June 2004.

5.2. DEFINITIONS

In this chapter, '**Laboratories**' are analytical or testing laboratories including microbiological (bacteriological) and radiological laboratories that carry out tests to establish the safety and the wholesomeness of food and the essential characteristics of food.

The key characteristic of these laboratories is that they carry out two types of measurements:

- Quantitative: finding the amount of a substance, a property or a micro-organism; or
- Qualitative: whether the substance or the micro-organism is present or not.

The word '**quality**' has many meanings (there is even a district in California called 'Quality'!), but in the context of testing laboratories it means that the laboratory provides test results that are '**fit for the purpose**'.

A test result that is 'fit for the purpose' is a '**valid**' test result which meets the requirements of the '**customer**'.

In the case of food testing laboratories the 'customer' can include the people who might want to consume the food product, the regulatory authorities who have the responsibility for ensuring the safety of the food, as well as the entity which requested the testing in the first instance.

A test result that is 'fit for the purpose' or is 'valid' is a test result which is the same as that which might have been obtained by another '**competent**' laboratory anywhere in the world within acceptable limits of '**measurement uncertainty**'.

'**Competent**' means having the demonstrable ability to carry out the test.

Measurement Uncertainty[110] (or Uncertainty of Measurement) is a parameter associated with the result of a measurement that characterizes the dispersion of values that could reasonably be attributed to the measurand. As will be explained later all test results have a measurement uncertainty associated with them. Such an uncertainty must be small enough to make the test results useful.

5.2.1. Quality management of laboratories

To ensure that a laboratory will always be capable of producing quality test results it has to have some kind of a '**management system**' in place to ensure this. A 'management system' is the framework of processes and procedures used to ensure that an organization can fulfil all tasks required to achieve its objectives.

5.2.2. Certification

Management systems for Quality in general have been standardized and the most commonly applied International Standard is **ISO 9001: Quality Management systems – requirements**. This standard specifies the requirements for all kinds of organizations who wish to have a quality management system in place which can be certified or registered by a third party, usually a certification body, also known as a **Conformity Assessment Body**. Conformity Assessment means that these bodies assess an organization to determine if it conforms (meets the requirements as intended) to the standard – in this case the ISO 9001 standard. CABs which are certifying bodies are often referred to as CBs rather than CABs which has a wider meaning and includes laboratories.

If, after assessing the organization, the CAB is satisfied that the organization complies with the requirements of the standard it issues a Certificate of Compliance and the organization is stated as an ISO 9001 certified or registered organization. Certification will be discussed further below.

As for other organizations a laboratory can put in place a quality management system that satisfies the requirements of ISO 9001 and be certified by a CAB as being ISO 9001 compliant: this means that the laboratory has a quality management system in place which meets the requirements of ISO 9001.[111]

It is important to note that compliance with ISO 9001 does not provide any assurance concerning the quality of test results.

110 Definition of uncertainty: "A parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand". Measurand: the particular quantity subject to measurement.

111 The IAF is the world association of Conformity Assessment Accreditation Bodies and other bodies interested in conformity assessment in the fields of management systems, products, services, personnel and other similar programmes of conformity assessment. Its primary function is to develop a single worldwide programme of conformity assessment which reduces risk for business and its customers by assuring them that accredited certificates may be relied upon. Accreditation assures users of the competence and impartiality of the body accredited.

International recognition of certification

For international recognition of the certification or registration of the quality management system of a laboratory, the Conformity Assessment Body (CAB) must itself be accredited by a body with multilateral recognition with other accreditation bodies such as the International Accreditation Forum (IAF).

5.2.3. Accreditation

The management system which most food control and food safety laboratories implement is specified in **ISO/IEC 17025 – General requirements for the competence of testing and calibration laboratories**. This standard has in addition to the management requirements (essentially the quality management system requirements of ISO 9001 as applied to testing laboratory activities) an extensive clause with technical requirements which specifies the requirements that a laboratory must have in place to demonstrate that it is capable of providing technically valid results ('fit for the purpose').

Certification vs Accreditation

Certification = Compliance	Accreditation = Competence

As the production of technically valid results is the objective of all laboratories it is evident that this is the standard they should follow, whether they are seeking certification under the ISO 9001 standard or otherwise.

Having implemented the requirements of ISO/IEC 17025 and the relevant technical requirements of this standard specific to the tests the laboratory can demonstrate to be technically competent to perform validly, a laboratory can request an Accreditation Body to assess its performance of the specified tests. If the Accreditation Body is satisfied that the respective laboratory meets the management requirements, demonstrates its competence for the specified tests and meets any special relevant additional requirements specified by the accreditation body, the laboratory can be accredited for those tests (described in a published "Scope of Accreditation").[112]

112 The accreditation of laboratories is based on a defined scope of accreditation which is clear and unambiguous, and provides the laboratory and other interested parties with a detailed list of the tests for which the laboratory has been accredited.

International recognition of accreditation

For international recognition of its accreditation the laboratory must be accredited by a body which has a Mutual Recognition Agreement (MRA) with other accreditation bodies, normally a member of the International Laboratory Accreditation Cooperation (ILAC).

It should be noted that while all the laboratory's activities may and indeed should be compliant with the management requirements of ISO/IEC 17025, only the tests specified on the Scope of Accreditation need to be compliant with the relevant technical requirements and any additional requirements specified by the accreditation body.[113]

5.2.4. Good Laboratory Practice

Laboratories that carry out the safety testing of novel food and new food ingredients for the purposes of approval by Regulatory Authorities are required to carry out their testing activities in compliance with the OECD Principles of Good Laboratory Practice[114]. These principles set down managerial concepts covering the organization of test facilities. As the testing is part of a special study of a novel product, there are specific requirements with regard to a Study Director, a Quality Assurance Unit, and more rigorous requirements regarding the control of records and of reference samples (of the material study).

GLP Study – a definition

'GLP Study': Non-clinical health and environmental safety study, henceforth referred to simply as 'study', means an experiment or set of experiments in which a test item is examined under laboratory conditions or in the environment to obtain data on its properties and/or its safety, intended for submission to appropriate regulatory authorities.

113 The ILAC Arrangement supports international trade by promoting international confidence and acceptance of accredited laboratory data. Technical barriers to trade, such as the retesting of products each time they enter a new economy would be reduced.

114 EU Directive 2004/10/EC requires Member States to take all measures necessary to ensure that laboratories carrying out safety studies on chemical products comply with the OECD Principles of Good Laboratory Practice.

Objective of OECD Principles of GLP

The primary objective of the OECD Principles of GLP is to ensure the generation of high quality and reliable test data related to the safety of industrial chemicals, pesticides, pharmaceuticals, food and feed additives, cosmetics, etc., in the framework of harmonising testing procedures for the Mutual Acceptance of Data.

Most GLP monitoring agencies expect laboratories to have a management system to the requirements of ISO/IEC 17025 in place (though not necessarily either certified or accredited). However for *in vivo* studies (testing with live animals or other biota) there are specific requirements under GLP.

5.3. ROLE OF LABORATORIES IN THE QUALITY INFRASTRUCTURE

The most important activity for the prosperity of every country is the exchange of goods and services. Nothing happens until one person or actor somebody sells a good or service to another. When a selling-buying transaction takes place, many positive things occur:

- the buyer obtains what he needs;
- the seller gains the money he can use to pay his suppliers and employees, to invest in new production, or to purchase other goods or services;
- the State gets taxes which can be used for many purposes, such as investments in roads and railways, social security and healthcare of citizens, culture, sports and so on.

So a strong and vibrant exchange of goods and services is in the best interest of every country. In order to facilitate this exchange, every country must ensure complete trust between sellers and buyers. The buyer must be convinced that what he or she buys is safe and of declared quality. A quality infrastructure is hence necessary for the smooth operation of trade.

5.3.1. The quality infrastructure

Quality infrastructure relates to the whole network of laws, regulations, standards, institutions and bodies organized in such a manner so as to support the trust of buyers and the exchange of goods and services. One of the possible ways to present the quality infrastructure is shown in Figure 2 below.

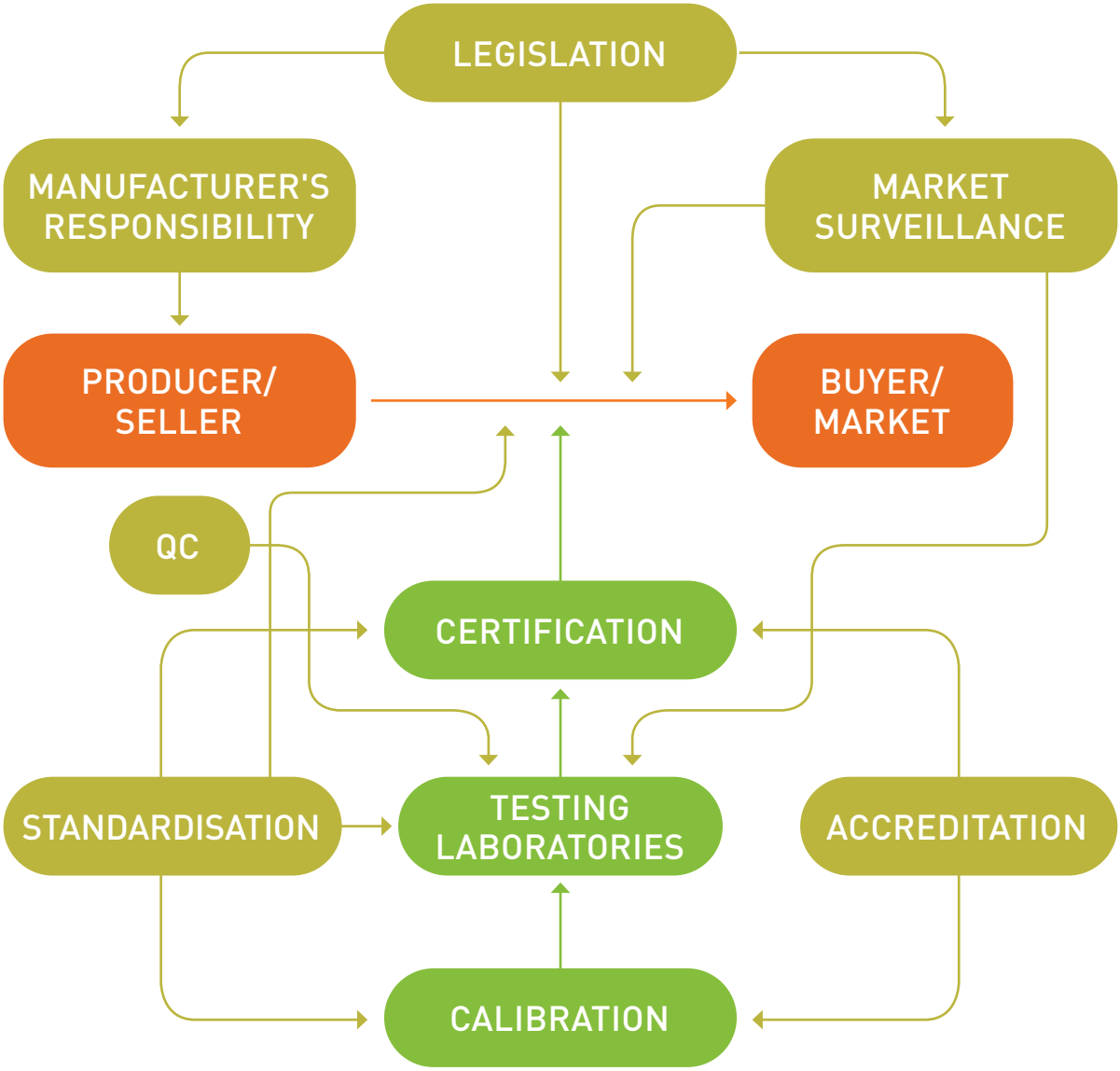


Figure 2 - The quality infrastructure

5.3.1.1. Underlying infrastructural foundations

Without sound and ideally efficient transport, communication, sanitation systems and power supplies that are uninterruptable and have steady voltage and frequency, it is very difficult for a laboratory to perform reliable testing acceptable to its clients. In the absence of any of these fundamental requirements, the laboratory itself has to make generally expensive provisions to substitute for them. Communication systems (Internet) can be particularly important for phytosanitary laboratories for the confirmation of the identification of pests.

5.3.1.2. Legislation

Legislation (whether national or international) is required to regulate safe production of food (the ***manufacturer's responsibility***, the ***market*** and ***market surveillance***).

5.3.1.3. Standardisation

Usually comprises nationally accepted international standards such as those provided by ISO or the *Codex Alimentarius* and accepted nationally by reference in legislation or by a national standards organization set up under specific national legislation. Standards provide internationally acceptable specifications for products, sampling and testing. Standards provide laboratories with specifications and guidance on the parameters to be tested for each raw material and product. Standards also provide guidance for sampling plans and for reference test procedures they might use or against which they might validate their own procedures.

5.3.1.4. Calibration

The availability of an accredited calibration laboratory is essential for laboratories. In the absence of such, the laboratory must set up its own calibration system. This implies that the laboratory has reference standards which must be reserved for the calibration of any measuring equipment it might use (for example, a set of high class masses for the calibration of analytical balances, thermometers, volumetric glassware etc.). The laboratory then also needs personnel trained in the calibration of these items, and of secondary items (such as incubators, ovens, centrifuges etc.). An accredited calibration laboratory which has accreditation for calibration on site (at the client laboratory) is essential for the calibration of balances. A calibration laboratory or a National Metrology laboratory is also essential for the calibration of measuring equipment used in production and trade.

5.3.2. The role of laboratories

As can be seen in Figure 2, testing and calibration laboratories are a key component of a quality infrastructure. Food Safety Testing laboratories are required for, *inter alia*:

- testing effectiveness of disease control in animals and crops;
- testing raw materials (meat, honey, fish and plants) for contaminants, residual pesticides and medicines, growth promoters, mycotoxins in plants, biotoxins in shellfish etc.;
- quality control of processed products;
- testing the presence or absence of Genetically Modified raw materials (generally plant products);
- testing water for production use and for human consumption;
- hygiene testing[115] in support of HACCP;
- market surveillance.

115 Microbiological tests of swabs of surfaces potentially in contact with food and process water.

Hazard Analysis Critical Control Points – HACCP

All food businesses are required by law (in the EU and most other countries) to have a food safety management system in place based on the principles of HACCP (Hazard Analysis & Critical Control Points). HACCP is a system that allows the identification and control of any hazards that could pose a danger to the preparation of safe food. It involves identifying what can go wrong, planning to prevent it and making sure that it is done. While HACCP is a legal requirement, it is also a benefit to the business of food production, storage, distribution and retail.

Effective Food Safety relies heavily on the support of testing laboratories. They operate at different stages and ways in food safety control:

- at State (and regional) level for monitoring of the national herd, crops and aquaculture;
- processing facilities for the control (QC) of raw materials and final produce;
- supporting market surveillance;
- private or State laboratories offering contract testing both to the state and the private sector;
- reference laboratories specializing in specific tests or contaminants or diseases and used to confirm the results of screening tests or in the event of disputes;
- laboratories providing testing services in support of HACCP.

Different types of laboratories require different facilities, environments and staff from different disciplines:

- metrology laboratories;
- calibration laboratories accredited for offsite calibrations that operate at client laboratory locations;
- chemical testing laboratories for wet chemical testing and sample preparation;
- instrumental laboratories for the more sophisticated equipment which requires special environmental conditions (clean and temperature conditioned), access to pure compressed gases (or pure gas generators), etc. This equipment is desirably provided with uninterrupted power supply units to allow for continuous and overnight use;
- microbiological testing laboratories;
- laboratories with facilities to carry out PCR[116] testing (often a suite of small rooms associated with microbiological or virology laboratories);
- virology laboratories;
- phytosanitary laboratories.

116 Polymerase Chain Reaction (PCR) is a highly sensitive and well recognized assay with extreme sensitivity for detecting the presence of pathogenic, bacteriological, viral, fungal and mycoplasmas DNA in any source.

These categories may be further subdivided into various specialisms which require special facilities and conditions. Special conditions include rigorous air-conditioning and temperature control, inward air purification, and slight positive atmospheric pressure, etc. Laboratories testing traces of residual pesticides and the like must be well segregated from areas where bulk pesticides are handled or tested. Most laboratories also require access to reliable power supplies of stable voltage and frequency.

Laboratories in general need access to internationally recognized accredited calibration services for any physical measuring equipment they make use of, such as analytical balances and temperature measuring devices, and secondary equipment such as incubators, refrigerators cold rooms etc., that have to be temperature controlled.

When the laboratory has sophisticated equipment it requires ready access to maintenance and service engineers. Such equipment may also need access to uninterrupted power supplies to avoid damage, or to allow its use with maximum economy.

All laboratories require ready access to laboratory consumables such as chemicals, media, high purity solvents, high purity compressed gases, as well as items such as pipette tips, filter media, etc. For island states the requirements for the international transport of dangerous materials may pose additional problems.

For the validation of testing, access to sources of Certified Reference Materials and Reference Standards and to Proficiency Testing Schemes is necessary.

Fundamental to the provision of laboratory services is the availability of well-educated and trained personnel. In some cases personnel will require training at centres of excellence elsewhere.

A State or Official Agency can assume an important role of coordination of limited resources. To cater for the demands of a country, this Agency needs to evaluate the cost-effectiveness of maintaining the required level in each discipline. In Europe, the various countries have recognized that, even in their environment, not every national laboratory can be everything to everybody and they have agreed to have different areas of speciality while maintaining the general requirements for their industry. In some developing counties, the national laboratory is no more than a ‘post office’ which co-ordinates the flow of work to suitable, accredited laboratories in other countries. The national agency also ensures that the work being performed meets the requirements of their customers and is recognized by the various regulating bodies of their country and of their trading partners.

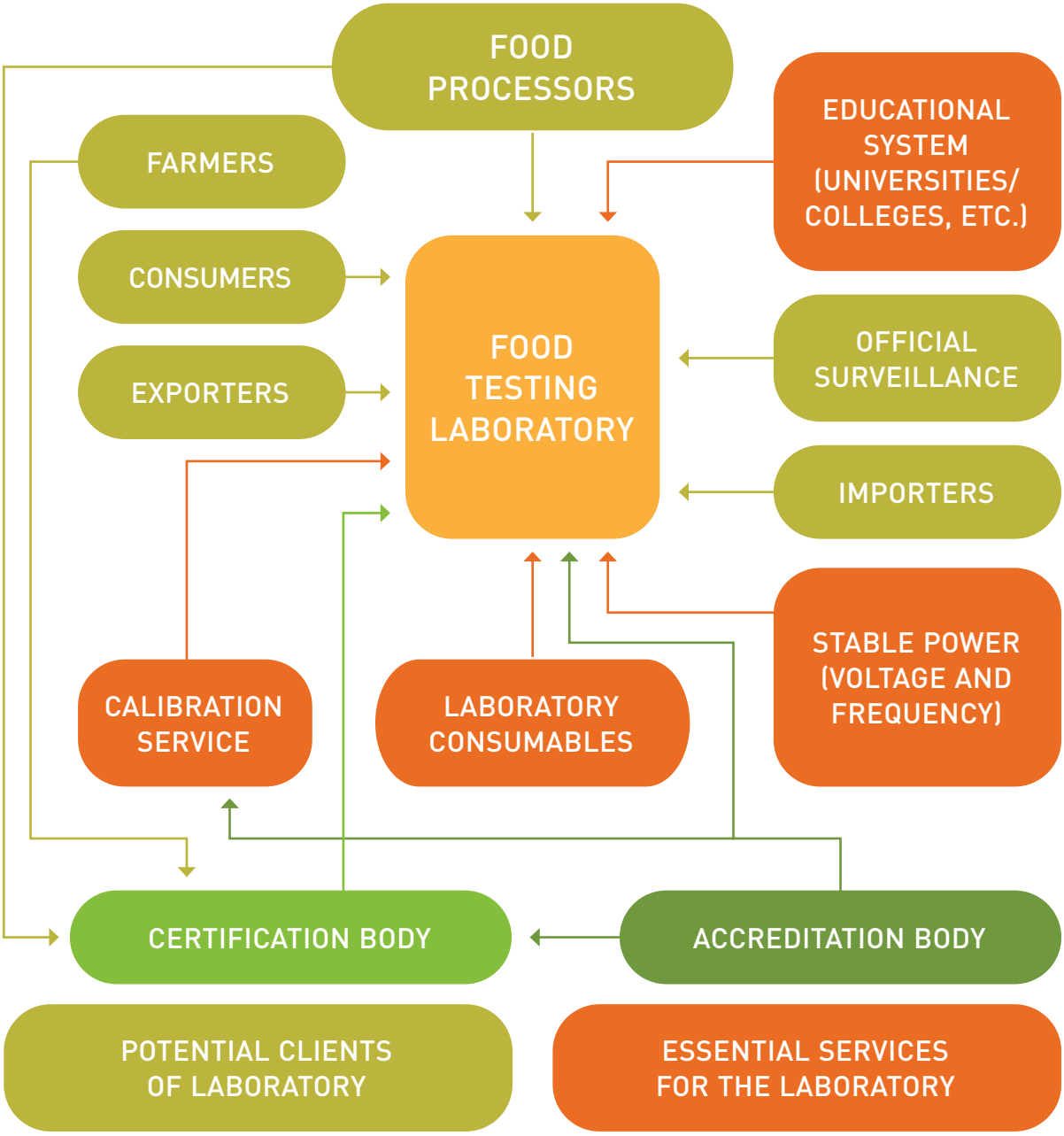


Figure 3 - The laboratory infrastructure

Figure 3 provides an overview of the resources necessary for a food laboratory to function:

- well trained staff – access to educational centres of excellence at post graduate, graduate, and technical levels or a source of suitably qualified staff;
- calibration laboratory or access to a supplier of internationally traceable measurement reference standards and an accredited calibration laboratory to recalibrate the reference standards periodically;
- access to a reliable source of laboratory consumables, capable of providing a wide range of test kits, chemicals (including high purity solvents, compressed

gases),[117] and media and other laboratory consumables (for example, disposable pipette tips, and pipettes, filter media etc.);

- steady uninterruptable power supplies.

In addition the laboratory which requires international accreditation will have to develop a relationship with an internationally recognized Accreditation Body.

Such laboratories are required to provide testing services to farmers (or their veterinarians), food processors, organs of the state monitoring food production and foodstuffs on the market, exporters and importers who might require certification of their products, and the certification bodies of products themselves.

5.4. CERTIFICATION

A laboratory may wish to be certified to a given management system standard to assure a customer that it is compliant with the requirements of this standard. The relevant standard for certification of a quality management system is the ISO 9001 standard, which provides for the implementation of a management system. In EU legislation this standard is referred to as EN ISO 9001 and it is supported by:

- ISO 9000:2005 – Quality Management Systems – Fundamentals and Vocabulary: covers the basic concepts and language;
- ISO 9004:2009 – Managing for the Sustained Success of an Organization – A Quality Management Approach: focuses on how to make a quality management system more efficient and effective;
- ISO 19011 – Guidelines for Auditing Management Systems: sets out guidance on internal and external audits of quality management systems;
- ISO 10010 – Quality Management – Customer Satisfaction – Guidelines for Codes of Conduct for Organizations;
- ISO 10002 – Quality Management – Customer Satisfaction – Guidelines for Complaints Handling in Organizations;

117 It is important that when a State is legislating for the transport of hazardous substances that safety legislation makes it impossible to import laboratory quantities of hazardous solvents and compressed gases. Suppliers may need to stock supplies imported by sea rather than by air of items prohibited for air transport under ICAO (International Civil Aviation Organisation) Codes.

- ISO 10003 – Quality Management – Customer Satisfaction – Guidelines for Dispute Resolution External to Organizations;
- ISO TR[118] 10013 – Guidelines for Quality Management System Documentation;
- ISO TR 10017 – Guidance on Statistical Techniques for ISO 9001:2000.

The standard "ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories", which has the most relevant management system requirements for laboratories, is not normally used for certification but can be a support for ISO 9001 certification when it applies to laboratories.

ISO 9001:2008 specifies requirements for a quality management system where an organization:

- needs to demonstrate its ability to consistently provide a product that meets customer and applicable statutory and regulatory requirements;
- aims to enhance customer satisfaction through the effective application of the system, including processes for continual improvement of the system and the assurance of conformity to customer and applicable statutory and regulatory requirements.

All requirements of ISO 9001:2008 are generic and are intended to be applicable to all organizations, regardless of type, size and product provided.

Laboratories can be audited and assessed and certified to ISO 9001 in their own right or as part of manufacturing or service organizations. While effective as a management evaluation tool, ISO 9001 **does not** evaluate the technical competence of a supplier and therefore of a testing laboratory as a supplier. The effect of this is that while **certification to ISO 9001 can assure a customer of compliance to a management system it does not assure that the test results, calibration or inspection data are valid or are accurate and reliable.**

118 An ISO TR is a Technical Report produced by the relevant ISO technical committee which might after 5 years be issued as a standard, but in any event is useful as a guidance document.

QUALITY MANAGEMENT SYSTEMS APPROACH (FROM ISO 9000)

An approach to developing and implementing a quality management system consists of several steps including the following:

a. determining the needs and expectations of customers and other interested parties **(The starting point)**;

b. establishing the quality policy and quality objectives of the organisation **(These need to align with [a])**;

c. determining the processes and responsibilities necessary to attain the quality objectives **(These need to align with [b])**;

d. determining and providing the resources necessary to attain the quality objectives **(Note the focus is always on objectives)**;

e. establishing methods to measure the effectiveness and efficiency of each process **(Note that it is the process that needs to be measured not simply the process outputs)**;

f. applying these measures to determine the effectiveness and efficiency of each process **(Note that it is both efficiency and effectiveness that should be determined)**;

g. determining means of preventing nonconformities and eliminating their causes **(This means reducing risks)**;

h. establishing and applying a process for continual improvement of the quality management system **(Improving the QMS is the same as improving the business because of where (a) comes from)**.

Such an approach is also applicable to maintaining and improving an existing quality management system.

An organization that adopts the above approach creates confidence in the capability of its processes and the quality of its products, and provides a basis for continual improvement. This can lead to increased satisfaction of customers and other interested parties and to the success of the organization.

This assurance can only be provided by testing laboratories that already follow the requirements of ISO/IEC 17025 for their testing activities. Therefore while it is possible to have the management system of testing activities certified, very few customers of their testing services are likely to be satisfied with this alone, and will require additional assurances of the competence of the laboratory to provide valid test results fit for the purpose. A laboratory that wishes to be certified and have its certification accepted needs to implement the ISO/IEC 17025 standard whether it wishes to have themselves accredited or not.

In its guidelines for the assessment of the competence of testing laboratories involved in the import and export control of food, CAC/GL 27-1997, the *Codex Alimentarius* Commission requires such laboratories for the purpose of fair trade to comply with ISO/IEC 17025 and specifically to participate in proficiency testing schemes and to have a system of internal quality control.

International certification bodies for food and food products such as GLOBALG.A.P.,[119] BRC Global Standards[120] require that any laboratories involved in the testing of food and food products meet the management and technical requirements of ISO/IEC 17025.

5.4.1. The certification process

All aspects of the ISO 9001 standard that are relevant need to be implemented before the laboratory can present itself for assessment by a certifying body. Some of the key issues that need to be addressed are:

- management including top management must be behind the project to achieve certification;
- a management representative (quality manager) must be appointed to drive the process of putting in place the management system and thereafter to ensure that it continues to be followed;
- a clear organizational structure needs to be in place;
- all procedures need to be documented. In a laboratory this includes test procedures, sampling procedures, calibration procedures and all other procedures that aim to ensure that whatever should happen does so every time.

The process in detail:

- **Step 1 – Decision and commitment**

  An informed decision must be made by top management to commit to the implementation of the ISO 9001 standard. This requires a good understanding of ISO 9001 from a business point of view.

- **Step 2 – Management representative**

  A member of management is appointed as management representative (or quality manager) to drive the system. This person is sent for comprehensive training in all the ISO 9001 requirements within the intent and purpose of the ISO 9001 quality management system. Job descriptions for this management representative and all other key managers must be written taking into account their responsibilities under the management system. At times it is useful to form a quality management team to assist the management representative. The management representative will develop a training plan for himself and all key staff.

---

119 GLOBALG.A.P. is a private sector body that sets voluntary standards for the certification of agricultural products around the globe. The aim is to establish ONE standard for Good Agricultural Practice (G.A.P.) with different product applications capable of fitting to the whole of global agriculture. GLOBALG.A.P. is a pre-farm-gate standard, which means that the certificate covers the process of the certified product from farm inputs like feed or seedlings and all the farming activities until the product leaves the farm. GLOBALG.A.P. is a business-to-business label and is therefore not directly visible to consumers.

120 The BRC Global Standards are a leading global safety and quality certification programme, used throughout the world by over 17,000 certificated suppliers in 90 countries through a network of over 80 accredited and BRC recognized Certification Bodies. The BRC Global Standards are widely used by suppliers and global retailers. They facilitate standardization of quality, safety, operational criteria and manufacturers' fulfilment of legal obligations. They also help provide protection to the consumer.

- **Step 3 – Implementation plan**

  Management facilitated by the management representative conduct a 'Gaps Analysis' against the requirements of the ISO 9001 standard to find what needs to be put in place to meet the requirements of ISO 9001. A plan is then developed to implement those elements not in place. This plan should identify the resources necessary to achieve full compliance, the persons responsible, and the time required to implement it. When this is implemented for all elements a full plan is prepared with milestones. It should then be possible to identify a date for ISO 9001 certification.

- **Step 4 – Employee introduction**

  As early as possible a training course should be provided to inform staff about ISO 9001, why the organization is embarking on its implementation and what this will involve for the organization and its staff.

- **Step 5 – Documentation**

  The organization's policy addressing all the requirements of the ISO 9001 standard must be written as well as the support procedures and work instructions necessary to implement it. This can be a daunting task for an organization, but many templates and the like are available. What is important, however, is that the policy reflects the reality of the organization itself. There is little value in writing aspirational policies and procedures. However the intent of the ISO 9001 standard must be achieved, which may require changes in the way the organization has been working in the past. An original copy of the standard should be available.

- **Step 6 – Putting the elements of ISO 9001 into practice**

  All new and amended procedures have to be put into practice throughout the organization.

- **Step 7 – Internal ISO 9001 audits**

  ISO 9001 requires that the organization periodically self-evaluates its ISO 9001 quality management system through internal ISO 9001 audits. These internal audits are also helpful during the ISO 9001 implementation phase. A complete and successful internal ISO 9001 audit is required before the organization can get ISO 9001 certified. Therefore, one or more employees will need to be appointed to serve as internal ISO 9001 auditors. They will require training and perform at least one complete internal audit.

- **Step 8 – Selecting a certification body and certification (or registration)**

  International organizations such as GLOBALG.A.P. and BCR Global Standards have lists of suitable accredited certification bodies. Suitable certification bodies (accredited by accreditation bodies with MLA with the IAF or otherwise acceptable to the customers of the organization should be selected.)

  Having selected the certification body, this body will send auditors to assess the organization's compliance with the standard. It may find some non-compliance which will need to be remedied before the organization is provided with a Certificate of Compliance with ISO 9001.

- **Step 9 – Maintaining ISO 9001 certification**

  The certification body will periodically (usually every 6 months or annually) carry out surveillances visits to ensure that the organization continues to comply with ISO 9001. In order to maintain the ISO 9001 certification, the organization needs to keep the ISO 9001 quality management system alive, use it in daily operations and continually improve upon it. In order to reap the marketing benefits of ISO 9001, the organization needs to properly publicize its ISO 9001 certification. In addition, the organization will only reap the many internal benefits of ISO 9001 by really utilizing the ISO 9001 quality management system in day to day business. Therefore, once the organization is certified it should properly leverage the certificate for marketing, use the ISO 9001 quality management system in daily business, and continually improve the ISO 9001 QMS in order to maintain their certification.

5.4.2. Implications for developing countries

To increase a developing country's capacity and opportunities for international trade it is important that effective systems of certification be in place throughout the entire process of food production from farm or aquaculture to export to the final consumer.

The laboratories' role in this is of key importance in the monitoring, testing and inspecting of raw materials including animal, animal products (such as milk and honey), through to final products.

Certification of the QMS of laboratories can be a stage (acceptable to some customers) on the road to full laboratory accreditation. However the international market and the full elimination of barriers to trade depend on testing, inspection and control carried out by testing laboratories accredited by an accreditation body with an MRA with ILAC for the relevant tests. Certificates of analysis or of testing from such an accredited laboratory are acceptable without further query in all markets.

5.5. ACCREDITATION

The standard which all accreditation bodies require a testing laboratory to have implemented is ISO/IEC[121] 17025 (in EU legislation referred to as EN ISO/IEC 17025).[122] It was developed by CASCO, the International Organization for Standardization's committee for conformity assessment, which for the purposes of this standard included members of ILAC.

5.5.1. The ISO 17025 standard

On its Web site ISO provides the following abstract of the standard:[123]

- "ISO/IEC 17025:2005 specifies the general requirements for the competence to carry out tests and/or calibrations, including sampling. It covers testing and calibration performed using standard methods, non-standard methods, and laboratory-developed methods;
- It is applicable to all organizations performing tests and/or calibrations. These include, for example, first-, second- and third-party laboratories, and laboratories where testing and/or calibration forms part of inspection and product certification;
- ISO/IEC 17025:2005 is applicable to all laboratories regardless of the number of personnel or the extent of the scope of testing and/or calibration activities. When a laboratory does not undertake one or more of the activities covered by ISO/IEC 17025:2005, such as sampling and the design/development of new methods, the requirements of those clauses do not apply;
- ISO/IEC 17025:2005 is for use by laboratories in developing their management system for quality, administrative and technical operations. Laboratory customers, regulatory authorities and accreditation bodies may also use it in confirming or recognizing the competence of laboratories. ISO/IEC 17025:2005 is not intended to be used as the basis for certification of laboratories;

121 The International Electro technical Commission (IEC) is the world's leading organization that prepares and publishes International Standards for all electrical, electronic and related technologies. As a member of CASCO it participated along with ILAC in the preparation of this standard.

122 Whereas calibration and testing laboratories are accredited against the standard ISO/IEC 17025, medical laboratories are accredited against the standard ISO/IEC 15189 and inspection bodies against the standard ISO/IEC 17020. Since this publication covers testing laboratories in the agro-food sector, only the standard IEC/ISO 17025 will be considered.

123 www.iso.org/iso/catalogue_detail.htm?csnumber=39883.

- Compliance with regulatory and safety requirements on the operation of laboratories is not covered by ISO/IEC 17025:2005".

For medical laboratories a different standard exists for accreditation. It is based on ISO/IEC 17025 and is called ISO 15189:2007 Medical laboratories – Particular requirements for quality and competence. This standard has no relevance to food or veterinary testing laboratories.

5.5.1.1. Accreditation bodies

Accreditation of laboratories is carried out by accreditation bodies that have in place a management system to **ISO/IEC 17011** Conformity assessment – General requirements for accreditation bodies accrediting conformity assessment bodies. For international recognition (for the purposes of international trade) the accreditation body is required to have a mutual recognition agreement, at a minimum, with the national accreditation body of the country where the products are traded.

Accreditation bodies are established in many countries with the primary purpose of ensuring that conformity assessment bodies (laboratory testing is a form of conformity assessment) are subject to oversight by an authoritative body.

Accreditation bodies that have been evaluated by peers as competent, sign bilateral or multilateral mutual recognition agreements to enhance the acceptance of products and services across national borders. This creates a framework to support international trade through the removal of technical barriers.

Internationally these accreditation bodies are members of the International Laboratory Accreditation Cooperation – ILAC. ILAC ensures through its procedures for peer assessment of its members that these members operate a uniform accreditation system in all countries. It operates through formally established regional accreditation cooperations.

5.5.1.2. Regional cooperation bodies

Regional cooperation bodies are formally established regional co-operations in accreditation, which tend to:

- have objectives similar to and compatible with those of ILAC;
- be committed to the obligations of the ILAC Mutual Recognition Arrangement; and

- consist of formally nominated representatives of the accreditation interests from at least four economies.

Recognized regional cooperation bodies have their regional Mutual Recognition Arrangements (MRA/MLA) successfully peer-evaluated by ILAC.

5.5.1.3. Recognized regional cooperation bodies

Asia Pacific Laboratory Accreditation Cooperation (APLAC), Secretariat – Australia

European co-operation for Accreditation (EA), Secretariat – France

Inter-American Accreditation Cooperation (IAAC), Secretariat – Mexico

5.5.1.4. Regional cooperation bodies

Southern African Development Community in Accreditation (SADCA), Secretariat – South Africa

African Accreditation Cooperation (AFRAC), Secretariat – Republic of South Africa

The European co-operation for accreditation operates under an EU regulation in the Member States of the EU and EFTA. It has 35 members and 13 associate members.

ILAC and EA produce useful guidelines for the application of accreditation in different types of laboratories. In addition when a laboratory has identified to which accreditation body it will apply, that body may have additional guidelines for the laboratory to follow. All these guidelines are free to download from the websites of these different organisations.

Who should be accredited?

Facilities involved with food safety and food production – All laboratories concerned with food safety! This includes:

- commercial laboratories;
- in-company laboratories;
- State and local health laboratories;
- Government laboratories performing food testing;
- regional laboratories.

Any laboratory supporting international food production safety programmes such as GLOBALG.A.P. or BCR Global Standards.

5.5.2. The accreditation process

As has been mentioned above a customer, a regulatory body or an importer (to meet its country's food safety regulations) may request that testing be carried out by laboratories accredited for the relevant tests. Calibration testing services may be required by its client laboratories to be accredited for the calibrations it undertakes.

When a laboratory considers itself as competent in the carrying out of specific tests or calibrations it may put itself forward for accreditation by an appropriate accreditation body, usually its own national accreditation body if it has an MRA with ILAC or one of its recognized regional accreditation bodies. If there is no national body it is free to choose another suitable accreditation body which has such a MRA.

Nevertheless, if such an application is made the laboratory has to be confident that it has implemented all the relevant requirements of ISO/IEC 17025 (see the road map for accreditation below) and that it meets any additional requirements that the selected accreditation body may require (for example, successful participation

in proficiency testing schemes, appropriate insurance cover etc.). These requirements will be specified by the laboratory accreditation body on initial application when the proposed scope of accreditation is known.

5.5.2.1. Accreditation application process

The laboratory should start by completing the selected laboratory accreditation body Application form. The laboratory accreditation body will provide an estimate of the cost of the accreditation based on the submitted information. This will not cost the applicant laboratory anything.

Accreditation costs are based on the required time to technically evaluate the tests or calibrations defined on the proposed scope of accreditation and the specific major field classification of the testing or calibrations. The number of locations to be accredited also contributes to the cost.

Laboratory accreditation bodies generally require that an *application fee* be paid which covers the cost of reviewing documents submitted by the client and assigning/coordinating the assessor assigned to the client. This is charged **after** client agrees to go forward with accreditation.

Afterwards there is *an annual fee* which covers the cost of maintaining the credentials, staff, relative committee participation and maintenance of the laboratory accreditation body website. The accreditation is usually valid for three to five years (this depends on the laboratory accreditation body chosen), and it is renewable based on the successful completion of a re-assessment. Annual fees are based on the number of major field technologies and laboratory locations to be accredited.

Some laboratory accreditation bodies will charge *a Preparation / Report Fee* which covers the cost of laboratory accreditation body staff and assessors or technical experts to review the relevant documents. This fee also covers the time needed to complete the concluding report.

Each assessment (or pre-assessment) visit is charged on the basis of assessment man-days and travel expenses.

5.5.2.2. Activities prior to assessment

The laboratory is required to own the most recent copy of ISO/IEC 17025 and must maintain a management system that complies with all applicable requirements of this standard. Prior to the initial assessment the laboratory must have completed at least one full internal audit and management review to the requirements of ISO/IEC 17025. If required, laboratories must also complete their best measurement capability and perform satisfactorily in one approved PT/ILC under their proposed scope of accreditation. The requirements for these are specified in the selected laboratory accreditation body's own regulations.

5.5.2.3. To start the accreditation process

The laboratory must sign a contract for accreditation and pay the initial charges.

5.5.2.4. Assessor assignment

Laboratory assessors are assigned based on their qualifications and technical competence as compared with the laboratories' scope of accreditation to be assessed. The laboratory accreditation body will allocate an assessor for the laboratory after all activities prior to the assessment have taken place and all the required documentation has been submitted to the laboratory accreditation body. It is normal for the laboratory accreditation body to communicate to the client laboratory the name and qualifications of their assessors. The laboratory may request a change of assessors where there might be a conflict of interest or the like.

5.5.2.5. Proficiency testing

Laboratories that wish to become accredited and maintain their accreditation are responsible for participating in a proficiency test, inter laboratory comparison or a round robin testing programme that will meet the requirements of the international accreditation community prior to the initial assessment. At the least, the PT/ILC must meet the requirements of ISO 17043.[124] Any proficiency test, inter laboratory comparison or round robin (PT/ILC) must be conducted by a provider approved by the laboratory accreditation body. Where such a provider is not yet approved by the laboratory accreditation body, approval must the obtained prior to the conducting of the test in order to have the results accepted as proof of compliance with the requirement.

5.5.2.6. Traceability and measurement uncertainty

The laboratory must follow the laboratory accreditation body policy to prove the traceability of its measurements throughout all steps of the calibration chain from BIPM/NIST (or other national equivalents) down to the laboratory. Best measurement capability must be calculated in accordance with regulations of the laboratory accreditation body.

5.5.2.7. Reporting uncertainty and traceability

Reporting on uncertainty and traceability needs to be in accordance with the Laboratory Accreditation Body's policies for Traceability and Uncertainty of Measurement. The following are the usual requirements:

- **Calibration and dimensional inspection laboratories**

  Laboratories must report their measurement uncertainty on all calibration and inspection certificates, unless the client does not require want it reported. Evidence that the client does not require the calibration uncertainty to be reported shall be available for an assessor to review at the time of an assessment. Regardless of whether the client requests the reporting of measurement uncertainty, the laboratory shall retain sufficient information to report the uncertainty.

124 The standard ISO ISO/IEC 17043:2010 refers to Conformity assessment – General requirements for proficiency testing.

- **Testing laboratories**

  Laboratories must perform and have available for the assessor a needs or a risk assessment, procedure(s) and calculated uncertainties for those tests that require to have it reported. Some laboratories may also be required to create uncertainty budgets and have these for the assessor to review during your assessment or surveillance visit.

5.5.2.8. Internal audits and management review

Prior to the initial assessment, the laboratory must complete at least one internal audit of its activities that covers their technical competence, compliance with ISO/IEC 17025, and one Management Review that is compliant with the requirements of ISO/IEC 17025.

5.5.2.9. Preparing the scope of accreditation

The laboratory is required to prepare a "Proposed scope of accreditation" as a part of its initial application process. The scope of accreditation is a formal document issued by the laboratory accreditation body to accredited laboratories. The scope is the expression of calibration parameters, testing technologies, ranges, parameters and uncertainties for which accreditation are to be granted. The laboratory accreditation body assessment process assures the technical competence of all parameters listed on the scope of accreditation.

5.5.2.10. Pre-assessment (optional)

A pre-assessment is an opportunity for the laboratory to have its quality system and technical activities evaluated by an assessor prior to the actual initial visit. This is an opportunity to point out areas of the quality system that may need improvement prior to the full assessment process. Most laboratory accreditation bodies recommend that laboratories utilize a pre-assessment to evaluate their preparedness for the accreditation process. Typically the same assessor that is assigned for the initial assessment will perform the pre-assessment in a one day onsite visit. Clients may request and contract for a more lengthy pre-assessment if they deem this necessary/beneficial.

5.5.2.11. Initial assessment

The laboratory must complete a detailed application form and submit this along with all supporting documentation to the laboratory accreditation body prior to scheduling the full assessment visit. The laboratory accreditation body will review the documentation, and resolve any issues prior to sending the detailed application form and supporting documents to the assessor for use during the full assessment. At the time of the full assessment visit, the quality management system will be assessed for implementation and compliance with ISO/IEC 17025. All equipment and tests/calibrations that are wished to be on the scope of accreditation will be verified for technical competence.

5.5.2.12. Relationship between the laboratory accreditation body and the laboratory

The laboratory must accommodate the laboratory accreditation body assessors during the accreditation process to assure that they are provided with the necessary materials. The laboratory also needs to appropriately arrange access to all areas of the laboratory necessary to assess its compliance (this can include ancillary rooms such as those for sample storage and archiving). These accommodations extend to surveillance, reassessments and for purposes of resolving complaints against the laboratory.

An accredited laboratory shall:

- at all times comply with the provisions of the accreditation programme, as defined in the accreditation programme documentation provided by the laboratory accreditation body;
- claim that it is accredited only for those services for which it has been granted accreditation and which are carried out in accordance with these conditions;
- pay fees assessed by the laboratory accreditation body;
- not use its accreditation in a way that brings the accreditation body into disrepute, and not make any statement relevant to its accreditation that the accreditation body may consider misleading or unauthorized;
- if the accreditation is suspended or withdrawn, the laboratory shall discontinue the use of all advertising materials that contain any reference to the laboratory accreditation body, and return any certificate of accreditation to laboratory accreditation body;
- not use its laboratory accreditation to imply product approval by the laboratory accreditation body;
- endeavour to ensure that no certificate or report, nor any part thereof is used in a misleading manner;
- make sure that its references to its accredited status comply with the requirements of the laboratory accreditation body in all communication media, such as advertising, brochures or other documents.

5.5.2.13. Granting of accreditation

Upon the completion of the initial assessment a technically competent individual will review the accreditation documentation. The decision to propose the accreditation of the laboratory will be made by the laboratory accreditation body's technical staff based on the laboratory's compliance with the accreditation requirements. Most laboratory accreditation bodies have a special "Accreditation Expert Committee" which reviews the proposal for accreditation and recommends the granting of the accreditation to its board. After granting of the accreditation the laboratory accreditation body will send a certification of accreditation along with an approved scope of accreditation to the laboratory.

5.5.2.14. Surveillance and full reassessment

Surveillance visits are conducted annually. These assessments are shortened versions of the initial assessment and typically cover half of the quality system and scope. Every three/five years (depending on the accreditation body) a complete reassessment is conducted.

5.5.2.15. Maintaining accreditation

The laboratory is required to comply with the requirements of ISO/IEC 17025 and the laboratory accreditation body and maintain technical competence for the items listed on its scope of accreditation. Annual surveillance assessment visits are performed each year, with a full ISO/IEC 17025 assessment being performed after the 3 to 5 years (as applicable) to assure compliance with all requirements. The laboratory must also participate in the appropriate proficiency testing and inter laboratory comparison programmes with satisfactory performance.

5.5.2.16. Extending accreditation

There are several circumstances that might require the extension of an accreditation. In each instance the laboratory accreditation body's technical staff will review all available documentation, which includes but is not limited to proficiency testing results, complaint files, and previous assessments, to determine whether the laboratory's accreditation may be extended. Where new techniques or equipment novel to the laboratory are introduced, the laboratory accreditation body may require to carry out a special assessment visit before the extension to the scope is approved.

5.5.3. Road map for accreditation to ISO/IEC 17025:2005

For a laboratory to achieve accreditation (by an ILAC or EA accredited National Accreditation Body) to the requirements of ISO/IEC 17025:2005, the laboratory needs to have in place a management system which meets the requirements of the standard.

5.5.3.1. Actions required for achieving accreditation

In order to put a quality management system in place the following is important:

- commitment to following those requirements at all times by top management, supervision and staff;
- adequate resources to produce technically valid test results which meet specifications or the customer (including regulatory bodies' requirements);
- regular organization of, or participation in inter laboratory comparisons or, even better, participation in Inter-laboratory proficiency schemes organized by a reputable body to the requirements of ISO 17043 where available;
- access to high quality or preferably Certified Reference Materials[125] (CRMs or SRMs);

125 A reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

- an effective system for the on-going quality control of test results as they are being produced;
- the validation of test methods as applied in the laboratory and of sampling taking procedures to demonstrate that technically valid results can be produced under all stated circumstances. This exercise also enables the laboratory to know the measurement uncertainty of their test results – and whether this meets their clients requirements;
- adequate attention to the calibration of all measuring instruments: the laboratory must ensure that, even when outside organizations carry out the actual calibration, that the measuring instruments are correctly calibrated by an organization which operates to an ISO/IEC 17025 management system and in particular provides the laboratory with calibration reports which meet the requirements of Clause 5.10 of ISO/IEC 17025:2005. The laboratory must also ensure that the reported uncertainty of measurement of the instruments is within the requirements for producing technically valid test results;
- the development of a "Quality Manual" in which the policy of the laboratory and the way it meets this policy is adequately documented. The provision of a road map to all the procedures necessary to ensure that at all times now and in the future the laboratory produces technically valid test results, which meet the requirements of the client;
- documented test and administrative procedures to ensure the uniform and continuing attention to the requirements necessary to produce technically valid results that are fit for the purpose;
- ensure that there is effective document control of both test procedures and of administrative and other procedures necessary to ensure an effective management system.

N.B.: Accreditation should be sought only for tests regularly carried out. Most laboratories require at least 18 months to put in place such a system and to audit it fully at least once a year.

5.5.3.2. The cost of having a laboratory that is capable of achieving accreditation

The total cost of achieving accreditation includes the following components which vary greatly depending on the scope of testing carried out by the laboratory:

- training of staff and management in the requirements of ISO/IEC 17025 ('the standard');
- training of staff in the application of the standard in their routine testing work;
- the calibration of equipment in accordance with the requirements of Clause 5.6 of the standard;
- the acquisition of suitable reference standards for internal calibration of test equipment. In the absence of a local accredited calibration service or an internationally recognized National Metrological Institute that might offer calibration services to laboratories for, at a minimum, analytical balances and laboratory thermometers, the laboratory needs to acquire suitable certified reference standards and to have these recalibrated periodically by an

accredited calibration laboratory. It is also necessary to train a member of the laboratory's own staff in the calibration of its equipment;

- the acquisition of Certified Reference Materials or Standard Reference Materials and where they do not exist high quality reference materials. Reference cultures and sera for microbiology testing;
- staff time and/or consultancy to prepare a "Laboratory Management Manual", and the associated procedures (administrative procedures in support of the policies enunciated in the management system manual, and test, calibration, equipment operating procedures);
- providing suitable worksheets/log books for the recording of data;
- setting up systems for record keeping, indexing, record storage etc.;
- staff time and laboratory consumables to validate all tests, samplings and other activities which might influence the validity of test results;
- staff time to set up a quality control programme (preparation of control samples, gathering data to set up the Shewhart[126] charts;
- staff time for internal auditing.

In addition to the above the following costs should be considered:

- recruiting sufficient specialized and qualified staff to meet the demands of the testing required of the laboratory;
- acquiring suitable equipment (if it is not already available) adequate for the quality and quantity of testing demanded of the laboratory;
- a budget for servicing and maintenance of equipment. This tends to be a high cost as it is unlikely that suitable service engineers will be locally available, the alternative of training instrument technicians capable of servicing and maintaining all types of equipment is also likely to be expensive;
- providing suitable premises and facilities which do not invalidate the test results;
- where air conditioning is necessary, provision for the power costs of running the laboratory on a 24 hour basis. An alternative which might be feasible is to validate tests in the range of temperatures and other conditions in which the equipment and testing is carried out to demonstrate that the effect any environmental conditions will not invalidate results or the resulting increase in the uncertainty of measurement is acceptable;
- the provision of uninterruptable power supplies for critical equipment.

All the above require to be addressed to ensure that the laboratory can be accredited for the scope of tests required.

126 Shewhart Charts (also known as Control Charts) in statistical process control are tools to determine whether ongoing testing is in a state of statistical control. ISO 8258 Shewhart control charts – Establishes a guide to the use and understanding of the control chart approach to the methods for statistical control of a process.

5.5.3.3. Proficiency testing schemes

A regular independent assessment of the technical performance of a laboratory is recommended as an important means of assuring the validity of analytical measurements, and as part of an overall quality strategy. It is a requirement by the laboratory accreditation body for many tests. A common approach to this assessment is the use of independent proficiency testing schemes. A proficiency testing scheme is a system for objectively evaluating laboratory results by external means, and includes regular comparison of a laboratory's results at intervals with those of other laboratories. International Schemes are available that conform to ISO/IEC 17043:2010 "Conformity Assessment – General requirements for proficiency testing".

Where funds are not available and where a number of laboratories actively test similar products, a local scheme might be organized. To achieve this, a scheme coordinator needs to regularly distribute homogeneous test samples to participating laboratories for analysis and reporting of the data. Each distribution of test samples is referred to as a round. The main objective of a proficiency testing scheme is to help the participating laboratory to assess the accuracy of its test results. In addition, participation in an appropriate proficiency testing scheme is recommended for laboratories seeking accreditation to the standard of ISO/IEC 17025. Indeed, for some types of testing such participation is mandatory.

5.5.4. Implications for developing countries

Smaller economies with very few laboratories cannot sustain a national laboratory accreditation system. Laboratory accreditation would have to be provided by overseas accreditation bodies with an MRA with ILAC. It is likely that only the accreditation bodies of the larger economies will have the resources to provide laboratory accreditation services to laboratories in the smaller developing economies.

The costs of the actual accreditation process are likely to be prohibitive except for laboratories of large commercial organizations. Government or state laboratories with a sufficient budget might also be in a position to fund the accreditation process. Laboratories that are subsidiaries of laboratories in larger economies and that have a common management system supported by the laboratory in the larger economy could perhaps reduce the cost of the accreditation process.

The actual accreditation, however, is not the only obstacle for smaller economies. It is unlikely that an internationally accredited calibration service exists locally (see above in the section discussing the costs of implementation of the management system capable of being accredited).

The costs associated with importing laboratory consumables (test kits, chemicals, media etc.) in small quantities often present problems. Some items may not be transportable by air to islands (compressed gases for gas chromatography, high purity solvents such as the highly toxic methanol need to extract mycotoxins and other contaminants from plant and animal produce, are examples). The cost of equipment maintenance also increases by the time needed to travel and the cost of travel itself, from the major service bases.

Nevertheless, the increased availability of internationally validated test kits for the monitoring and screening of contaminants and diseases (and indeed even for confirmation of tests in some instances) can reduce the costs of testing. Developing economies can also benefit from the use of digital cameras and the Internet for identification and confirmation of phytosanitary pests and organisms by ready access to international expertise and reference data, which was not previously possible.

It is likely that to sustain international trade from smaller/developing economies solutions based on local co-operation between countries will be necessary even in the longer term. Larger developing economies may with time develop the necessary infrastructure with the assistance of the developed economies to which they export. It is possible that the developed economy will provide much of the testing services required.

The lower costs associated with certification schemes such as those operated by the British Retail Consortium and GLOBALG.A.P. or by the larger retail conglomerates such as TESCO and Metro might be an acceptable solution for a lot of products as these schemes are less dependent on accredited laboratory testing.

5.6. GOOD LABORATORY PRACTICE

The Good Laboratory Practice regulations originated in the 1970's as a consequence of malpractice in research and development activities of pharmaceutical companies and in contract facilities (mainly toxicology laboratories) used by them. While some of the malpractice arose from fraud the most important failures were the consequence of lacking proper management and organization of studies used to complete regulatory dossiers[127] for the market authorization of new products.

To deal with this, the US Food and Drug Administration imposed what are known as the GLP regulations. In 1981 the OECD[128] published GLP principles and many countries (member states of the OECD, including the EU) have signed agreements that make the OECD GLP Principles binding on them. This has made the OECD Principles an international text.

The OECD recognizes that not all parts of the GLP Principles are easy to interpret. This is why the OECD has instituted a series of advisory documents on various aspects of GLP organization. There are seven consensus type documents. They have mostly been derived through discussion between the regulators and industry during consensus workshops. The OECD has a GLP Group made up of senior members of the respective member states' GLP monitoring authorities. This group oversees the GLP activities of the OECD. These activities include the organization of training courses for GLP inspectors from all over the world and the organization of joint inspections, which are performed with a view to harmonizing the approach of various member states to GLP inspections.

127 'Regulatory Dossier': a dossier with data proving that the pharmaceutical, medical device, novel food, pesticide, cosmetic and the like has the quality, efficacy and safety properties suitable for the intended use.

128 The mission of the Organization for Economic Co-operation and Development (OECD) is to promote policies that will improve the economic and social well-being of people around the world.

5.6.1. Outline of GLP

While the original regulations related to pharmaceutical products their scope now includes all safety studies of consumer products including novel foods, food ingredients, agricultural chemicals (indeed all chemical products).

The intent of GLP was to regulate the practices of scientists working on the safety testing of prospective drugs. With the obvious potential impact on consumers and patients recruited for clinical trials, the safety of drugs became a key issue and GLP was seen as a means of ensuring that scientists did not invent or manipulate safety data and that GLP compliant studies were properly managed and conducted. Hence GLP became the champion of the consumer, the regulatory safeguard, the guarantee that the safety data were being honestly reported to the registration or receiving authorities as the basis of a decision on whether or not to allow a new drug onto the market. GLP was imposed on the industry by regulatory authorities, in the same way as good manufacturing practice had been before, and good clinical practice was to be afterwards.

All the Member States of the OECD have a GLP monitoring authority which audits GLP facilities and Studies for compliance with the Principles of GLP.

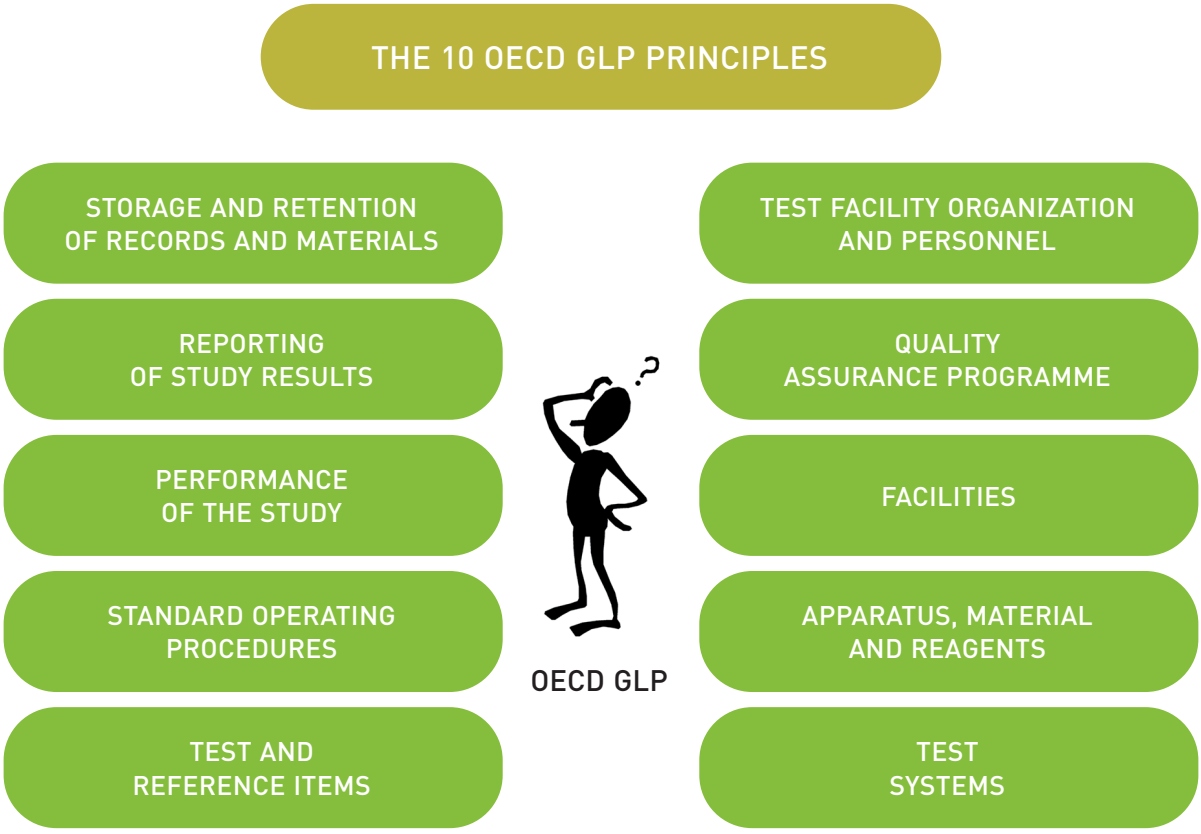


Figure 4 - 10 OECD GLP principles

There is little difference to the actual work of a chemical and microbiological testing laboratory to the requirements for accreditation to ISO/IEC 17025. However the testing work is 'study' based, the overall management of a study, record keeping and retention and storage of test materials etc., are more strictly specified. Most laboratories conduct toxicology studies within the spirit of the GLP guidelines even if the studies are not going to be used for regulatory purposes.

A study usually has a 'sponsor'. 'Sponsor' means an entity which commissions, supports and/or submits a non-clinical health and environmental safety study.

A sponsor can include:

- an entity which initiates and supports, by provision of financial or other resources, non-clinical health and environmental safety studies;
- an entity which submits non-clinical health and environmental safety studies to regulatory authorities in support of a product registration or other application for which GLP compliance is required.

DOCUMENTS AVAILABLE TO DOWNLOAD FREE FROM THE OECD WEB SITE

www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/oecdseriesonprinciplesofgoodlaboratorypracticeglpandcompliancemonitoring.htm#GLP_consensus_documents.

No. 1: OECD Principles on Good Laboratory Practice (also available as an Annex to the EU Directive 2004/10/EC for Good Laboratory Practice, see Chapter 1)

GLP CONSENSUS DOCUMENTS

No. 4: Quality Assurance and GLP (revised 1999);

No. 5: Compliance of Laboratory Suppliers with GLP Principles (revised 1999);

No. 6: The Application of the GLP Principles to Field Studies (revised 1999);

No. 7: The Application of the GLP Principles to Short Term Studies (revised 1999);

No. 8: The Role and Responsibilities of the Study Director in GLP Studies (revised 1999);

No. 10: The Application of the Principles of GLP to Computerized Systems (1995);

No. 13: The Application of the OECD Principles of GLP to the Organization and Management of Multi-Site Studies.

GUIDANCE DOCUMENTS FOR COMPLIANCE MONITORING AUTHORITIES

No. 2: Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice (also available as an Annex to the EU Directive 2004/9/EC for the Monitoring of Good Laboratory Practice See Chapter 1);

No. 3: Revised Guidance for the Conduct of Laboratory Inspections and Study Audit;

No. 9: Guidance for the Preparation of GLP Inspection Reports.

ADVISORY DOCUMENTS OF THE WORKING GROUP ON GLP

No. 11: The Role and Responsibility of the Sponsor in the Application of the Principles of GLP;

No. 12: Requesting and Carrying Out Inspections and Study Audits in Another Country;

No. 14: The Application of the Principles of GLP to in vitro Studies;

No. 15: Establishment and Control of Archives that Operate in Compliance with the Principles of GLP.

POSITION PAPERS

- The Use of Laboratory Accreditation with reference to GLP Compliance Monitoring (1994);
- 'Outsourcing' of Inspection Functions by GLP Compliance Monitoring Authorities (2006)

5.6.2. Implications for developing countries

If a sponsor in a developing country were interested in submitting a novel food product or food ingredient to a regulatory authority for market approval, it would be necessary for that sponsor to have the study for the regulatory dossier for the market authorization carried out in compliance with GLP regulations. This would entail appointing a suitable study director who would then be responsible for all the testing carried out and for ensuring that it was in compliance with GLP Principles wherever it was carried out. An independent quality assurance function would also be necessary to audit all aspects of the study periodically and to sign off that the study was carried out in accordance with GLP. A monitoring authority from one of the 34 Member States of the OECD would then have to carry out a thorough inspection of the study and be satisfied that it was fully compliant with the Principles of GLP.

A more likely scenario is that an entity in a larger developed economy might be the sponsor and that the Study be carried out in existing GLP compliant laboratories in the developed states. Some elements might be subcontracted to potentially GLP compliant laboratories in the developing country.

A further possible scenario is that a highly competent laboratory in the developing country puts in place all the requirements for GLP compliance for specific types of testing work and requests a monitoring authority from one of the 34 Member States of the OECD to certify its GLP compliance for specific testing work. It could then promote itself as a subcontractor for suitable studies.

5.7. CONCLUSION

Laboratory testing is an essential aspect of food safety. The results of laboratory tests can result in food products being approved or withdrawn from sale or export, being prohibited or permitted on the market. Considerable reliance is therefore placed on test results (of correctly taken and representative samples).

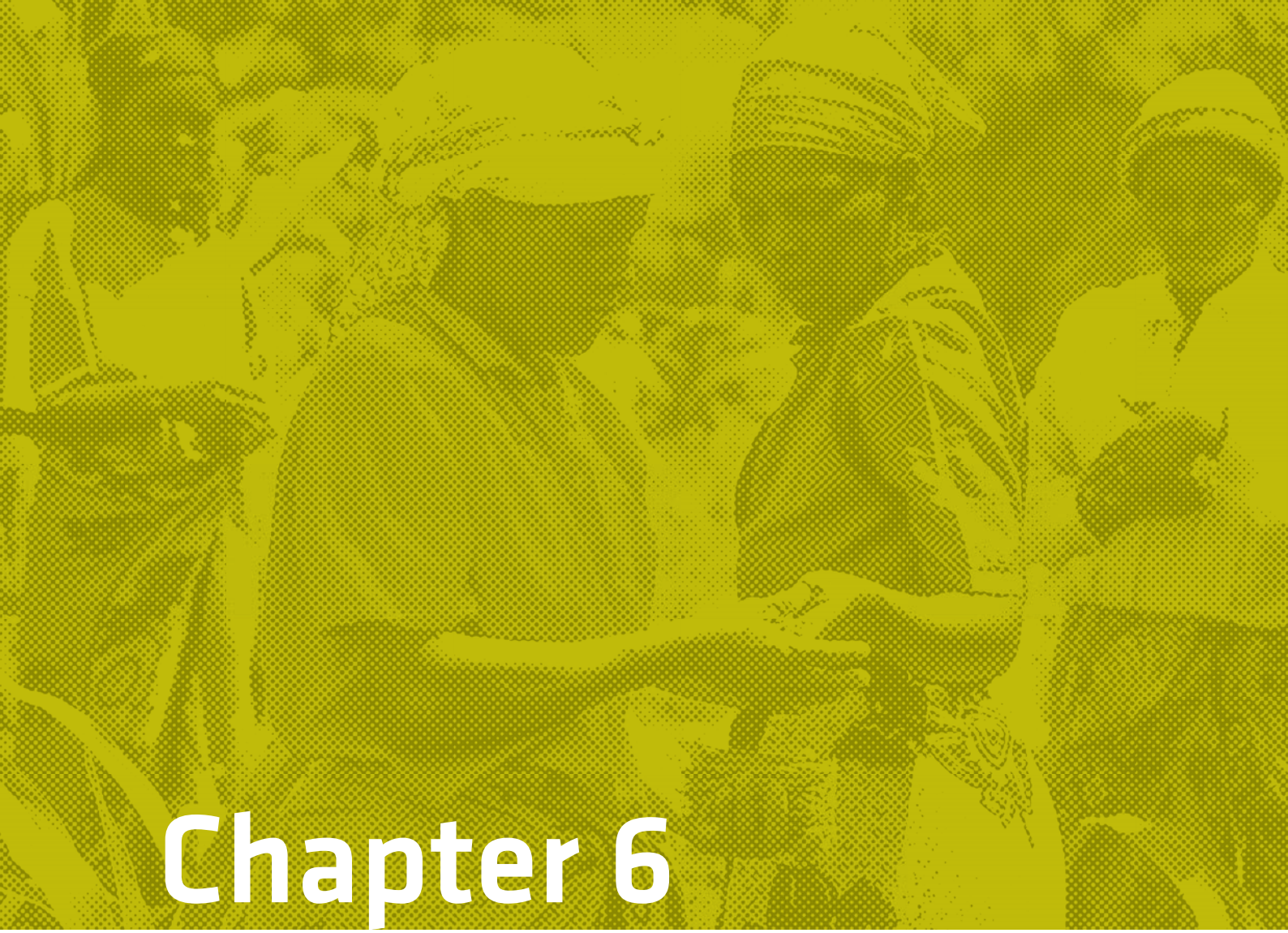
Unless the test results obtained by a laboratory are demonstrably valid and fit for purpose, the resources utilised to obtain them are wasted. However, the costs associated with full accreditation of all the tests required to ensure a food product is safe, meets quality standards, and is correctly described (e.g. genetically modified or not, heat treated or not etc.) can be high, in addition to the costs associated with carrying out the tests itself (trained staff, proper facilities, equipment, consumables, test kits, suitable environmental conditions etc.).

When laboratories are accredited for specific tests they can be used with confidence by others. Pooling the resources of all the laboratories in a state or region for the various tests required can be a viable solution for a developing economy.

In the short term, where such a national laboratory infrastructure has not been developed, those laboratories that do exist should be supported and encouraged to implement, in so far as they can, all the requirements of ISO/IEC 17025, even if they do not expect to be able to obtain accreditation in the immediate future.

Certification of laboratories to ISO 9001 (while operating in so far as possible to the requirements of ISO/IEC 17025) could be a valuable first step along the way and might be sufficient for some of the international trading organisations (generally for the non-meat containing products).

Ensuring that a pool of well-educated potential staff at all levels for laboratories should be a national aspiration. Gradually building up a national or regional infrastructure of inter-laboratory comparisons to ISO.



Chapter 6

Business plan for laboratories

6.1. INTRODUCTION

6.1.1. Context

A **business plan** describes (analyses and discusses) how to conduct a business, in this case how to establish and run a laboratory. The business plan relies on a business model describing how to carry out its operations. In other words: a business plan helps to implement a **business model** that **meets market needs, is profitable and remains profitable**. It addresses three fundamental strategic questions:

- **What are you going to do (sell)?**
- **Who will benefit (use) from your services (buyers)? Which needs are addressed by the food control laboratory?**
- **How are you going to do it (business model and business implementation)?**

It is important to ensure a business plan pursues realistic goals and objectives. One method often applied to safeguard this is the 'SMART' approach, which suggests a business plan needs to be structured following five characteristics: Specific, Measurable, Attainable, Relevant, Timely.

Table 1: Diagnostic questions for 'SMART' business plans

Acronym element	Description	Diagnostic questions
Specific	Specific means that the objective is concrete, detailed, focused and well defined. The objective must be straightforward and emphasize the action and the required outcome. Specific also means that it is result and action-orientated. Objectives need to be straightforward and to communicate what you would like to see happen. To help set specific objectives it helps to ask: • WHAT am I going to do? This is best described using strong, action verbs such as conduct, develop, build, plan, execute, etc. This helps your objective to be action-orientated and focuses on what is most important; • WHY is this important for me to do? • WHO is going to do what? Who else needs to be involved? • WHEN do I want this to be completed? • HOW am I going to do this?	• What exactly are we going to do, with or for whom? • What strategies will be used? • Is the objective well understood? • Is the objective described with action verbs? • Is it clear who is involved? • Is it clear where this will happen? • Is it clear what needs to happen? • Is the outcome clear? • Will this objective lead to the desired results?

Acronym element	Description	Diagnostic questions
Measurable	If the objective is measurable, it means that the measurement source is identified and we are able to track the actions as we progress towards the objective. Measurement is the standard used for comparison. For example, what financial independence means to one person may be totally different compared to what it means to another. If you cannot measure it, you cannot manage it. It is important to have measures that will encourage and motivate you along the way as you see the change occurring, this may require interim measures. Measurements (and visible progress) go a long way to help us to know when we have achieved our objective.	• How will I know that the change has occurred? • Can these measurements be obtained?
Achievable	Objectives need to be achievable, if the objective is too far in the future, you'll find it difficult to keep motivated and to strive to attain it. Objectives, unlike your aspirations and visions, need to be achievable to keep you motivated. Objectives need to stretch you, but not so far that you become frustrated and lose motivation. In some cases it may be advisable to set up intermediate aims.	• Can we get it done in the proposed timeframe? • Do I understand the limitations and constraints? • Can we do this with the resources we have? • Has anyone else done this successfully? • Is this possible?
Realistic	Objectives that are achievable may not be realistic, however realistic does not mean easy. Realistic means that you have the resources to get it done. The achievement of an objective requires resources, such as skills, money, equipment, etc. for the task required to achieve the objective. Whilst keeping objectives realistic, ensure that they stretch you. Most objectives are achievable but may require a change in your priorities to make them happen.	• Do you have the resources available to achieve this objective? • Do I need to revisit priorities in my life to make this happen? • Is it possible to achieve this objective?
Time	Time-bound means setting deadlines for the achievement of the objective. Deadlines need to be both achievable and realistic. If you don't set a time you will reduce the motivation and urgency required to execute the tasks. Agreed Time frames create the necessary urgency and prompts action.	• When will this objective be accomplished? • Is there a stated deadline?

A business plan needs to leave room for unforeseen factors and thus not get carried away focusing solely on opportunities (or risks). Also, even the most detailed and exhaustive business analyses cannot cover all aspects and risks. Therefore a business plan has to be seen as a dynamic document that requires constant re-evaluation to adapt to any changes in the internal and external environment.

To develop a feasible business model it is necessary to collect a variety of data and to analyse this information. The outcome of this analysis should be documented and will become the input for the business plan. Table 1 outlines a typical structure of a business plan.

Table 2: Structure and content of a laboratory business plan

Chapter	Chapters in the business plan	Content
0.	Executive summary – should be written as the last step and is a brief 2 page summary of the business plan	Objectives, products and services, target markets, financial projections, investment required
1.	Description of services offered and of business model	Pricing, ownership and possible affiliation to private or governmental institutions. Brief description of strategy
2.	Internal analysis	Organization, responsibilities, operation, service delivery, human capacities
3.	External analysis	Risk analysis and risk management plan, stakeholders, regulatory demands and market analyses: who has interest in food analysis and what is needed in the particular region
4.	SWOT analysis	**S**trengths, **W**eaknesses, **O**pportunities, **T**hreats
5.	Strategy and laboratory set-up	Laboratory vision, strategy, business set-up: Infrastructure, equipment and basics operations plan
6.	Management and staff expertise (provide CVs of key persons in the appendix) and include plan for further training if necessary.	Staff and competences needed. Check if staff and logistics will match the objectives of the laboratory.
7.	Performance Indicators and GANTT Plan	Milestones and timelines
8.	Financial plan	Financial plan: pricing and funding
9.	Appendix: Optional supplementary material for larger business plans	Organigram, list of planned investments, table of training needed, table of staff (existing and to be hired) with qualifications, CV of laboratory head and management, room plan of laboratory, map of region to illustrate logistics, LOI of stakeholders and partners

6.2. EXECUTIVE SUMMARY

The executive summary is a brief outline of the business plan comprising all its key elements. A good executive summary highlights the overall need for funding or loans and presents a positive outlook about the planned laboratory towards stakeholders and institutions which may lend financial support to the project. It will comprise the following:

- potential markets for analytical services – the market needs (type of services needed and how are they currently served);
- competitors – who are the competitors that can enter the market or supply your laboratory service;
- decision makers and stakeholders – who will decide which analyses should be done and by whom, who has the primary interest in the analytical service and who is paying;
- market potential (volume and revenue potential) – what types of analyses are needed and what is the expected volume seasonal variations;
- summary of planned investments;
- political and organizational interests – are other interest that may influence the need for and analytical service e.g. from government and organizations;
- vision and strategy of the laboratory;
- financial plan.

6.3. DESCRIPTION OF SERVICES OFFERED

This chapter of the business plan describes the services to be offered by the laboratory. By briefly describing the business model of the laboratory it serves as an introduction to the business plan.

The business model is a description of how the laboratory operates, thus how services are provided, how they are financed, who the customers are and what revenue are achieved. For a business plan both the actual, currently present (in the case of an already existing laboratory) and the future situation need to be taken into account. The focus is on how the laboratory delivers services and makes revenue, which includes the following questions:

- Services: what services and analyses are offered?
- Volume of business and turn-over: how many samples, prices income, revenue etc.?
- How do you sell/market your services? How do you reach your customers and stakeholders? Who orders analyses and who pays? How do you differ from your competitors?
- How do you operate: getting the samples, deliver results, getting payment?

6.4. INTERNAL ANALYSIS

The internal analysis serves to establish the institutional capacity of a laboratory. It will define a clear map of the organization of a laboratory, and its human and logistic capabilities. This analysis can be done by reviewing documentation; however these findings need to be validated by an on-site visit.

6.4.1. Assessment of the overall organization

This assessment should be done together with the managers (top management and division/department managers). Below an indicative outline of fields to analyse is provided.

In order to establish ownership and responsibilities within a laboratory, the following points need to be addressed, *inter alia*:

- **Ownership and board of directors** – Who owns the laboratory and has the overall responsibility? Is the laboratory part of a national institution (official authority laboratory with national responsibilities) or a local laboratory of a private international company? Is the laboratory part of a public-private partnership organization, e.g. is operated by a food processing and distributing organization?
- **Who defines the vision and strategy** – Does the laboratory have an individual vision and strategy? Or are its activities and strategy part of a larger organization also comprising non-laboratory institutions? Should the latter apply, what is the role of the laboratory in that a larger organization?
- **Decision processes** – Map the business decision processes, thus who and how the various decisions are made on each level? Where do competencies and responsibilities lie?
- **Budgeting, allocation of resources and procurement, purchase etc.** – Map the key steps in the process.

In order to establish operation, company culture and service processes of a laboratory, the following points need to be addressed, *inter alia*:

- **Operation, company culture and service processes** – What are the current services provided, how are they offered and who are the customers? How is the daily business conducted?
- **Funding** – does the laboratory have financial resources for investments?
- **To whom does the daily management report and to whom does the top management report?** An organization diagram/organigram should be developed for this purpose;
- **Shortcomings in empowerment and regulation as judged by management and laboratory staff** – What are the key barriers in the daily operation (e.g. flexibility in working time, bureaucratic procedures for procurement, recruitment etc.)? The objective is to collect a staff perspective on the current situation;

- **Culture** – Is laboratory staff satisfied by their workplace and do they excel in what they are doing? Is the laboratory a reputable organization? Is the company vision communicated and understood by staff?
- **Services to customers** – Does the laboratory keep deadlines? Are response times reasonable, realistic and comparable to other laboratories? Does the laboratory act immediately on important results that may require immediate action?
- **Customer satisfaction** – Dissatisfactions that may motivate customers to change laboratory should be identified;
- **Unique competencies/capabilities** – Is the laboratory exclusively providing certain analyses in the country/region, having a specific piece of equipment, etc.?

6.4.2. Audit of current capability and resources

The objective of this part is to compile a description of tangible and intangible resources available and how they are managed. This is most efficiently done by a visit to the facilities and through on-site interviews with those responsible.

6.4.2.1. Tangible factors typically assessed also in formal quality system audits

Is a modern quality assurance system in place and is the staff trained using the system? Is the laboratory certified to ISO 9001, ISO 14000 and/or accredited to the ISO 17025 or GLP standard? In the absence of formal certification and accreditation, how well does the laboratory already comply with those standards?

a. ISO 9001:2001 and ISO 14000 issues:
   - Buildings and facilities: who checks general assessment of facilities, maintenance, security etc.?
   - Who is in charge of the logistics for receipt of samples?
   - Localization and infrastructure – roads and transport, utilities (power, water, phone lines, Internet etc.), supplies and waste management (ISO 14000): how is this handled? Do they operate satellite reception points?
   - Instruments and equipment – What is available and what is the status, in operation, maintenance status quality etc.? Plans for renewal and acquisition of necessary equipment to meet expectations;
   - Are calibrations performed regularly and documented properly?
   - Personnel competencies and training – This should include a general assessment of staff competencies and their training level. Are programmes in place to ensure continued training of the staff?
   - Is the QM structured logically and easy to revise? Is the Quality System sufficient without deficits or 'above level' with unnecessary resources to maintain it? Are independent audit and proficiency tests performed and what are the results? Are adequate actions taken in case of non-compliance?

b. ISO 17025 and GLP issues:

- Are prerequisite programme and GHP elements being followed, what is the documentation standard?
- Are measurement routines and tests documented and validated?
- Who is in charge of the documentation of test measurements?
- Is there a staff training in GLP?
- Is there a control and monitoring of humidity, temperature and if appropriate electric shielding.

6.4.2.2. Additional (soft) factors

Additional factors to consider include:

- Motivation and performance – The degree of absence (illness, substitution for pregnancies etc.)? What incentives are in place e.g. remuneration, social security and other factors to retain staff and increase the work performance?
- Staff resources – Are staff capacity and the present training level appropriate considering the activities and budgets? Is it possible to recruit new staff if urgently needed?
- Methods – Which methods are being used? Are they up-to-date and is their performance adequate?
- Development – Does the laboratory have the capability to develop methods and participate in research, teaching and training of others?
- Agility – what is the ability to change direction as a response to a new situation or a change in environment or technology? Or new demands (new methods/ samples)?
- Significant shortcomings/lack of equipment or competencies as judge but the laboratory?

6.5. EXTERNAL ENVIRONMENT

The analysis of the external environment evaluates the business environment in which the laboratory operates. This may focus on a vast amount of issues, which have been separated into political, market and stakeholder analysis below.

6.5.1. Political concerns

Political concerns may have a large impact on a laboratory. They are probably among the factors which may be least influenced by the laboratory itself. Factors to consider for the political concerns include:

- trading policies;
- funding, grants and initiatives;
- home market lobbying/pressure groups;
- international pressure groups;

- wars and conflict;
- government policies;
- government term and change;
- inter-country relationships/attitudes;
- political trends;
- governmental leadership;
- government structures;
- internal political issues;
- ecological and environmental issues.

6.5.2. Market analysis

The results of the market analysis are particularly important in a business plan. It is also highly recommended that actual discussions, negotiations and possibly letters of intent (LoI) are included in business plan writing.

The nature of regional food production will influence the need for corresponding analytical services. The following factors should be analysed, *inter alia*:

- Food risk profiles – give an assessment of what the needs are in the food safety market based on product risk profiles and development perspectives of local food industry.
- Regulatory demand of countries to which agricultural products, fish and meat are exported, e.g. EU Regulation (EC) No. 882/2004.
- Market structure – Does the laboratory service individual costumers or organizations? Does it work in partnerships? How is the structure of the market for laboratory services organized? Government controlled, private or a mix of the two?
- Barriers both legal and political – Local, regional, national and international regulation, interest groups, unions, NGO and others with interest in this food business and food safety area.
- How is the financial situation among key customers?
- How is the financial situation at a national, macroeconomic level (deflation, recession)?
- Cooperation with customers – are customers dependent on other laboratory services (advice, can the laboratory offer training in sampling, evaluation of results etc.)?
- What accreditation expenses may be required: local accreditation body or use of accreditation from far away?
- Is the laboratory depending on buying consumables abroad in foreign currency?
- Local or regional laboratory capacity – is there a need to focus, merge or spilt of laboratories in the area?
- Who are competitors – intrusion of new laboratories into the region/market, e.g. sending or receiving samples to/from other countries?

Food safety regulations

The EU, the FDA and other national authorities have developed a series of food standards and control systems to be applied throughout the production chain to secure safe food for the consumer. A core principle for example in EU food control is to enforce control closest to production, applying an audited self-control system at all stages in the production chain and full traceability throughout the production chain.

To document the quality and compliance of food for export or local sale, requires that representative samples of the food are analysed. The overall objective is to secure the properties and integrity of food products so that the food is what it claims to be and that it does not contain contaminants, additives, residues or microorganisms above permissible limits. These requirements may vary from region to region.

An example is EU Regulation (EC) No. 882/2004 *on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.*[129] All EU countries operate a control system in compliance with this regulation. Also important is EU Regulation (EC) No. 2073/2005 *on microbiological criteria for food stuffs*, which states the allowed limits, analyses, methods and sampling for certain microorganisms in food produced in and exported to the EU.[130]

Combining local needs with risk analysis will highlight the most important issues in each country / region looking at the market structure and food production from farm to vendor or to export:

- farm structure (big or small, many or few products);
- food commodities relevant;
- size of food production;
- the dynamics of primary food production;
- local buyer and wholesale structure;
- transport and storage;
- food processing and production industry scale (small or large-scale), number of plants, local or international scope etc.;
- export, types of products, structure of export industry, target countries;
- chain stores and markets;
- integrity issues (fraud/authenticity).

Risk analysis at this stage focuses on risks to the end-consumer and the risk of non-compliance to producers (e.g. local ACP food producers). It should be done both in the form of a SWOT (qualitative analysis of strengths, weakness, opportunities,

129 See eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32004R0882:EN:NOT.
130 See eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32005R2073:EN:NOT.

threats) as well as an HACCP assessment of the whole value chain. A high risk of non-compliance and a large export volume means – in the absence of other laboratories – a large need for laboratory services. Thus such risk analysis is part of the market analysis.

A large need for the services may trigger governmental support and/or the support of external funding organizations. It has to be taken into account, however, that the laboratory services need to be affordable within the pricing strategy available for the total value chain of the product.

6.5.3. Stakeholder/consumer analysis

To determine the need for food analyses, it is necessary to understand the stakeholders who have interest in food safety and what they require from the analyses.

Analysing the food production chain there are many steps involved, starting from primary production and continuing with processing, wholesales, export, imports, (chain) stores and finally consumers. Stakeholder characteristics differ but all share an interest in price and value as well as in food quality and safety. At all stages except at the two ends of the chain the key interest lies in achieving a revenue through getting the best (specified) quality at the lowest price. Food safety, however, is mostly a matter of being in compliance with regulations – or maintaining a reputation. The interest of producers lie in getting the highest price for the quality produced; for consumers they lie in getting safe food of high quality at a low price. Food safety is primarily a concern for the end consumer who relies on authorities to secure food safety through regulation and control.

A clear understanding of who has a stake in food analysis is a fundamental part of developing a business plan. Two key questions that need to be addressed are:

- Who requests analyses?
- Who needs the results?

Authorities may request analyses to allow the export of a product; thus authorities request analyses but the exporter needs them. Further questions which need to be addressed include:

- Who is going to pay?
- Who will get the results?
- What are the general analytical and laboratory requirements (e.g. accreditation, independence, ownership)?

By combining this information it is possible to attain a broad overview of the analytic market potential, the food types to be analysed and the analytical requirements needed. As food in general has a short shelf life there may also be time constraints that need to be considered, such as planning analyses for export certificates. An example is that most microbiological analyses have a short deadline of 24 hours from sampling to analysis.

Finally, it is necessary to identify the gatekeepers, those who decide type and to some extent number of analyses and parties to sell the analytical service to. As discussed

above the first are often the authorities while those who need the results may be the producer, wholesaler, exporter or other link in the chain. The outcome of this assessment is an understanding of the mechanisms that governs food control in a particular region.

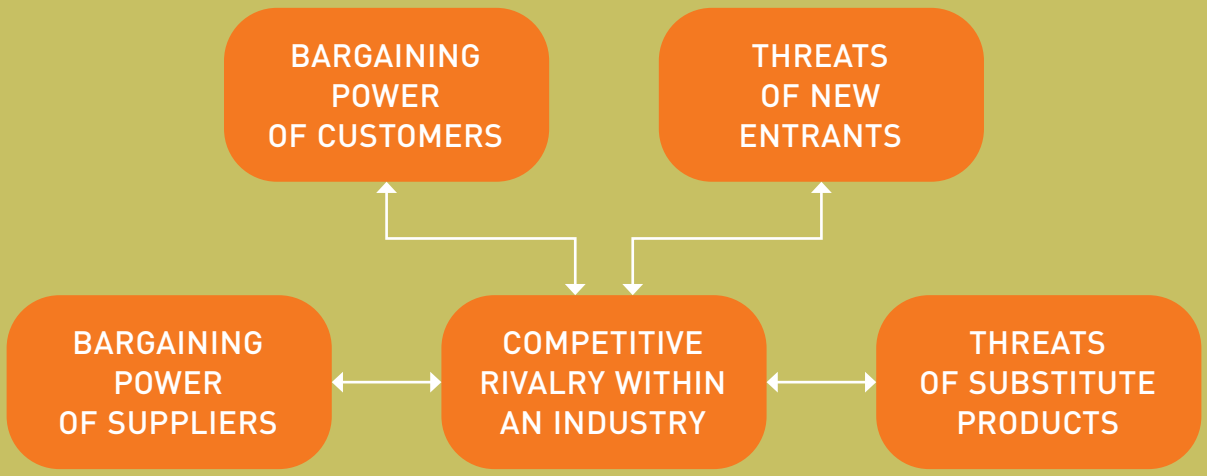
It is highly recommended that communication with stakeholders takes place during the process of business plan writing.

Analysis of the external environment: Porter's Five Forces

When analysing the external environment Porter's five forces may be a useful tool. Porter's five forces is a classical model, which in a simple way illustrates the effects that govern competition in the market for a specific business.

The Five forces to be considered in the laboratory environment can be illustrated as following:

- **Competition** within the existing business thus other laboratories that can supply the same analyses and are within the transport range of samples. And how is the competition: on price, speed, volume, added services etc.
- **Supplier.** Covers two different effects: the power of suppliers to deliver material and equipment at a competitive price and access to competent staff with the right skills and with a reasonable wage demand.
- **Substitute.** Can your offering (*i.e.* analysis) be made obsolete e.g. by introducing new technology like classical microbial analyses are being replaced by molecular methods or are legislation changes not requiring the same analyses anymore.
- **Buyer.** If there is more than one customer they may form an alliance to put pressure on price, which can have a strong effect. Also a single big customer may be that important that he can ask for discount not to find alternatives.
- **New players.** New laboratories that may enter the market, maybe with new technology or perhaps a customer will establish their own laboratories



6.6. SWOT ANALYSIS

To give a clear picture of the laboratory as a business the outcome of the assessment of the internal and external environments is synthesized into a SWOT analysis. Other analysis methods exist (for example the PESTEL method and a Checklist for the establishment of a new service, both of which are included in the Annex of this chapter). But due to its wide and easy application the SWOT analysis is used in this chapter. A SWOT analysis looks at the internal and external environment impacting on a business entity as follows:

- Internal environment: Strengths, Weaknesses;
- External environment: Opportunities, Threats.

A simple SWOT model can be formulated as a table where strengths and opportunities are balanced against weakness and threats. Strength and weakness are internal factors whereas threats and opportunities are external factors. Figure 1 illustrates a template SWOT plot. An alternative template can be found in the Annex of this chapter.

It is important to be honest and specific when doing the SWOT analysis. Adding a weakness and suggesting a way of dealing with it is much better than to omit it and fail at a later point.

Table 3: Template SWOT Plot

Strengths	Weakness	Compensatory actions
• Internal resources and capabilities available at the laboratory • Existing cooperation with external partners • Existing accreditations and quality certifications	internal weakness: • e.g. training needs, financial or infrastructural constraints	• List here planed actions, e.g. training plans planned improvements in infrastructure, quality system or equipment addressing the listed list of weakness
Opportunities	**Threats**	**Risk management**
Positive external influences: • Market needs • Local safety risk issues • Upcoming changes in regulatory guidelines or market potential • Opportunities due to changes in the value chain	External threats: • competing laboratories, but also financial risks, risk because laboratory may not be compliant to new regulatory requirements	• List here planned and possible actions which are within the scope of action against threats. • Try to build on strengths to counter threats.

The SWOT analysis should be detailed highlighting current strengths and opportunities while acknowledging weakness and threats. A reliable and realistic picture of the current situation needs to be presented (*i.e.* no wishful scenarios) to assess capability and capacity to create a new laboratory or go into new business areas. All relevant staff and laboratory departments need to be included (not just managers) in order to validate the data.

An overall SWOT plot may be developed for the laboratory giving an overview of the whole laboratory. It is advisable however to carry out the SWOT analysis with key laboratory counterparts. Different SWOT plots may be developed for selected issues, for example:

- Vision and strategy: do the vision and strategy fit with the resources and capability available? What is done to develop the organization?
- Culture: what is the current culture, are staff motivated and with a sense of purpose, is collaboration encouraged?
- Product and service portfolio: how do services perform in the current set-up and can they be sustained (analysis performed and results delivered on time)?
- Are the necessary resources (financial, human, infrastructure and supplies) available to start or continue operation?
- Have goals been set and are the performance being measures (e.g. KPI)? Is the laboratory operating under a set of performance measures, who is defining the KPI and who do the follow-up?

Table 4: SWOT Plot example for a food control laboratory in an ACP country focused on the analysis of fresh fish

Strengths	Weakness	Compensatory action
• Lab has 10 rooms, 4 fully air-conditioned, staff of 18 technicians, 4 microbiologists • 250 customers from regional fish producing and processing organization • Laboratory has ISO 9001 certification • Lab can be extended to triple capacity it all equipment is used and a 2-shift system is implemented • 140,000 € subsidies • Permission to build a 2nd lab	• Cooling room capacity is reached (bottleneck) • Documentation is done with old computer system which frequently breaks down • No validation expertise • No ISO 17025 accreditation	• Put second cooling room into a new lab building • Validation training plan • Time plan and project plan to obtain ISO 17025 • Install a new documentation system
Opportunities	**Threats**	**Risk management**
• Local safety risk issues • Need to be registered as regulatory guidelines or market potential • Lab has been approached to offer service to meat (deer) producers as well	• 2 laboratories in the region with similar customers • Tax exemption will expire in 2020 • New harbour will mean that transport route will be changed	• Marketing plan and initiative to expand testing to new value chain • Make long time agreement with current customers • Make cost reduction plan (waste, energy cost etc.)

Essentially the SWOT analysis carries out a risk analysis to identify the most important weakness and threats. As a last step preventive actions and risk mediating actions and plans should be added.

It is not possible to foresee all risks but it is important to look at the business critical risk from two perspectives: the threat to the business, and the operational risks. There is some overlap between to two main risk perspectives. However, business risks need to be dealt with on the management level, whereas operational risks are often dealt with by the daily technical operating staff.

Risks should be ranked according to how critical they are to the business (analytical services) and what part they may affect. Finally, for all risks characterized it is critical to develop a contingency plan describing "what to do if..." and who is responsible for the actions.

6.6.1. Business risks

Business risks are major events that affect the whole laboratory and may be beyond the control of the management and the laboratory as a whole. A reality check is needed to see if all risks have been identified. Furthermore, it is important to have a plan of what to do if additional risks are discovered. To illustrate the topics to consider some examples of business risks are given below, most of which have already been mentioned in sections 4 and 5 of this chapter:

- competitor enters the market, regional and international competition;
- the services/analyses are not needed anymore, the market disappears (change of regulation, food production is changed);
- new technology calling for significant investments (e.g. equipment, environment);
- new legislative requirements e.g. analytical quality and accreditation but also to operate a business: environmental, health and safety, financial, operational, regional etc.;
- political factors – privatization, regional vs. national political interests etc.;
- taxes and other general business related cost;
- lacking access to core technologies;
- liability and breach of integrity – can errors or operation failures result in a liability;
- financial:
  - access to liquid capital (e.g. foreign capital);
  - customers cannot or will not pay the real cost;
  - funding and investment;
  - change of interests and other capital costs;
  - salaries and other personnel related costs;
  - financial support and grants;
  - exchange rate fluctuations that may affect the procurement etc.

As conditions and the business environment can change rapidly, it is important to constantly monitor and adapt to the risks identified and adjust the business plans.

6.6.2. Operational risks

Operational risks have effect on the day-to-day ability of a laboratory to deliver services as expected or promised. These range from lack of supplies to major events like a local disaster. Some examples are listed below; however these depend very much on local conditions and should be supplemented by a brainstorming session with laboratory staff and persons with extensive local knowledge:

- lack of supplies – materials and supplies not arriving as planned;
- instrument failure and lack of spare parts or unavailable services;
- Infrastructure failure – lack of power, water and other supplies, blocked drains etc.;
- staff – if you can't get sufficient staff with the right skills;
- weather and environmental effects on the transport infrastructure – getting samples and supplies to the laboratory and results to the customers;
- serious disturbance to the laboratories – fire, water damage, weather, war etc.;
- IT problems;
- etc.

As with business risks these risks shall be ranked according to their impact on daily operations. For all critical risks, a contingency plan must be developed.

6.7. STRATEGY AND BUSINESS SET-UP

Based on the observations made in the SWOT analysis the laboratory strategy needs to be developed. Normally a vision underpins a laboratory strategy.

In general there are two key drives to sustain a business or venture in new activities:

- filling market needs, there is an unfilled need for analytical services;
- exploiting strengths and capacity by creating a new market.

To meet these market needs it is necessary to have a coherent vision and strategy. If new analyses or services are added to existing activities these activities must fit the current strategy or the current strategy has to be revised. A vision and strategy are central elements in a business plan, the '*raison d'être*' of the laboratory.

While many laboratories and businesses operate without a written-down vision and strategy, there is an understanding of what is being done. However, to develop an efficient and feasible laboratory it is very important to develop and share the vision and strategy with all staff. They should also be involved in the process of solving problems as they tend to know the every-day problems. Hence they may contribute to problem solving and become motivated by doing so.

The vision and strategy is typically supplemented with a mission, some strategic goals and a set of values. The better they are formulated and shared by all, the better the chances of success.

A management and organization plan transposes the strategy into action ensuring that whatever the laboratory decides to do, makes sense and has a long-term perspective. More importantly the organisational plan also outlines the resources and competences needed to reach the goals and how to organize the laboratory.

On top of the organizational perspectives there should be an operational plan describing how business is carried out. This outlines all procedures and processes from sample handling to accounting and maintenance but also controlling costs, quality, development and treatment of the customers. This perspective is a mandatory part of the ISO 17025 accreditation system.

VISION, MISSION, STRATEGY

What – who – how

Describe the following in some detail:

- What are we trying to deliver? Be specific in terms of products but also look beyond the obvious. For example, the lab can deliver security and proof of integrity thereby increasing the value of products.
- Who do we see as our customers? Look beyond the obvious provider of samples or authorities. The laboratory may be as much an independent partner for the food producers benefitting from documented quality, exports companies etc. as it may be an official control body.
- How can this be done? The required setup, proof of independence, accreditation and audit, transparency?

Mission and vision

- Mission What are the reasons we are in business / exist as a laboratory?
- Vision What and where do we want to be? Envision the future of the laboratory;
- Strategy How do we get there?
- Measure How do we know when we are there?
- Values What do we believe?

6.7.1. Laboratory set-up

The laboratory set-up plan is an important part of the business plan. It should list all necessary investments. For the final time plan (GANTT or other) it is useful to ask suppliers not only for prices but also for their time of delivery. The following paragraphs outline the most important topics to consider.

A food control laboratory needs to comply with the standards of quality management systems and may aim to become accredited to the norm ISO 17025 at some point in the future. Thus it is highly advisable to become well acquainted with the requirements of key norms such as ISO 9001, ISO 14000, ISO 17025 and the basics of Good Hygiene Practice and Good Storage Practice. Figure 3 depicts key norms relevant to laboratories.

Table 5: Quality Systems relevant to laboratories

Quality systems – laboratory	
ISO 9001:2008 • Quality management • Adequate training • Communication w/customers • Documentation & traceability • Audits	**Good hygiene praxis (GHP)** Product dependent guidelines to observe in the laboratory, e.g. pest control
FOOD CONTROL LABORATORY	
ISO 14000 • Environmental management • Waste regulations • Legal compliance	**ISO 17025** **Good laboratory practice** Similar to ISO 9001:2008 plus: • Calibration and test method validation • Collaboration w/reference laboratories • High standard of laboratory facility • Very high standard of documentation of traceability
HACCP Risk analysis All test storage and methods in the laboratory, e.g. contamination w/microorganism, chemical contamination, etc.	

The facilities of the laboratory need to be suitable for the planned operations. This means that sufficient space for the registration of incoming samples and the storage and disposal of samples needs to be provided. For samples which need cooling a continuous electric power supply and cooling facilities need to be provided. Thus it is useful to include a floor plan of the laboratory to the business plan.

As many analyses are temperature dependent, temperature and humidity control needs to be installed where appropriate in the laboratory facilities. Records of this monitoring have to be kept and archived for audits.

Floors, walls and ceilings should be of material that can be easily cleaned, the latter being absolutely mandatory for laboratory surfaces of tables, benches, drafts and the floor.

It is advisable to have rounded edges at the interface between floors, walls and ceiling. For some analyses a full clean-room environment is advisable. For other analysis separate clean-benches or parts of the laboratory with laminar flow and separations made out of flexible separation sheets may be sufficient.

The staff needs to have access to facilities to wash and clean themselves and separate rest areas in order not to contaminate samples.

Documentation of samples needs to be kept safe from pests, humidity, flooding and in the case of electronic storage magnetic fields. Thus the business plan also should address how the documentation will be handled.

Prior final laboratory installation but also during the drafting of the business plan it is thus highly advisable to discuss the layout and the installation plan with protagonists of the official accreditation organization and/or an international certification body.

6.7.2. Laboratory organisation, capacity and logistics

Food analyses range from simple procedures to complex and elaborate procedures requiring very expensive equipment. The laboratory business for food analysis is therefore governed by both the requirement for proximity to the sampling point and access to advanced equipment.

Some of the main factors needing to be considered in the laboratory strategy are:

- **Proximity:** some of the analyses need to be performed shortly after sampling whereas other types allow long transport of samples. Many simple quality assessment analyses and microbial analyses are in the first group, whereas many chemical analyses in general belong to the second group.
- **Location:** ensures that providers of samples, consumables, spare parts, services etc. have easy and reliable access to laboratories and to local knowledge about food production. Both the kind of analysis and the proximity to producers, processors and distributors is important, as not all types of samples can be transported over longer distances.
- **Expertise:** Laboratory staff needs adequate education and training. The staff of a food control laboratory, however, needs also continuous training, which is mandatory for all mentioned quality systems. These trainings range from annual safety trainings, regular hygiene and GLP trainings to specific trainings for the use of instrumentation equipment. A plan which specifies the names and functions of the staff and the planned training needs to be written. Such plan is also required for example by the quality management system ISO 9001:2008. A person which is responsible for quality assurance needs to be appointed and needs to write regular quality reports which have to be reviewed by the laboratory management.
- **Procedures and methods:** All methods need to be validated. This means that the laboratory needs to prove that the results of its analyses are reproducible and valid with the very equipment present in the laboratory. All test methods need to documented in standard operations procedures (SOPs). All validations need to be documented as well and the documentation archived.

- **Number of samples:** some procedures require a minimum number of samples to be viable either to secure sufficient utilization of expensive equipment or because chemical and regents expire rapidly. The number of samples may be standardized by a norm or publically available specification, but can be also dependent on the type of equipment used in the laboratory. In this case the number of samples may be determined during the method validation process.
- **Equipment and method validation:** some analyses, in particular chemical and rapid microbiological analysis require complex and expensive analytical instrumentation and advanced laboratory facilities. All equipment needs to be calibrated and regularly monitored and maintained.
- **Audits and risk analysis:** a risk analysis about the technical operations of the laboratory should be conducted. In all quality systems described self-audits need to be carried out annually – in addition to the regular audits by governmental authorities and certification organizations.
- **Communication with authorities and stakeholders:** some samples/analyses require fast results for the authorities or customers to act, for instance to withdraw a batch from the market because of risks (e.g. pathogens in ready to eat food or methanol in liquor) or to release a detained import/export batch.

The business plan should mention why location, organization structure and the products which are to be tested were chosen and state that the chosen business model (method how the laboratory and its staff are financed) will work. The financial details should be given in the financial part of the business plan. The time which is needed for validation and installation needs to be incorporated into a GANTT time chart.

For each point listed above it is useful to set a target that should be met, for example number of staff and its education, duration of validation and time when validation is finished.

Many of the factors listed above influence each other. The number of analyses influences the storage space, cost of analysis (salary of staff, amount of equipment and consumables needed for the laboratory analysis) and even the requirements on staff training and education. It is thus useful to use number of analysis per method as the starting point to calculate different financial and/ or capacity scenarios for the business plan.

6.8. STAFFING AND TRAINING

This section of the business plan outlines staffing needs of the laboratory with concrete job descriptions. In a form of training needs analysis it compares the staffing needs with the personnel available determining where gaps need to be filled with new personnel and where training and/ or coaching of existing staff will be sufficient. As much as possible trainers / training institutes should also be identified.

Profile descriptions of the personnel should be included in this section, with detailed CVs in annex of the business plan.

6.9. PERFORMANCE INDICATORS AND TIME PLAN (GANTT)

In order to render laboratory performance (and thus its business plan) measureable it is important to define performance indicators and a time plan with milestones.

6.9.1. Performance indicators

To evaluate the performance of a laboratory or a new analytical service it is important to select a set of factors that can be measured to judge the performance. In general two different sets of factor are used:

- Critical Success Factors (CFSs) are elements that are vital for a given strategy to be successful. These factors can be both quantitative and qualitative and are used to describe if a business is successful;
- Key Performance Indicators (KPIs) are measures that quantify objectives and enable the measurement of strategic performance. In general KPI are quantitative parameters that can be measured on scale and compared, e.g. number of analysis per week.

CSF's have five primary sources, and it is important to have a good understanding of the environment, the industry and the business in order to be able to describe them well. These factors are customized for companies and individuals. The customization results from the uniqueness of the organization.

It is important to build on knowledge of competitors in the industry. It is worth highlighting this principle separately as it is critical to have a good understanding of competitors when identifying an organization's CSF's. Knowing where competitors are positioned, what their resources and capabilities are, and what strategies they will pursue can have an impact on an organization's strategy and also resulting CSF's.

CSFs should be developed leading to observable differences. A key impetus for the development of CSFs is the notion that factors measured are more likely to be achieved versus factors that are not measured. Thus, it is important to list CSFs that are observable or possibly measurable in certain respects such that it will be easier to focus on these factors. These do not have to be factors that are measured quantitatively as this would mimic key performance indicators. However, writing CSFs in observable terms will be helpful.

CSFs are often developed for each segment: customers, finances, internal processes, developments, innovation, method development and quality assurance.

The performance indicators, KPI metrics, measure the performance and are often developed for:

- recruitment;
- employee development;
- finance and business;
- Health and safety;
- Environmental.

Numerous examples of CSFs and KPIs can be found in business literature but the most importantly they need to be clear, specific and realistic/relevant.

6.9.2. Time plan

The scheduling of a laboratory implementation process depends crucially on the existing background. In many cases laboratory facilities may already be present or funding may already have been approved. In other cases, when operating from scratch the process of laboratory facility planning will take significantly longer. Funding may have to be obtained, banks may ask for many documents and/or securities, the permission to build may have to be obtained and a marketing plan needs to be decided upon – including a budget for such.

The GANTT time plan used in this section assumes that laboratory facilities of some sort exist. Furthermore, the political and economic environment is ready – including governmental funding – for the installation of the laboratory. Generally speaking, the greater the laboratory infrastructure available, the earlier operations may start.

In all cases, however, time for validations of methods, calibrations of equipment, training of staff, preparation of documentation as well as for internal audits need to be included into the GANTT time schedule.

6.10. FINANCIAL ANALYSIS

Financial analysis covering all income, expenses, value stream and cash flow can be complex. Several models can be found in business literature in this field. For the purpose of a cost analysis of a laboratory and laboratory services in the ACP region it is suggested to use a simple model that is still accurate.

Put very simply the financial analysis can be reduced to accounting for all:

- income and expenses;
- assets and debts (liabilities);
- investment and depreciation.

However, to evaluate the feasibility of a laboratory or an analytical service a somewhat different perspective is needed with a more detailed focus on expenses, income from the service and funding for development and investments. As many food control laboratories are publicly owned they operate under different financial conditions than privately held businesses.

The starting point in the evaluation of an existing laboratory is the complete budget and the latest financial balance sheets and statements from which some of the basic expenses and income can be deduced. These documents should be able to give an overview of the financial structure so as to learn how budgeting and accounting works and the core principles used.

For a new laboratory accounting documents should be simulated, including at least a balance sheet and profit and loos statement. If possible three scenarios should be included in the calculation: best case, medium case, very conservative (worst) case. Table 6 below provides a template example of a financial plan of a new laboratory set-up while Table 7 presents a template profit and loss statement.

Table 6: Financial planning for the set-up of a laboratory

LABORATORY COSTS	Year 1	Year 2	Year 3
1. Investments			
General infrastructures			
Building			
Electricity generator			
Computers (hardware)			
Vehicle			
Furniture			
Laboratory infrastructures			
Cost of laboratory furniture (tables, shelves, special cupboards, lab benches, fume hoods)			
Cost to fit out storage area (freezers etc.) and for air conditioning (control of temperature, humidity)			
Cost of larger laboratory equipment			
Cost of smaller laboratory equipment			
Other cost			
2. Operational costs			
Cost for running the laboratory			
Cost of disposables (reagents, chemicals, test kits) for the laboratory			
Cost of glassware			
Cost of software *e.g.* analysis software			
Server cost			
Calibration costs			
Other cost			
Staff, management and outsourced services			
Salary management			
Salary Staff			
Salary subcontractors			
Security costs			
Legal, tax advice, consulting fees			
Cost of other outsourced activities			
Overhead costs			
Insurance			
Rent			
Communication fees (phone, Internet, mail)			

LABORATORY COSTS	Year 1	Year 2	Year 3
Server fees			
Water			
Electricity			
Fuel, heating			
Waste disposal			
Travel			
Membership fees in organizations			
Fees for training of staff			
Advertising costs			
Other costs			
3. Capital costs (interests, loans)			
Capital costs (interests, loans)			
Depreciation			
Sum (Total cost)			

Table 7: Profit & Loss Table

PROFIT AND LOSS	Year 1	Year 2	Year 3
Revenues			
Revenues from analytic services			
Revenues from training			
Revenues from consulting			
Income from governmental grants			
Income from stakeholders (membership fee and other contributions)			
Loans			
Revenues from analytic services			
Expenses			
Cost for tangibles (investment into infrastructure, large laboratory equipment etc.)			
Operation cost			
Salaries (management, staff, contract services)			
Overhead costs			
Capital costs			
Resulting Profit/Loss			
Cash			

The financial analysis should strive to include the following perspectives for the laboratory:

- the overall laboratory costs independent of activities (indirect cost);
- the cost of the specific activity. e.g. analyses and services (activity cost);
- investment (invested capital and funding) and the cost of capital for investment;
- depreciation on investments;
- liabilities.

It can be quite difficult to estimate all costs and revenues. It is however important to achieve a good estimate on key numbers, followed by a reality check by people with relevant on site experience (ideally working in the laboratory if the business plan is being developed for an already existing establishment). Some of these costs can be very difficult to obtain, in particular indirect costs in large organizations. However a local estimate may be sufficient as the overall budget is often also discussed locally.

6.10.1. Indirect costs

Indirect costs include many of the things that are taken for granted – from buildings to furniture. However, these represent significant investment and require running costs to maintain. Some of the indirect costs are directly associated with tangible assets, whereas others are related to developing current capability (expertise and know-how). Examples of indirect costs are (this is not an exclusive list; many more may be relevant depending on country, local environment, laboratory status etc.):

- Tangibles (physical objects that represent a value):
  - buildings;
  - building infrastructure (power supply, IT infrastructure, water supply, gates, gardens etc.);
  - computer servers, libraries;
  - inventory, appliances, furniture and facilities;
  - laboratories and offices;
  - laboratory support facilities (water purifying systems, storage, cold storage, freezers, and dishwashers);
  - basic general laboratory equipment (benches, fume hoods, incubators, autoclaves, balances etc.);
  - general laboratory installations (ventilation, air conditioning, cooling water, special gasses, vacuum systems, steam etc.);
  - etc.
- Intangibles (non-physical things like knowledge, methods, organization that has a value):
  - organization and operational knowledge (know-how);
  - knowledge and archives e.g. methods developed;

    - quality assurance system;
    - a trained staff;
    - etc.
- Operation costs/Services:
    - management, HR, finance and accounting, safety and security etc.;
    - general maintenance and service (building, infrastructure etc.);
    - supplies (power, water, energy etc.);
    - security;
    - staff training programmes;
    - procurement;
    - insurances;
    - taxes, liabilities etc.;
    - legal assistance;
    - accreditation and audit costs;
    - cleaning of rooms and lab ware;
    - etc.
- Other indirect cost (everything else that doesn't fit in the above):
    - idle time on instruments and non-productive working hours (e.g. for training courses, updating or quality assurance);
    - sponsorships, contribution to local activities;
    - staff benefits.

The overall indirect cost may be calculated as the sum of the above-mentioned indirect costs. For some laboratories in ACP countries staff remuneration are not a part of the laboratory budget as they are included in a large governmental organization. They may hence not be easily estimated. In this case the known indirect costs should be calculated and the hidden costs listed.

6.10.2. Costs of a specific service (activity costs)

The most comprehensive way to estimate the cost of a service is to use an Activity Based Costing system, ABC. However this is rather complex and requires a very efficient overall accounting system. A more practical approach is to consider three types of costs related to an activity:

- costs that depend directly on the activity (for example the number of samples analysed), e.g. consumables and chemicals used for the activity and perhaps special equipment for that particular purpose;
- costs of developing the specific activity – e.g. time for method of development and validation;
- share of the indirect costs as described above corresponding to the activity.

6.10.3. Funding, investment and revenue

Many food control laboratories are publicly owned and are therefore funded, at least partly, by government. These laboratories may be required to generate revenue from sales of services or to supply a service for free, or any combination of these. It is critical to understand under which condition the basic funding is given and what specific deliverables are expected.

The assessment is most efficiently split into the following groups:

- funding to build the laboratory (to what purpose was it built), typically a "one-off" investment;
- funding to cover the basic indirect costs, on a yearly basis to cover the basic running cost (indirect costs, see above);
- funding to develop a service (analysis);
- funding to provide a service (activity cost), e.g. payment to do a certain number of analyses;
- funding of staff provided (in case a certain number of staff is approved of and funded by government).

These types of funding may be provided independently of each other. However food control laboratories are often established by authorities while the operation (indirect and activity based costs) may be a mixture of public funds and payments for delivered services (analyses). This situation can be quite challenging. While the initial investment may be sufficient to set-up a laboratory and get equipment, it can be difficult to get sufficient funds to cover maintenance, and even more so, to constantly replace equipment that has failed, is worn-out or is obsolete. All these costs are a part of the indirect costs that has to be covered when determining the prices of a service. At the same time there may be other restraints on the cost that can be demanded for a service (an analysis) creating a very difficult financial situation.

Finally, in some cases it is expected that the analytical services generate revenue, which have to be added to the above-mentioned costs particularly for privately held laboratories.

6.11. APPENDICES

A.1. PESTEL analysis

What if the agricultural praxis changes or there are environmental changes, market changes, rapid changes.

To provide a picture of the environment in which the laboratory is going to operate: Political factors, Economic, Social, Technological, Environmental factors – both business and industry environments and the external environment, Legal considerations. The scheme below can be used to assist a PESTEL analysis where numerous factors can be addressed and rated according to importance. Focus should be on those that are rated as important.

	Examples to consider	Effect level	How critical
POLITICAL	• Trading policies • Funding, grants and initiatives • Home market lobbying/pressure groups • International pressure groups; • Wars and conflict • Government policies • Government term and change • Inter-country: relationships/attitudes • Political trends • Governmental leadership • Government structures • Internal political issues • Shareholder/ stakeholder needs/ demands	• Local: Provision of services • National: Government policy on subsidies council • International: World trade agreements	
ECONOMIC	• Home economy situation • International economies and trends • General taxation issues • Taxation changes specific to product/services • Seasonality/weather issues • Market and trade cycles • Specific industry factors • Market routes and distribution trends • International: trade/monetary issues • Disposable income • Job • Growth/unemployment • Exchange rates • Inflation • Interest and exchange rates • Production level • Internal finance and cash flow	• Local: salaries • National: Taxes International: world market prices	

	Examples to consider	Effect level	How critical
SOCIAL	• Consumer attitudes and opinions • Media views • Law changes affecting social factors • Brand, company, technology image • Major events and influences • Buying access and trends • Ethnic/religious factors • Ethical issues • Demographics (age, gender, race, family, size) • Population shifts • Education • Immigration/emigration • Health • Housing trends • Attitudes to work • Attitudes to people doing certain types of work • Earning capacity • Staff attitudes • Management style • Organisational culture • Changes to education system	• Local: education and language skills • National: ethnics issues • International: migration	

	Examples to consider	Effect level	How critical
TECHNO-LOGICAL	• Competing technology development • Research funding • Associated/dependent technologies • Replacement technology/solutions • Maturity of technology • Information and communications • Technology legislation • Innovation potential • Technology access, licensing, patents • Global communications • New discoveries • Research • Energy uses/sources/fuels • Rate of obsolescence • Health (pharmaceutical, equipment etc.) • Information technology • Internet • Transportation • Bio-tech • Waste removal/recycling • Email • M-learning • E-learning • Collaboration tools • Software changes	• Local: improvements in technologies • National: technology development; • International: breakthrough technology	
ENVIRON-MENTAL	• Ecological and environmental issues • International, national, local • Environmental regulations • Customer values • Market values • Stakeholder/ investor values • Staff attitudes • Management style • Organizational culture • Staff morale • Staff engagement • Global factors • EU based factors	• Local: waste issues • National: infrastructure and logistics • International: climate change	

	Examples to consider	Effect level	How critical
LEGAL	• Current legislation home market • Future legislation • European/international legislation • Regulatory bodies and processes • Environmental regulations • Employment law • Consumer protection • Industry-specific regulations • Competitive regulations • Liability	• Local: planning permission • National: regulation, accreditation and audits • International: treaties and agreements (e.g. human rights or environmental policy)	

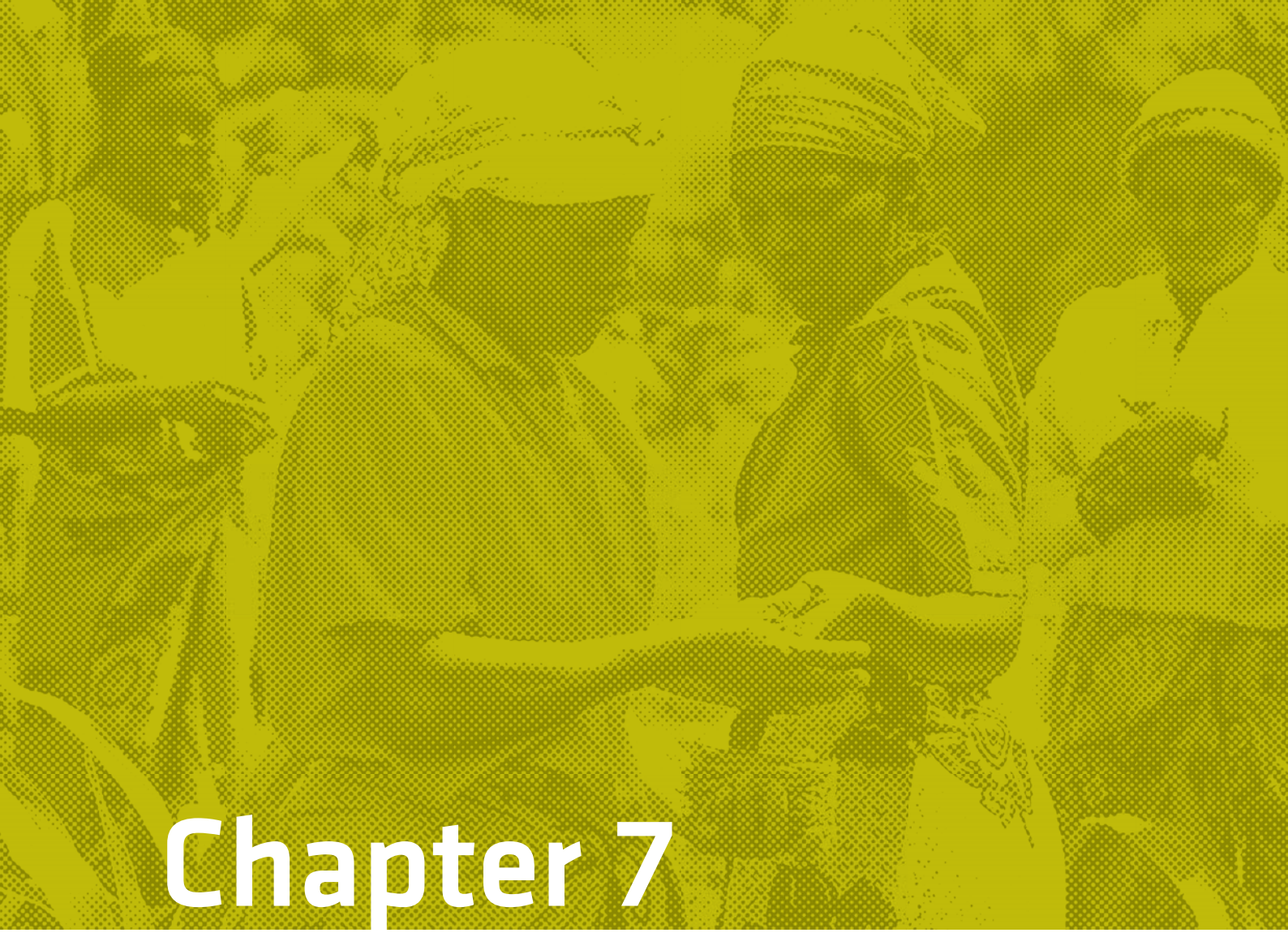
A.2. Risk assessment of a new service

CHECKLIST: Assessment for the need of a test		
Demand of the test	**Yes/No**	**Comment**
Is there a specific health risk for customer, which can be named?		
Is there a regulation that prescribes the test?		
How large is the production of the food products, the test can be applied to in the region?		
Is there a competing test technology, which is already used by a competing laboratory?		
Why is the method that is considered better: faster, cheaper and more reliable? Is it accepted by the importing countries organizations as a valid test method?		
How many tests can be carried out in this laboratory, can the laboratory meet the estimated demand?		
Sort the health risks and the current market price for associated tests in order of magnitude (separate sheet)		
Multiply each of these values with first the value of food product produced in the region		
If the test is implemented, can it be easily transferred to other kind of food product, is extra equipment needed for sample processing and storage, how are the logistics for sample transfer?		
Pricing	**Yes/No**	**Comment**
What is the standard price for the test, as it is offered by other testing laboratories?		
Are there pricing limits imposed by governmental rules?		
Are there pricing limits imposed by economic constraints in the value chain?		

A.3. Alternative SWOT plot

The results and conclusions of the internal and external analysis can also be presented in the grey area of the other SWOT plot sheet given below. Strengths and opportunities are matched, also counteractions of weakness and opportunities and possible countermeasures to threats and weakness are presented as conclusion in the four white areas.

		EXTERNAL	
		Opportunities *What are the market needs and local food safety risk profiles that can be addressed*	**Threats** *List the risk factors and score these according to their importance*
INTERNAL	**Strengths** *List all the strengths, resources and capabilities available at the laboratory.*	Describe how the opportunities can be develop into new business exploiting strengths in new areas.	Describe how the threats can be met using the strengths, what further training and resources are needed to fight of the threads.
	Weakness *In this box put all the points that need to be developed.*	Describe what training is needed to develop the weakness into strengths that can be used to meet opportunities.	If the laboratory has activities that fall into this area they should be considered for termination. Otherwise an extensive training plan should be developed.

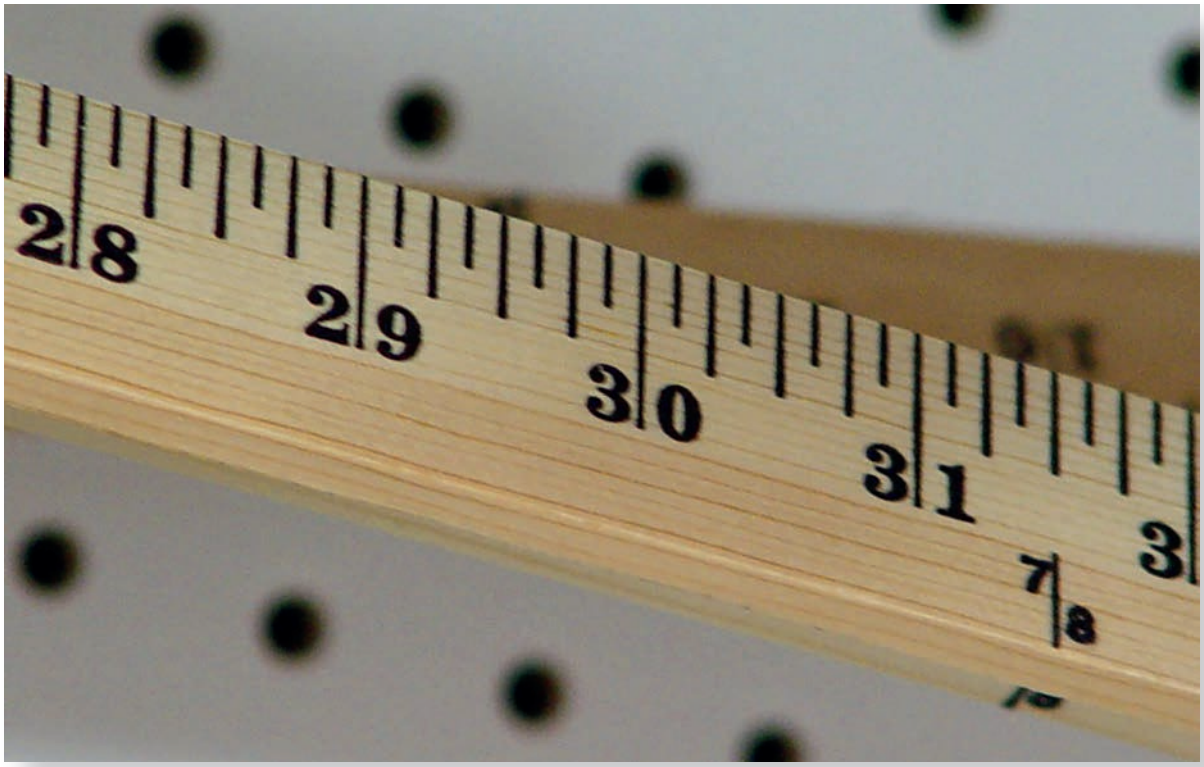


Chapter 7

Metrology and the metrological traceability chain

7.1. INTRODUCTION: DEVELOPMENT OF METROLOGY IN SHORT

7.1.1. Introduction



The aim of this chapter is to inform about the concept of achieving reliable and trustworthy measurement results. This concept is not specific for food safety although certain specific aspects have to be considered in this field. After a short historical overview of the development of the art of measurement and the driving forces behind, the principles to be followed when measuring are described. Confidence in correct measurements is of high importance because it is essential for checking the conformity of products with standards and regulations and the acceptance of certificates. The concept to ensure mutual acceptance of measurement and calibration results is explained as well as the role that national, regional and international metrology organizations have to play. Although it will not be possible to provide answers to all questions, hints are given how to tackle problems and whom to contact.

Since every discipline has its special terminology. But for the better understanding and readability of the following two clarifications are given here.

The term 'metrology' is relatively new compared to what it describes: all activities concerned with measurements. The internationally accepted definitions of this term as well as others related to metrology are given in the *International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM)*, 3rd edition 2012.[131] Taking into account that these definitions are academic and sometimes

131 *International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM)*, 3rd ed., 2012, available from: International Organization for Standardization, 1, rue de Varembé, CP 131, CH 1211 Geneva 20, Switzerland or www.oiml.org/en/files/pdf_v/v002-200-e07.pdf/at_download/file.

very lengthy the terms used in the text are sometime simplified for the better understanding. Nevertheless, the definitions given in the *VIM* should be considered as the relevant ones.

The term traceability has different meanings depending on the context in which it is used. In this document traceability is always used in the sense of metrological traceability even without mentioning metrological explicitly.

7.1.2. Development of metrology in short

Metrology started in the very early days of mankind. Natural phenomena were used as measures. The time between two consecutive sunrises marked one day, one month was measured as time between two full moons. Shorter and longer time intervals could also be measured either by simple devices using the principle of sand glasses for example or other astronomical phenomena. The need for measuring quantities other than time evolved with the development of civilizations. To measure means to compare an unknown quantity with a defined unit for this quantity. The ratio gives the result of the measurement in terms of the applied unit. For example, the weight of a load on one site of an equal arm balance is compensated by the number of unit weights which are needed to achieve equilibrium. Nowadays, the unit of weight is the kilogram, kg.

In ancient times other units were used which usually varied locally. Very often the length of the forearm of the local ruler or king was used as the unit of length, commonly named 'Cubit'. Based on this unit artefacts made of wood or stone with subdivisions were produced representing the unit of length and enabling measurements at different locations. In ancient Egypt these reproductions of the unit length had to be compared with the official reference unit at every full moon. The principle to compare measuring instruments with standards of higher accuracy is still used today. In ancient times, violation of the obligation for verification caused severe punishments. The construction of buildings such as the pyramids and temples and the land measurements after the yearly Nile floods required a well-organized and technically advanced measurement system.

One of the activities which stimulated and still stimulates measurement is trade. In order to facilitate trade, rulers prescribed the use of standardized units within their empire and imposed sanctions in case of violations. Regular periodic verifications assured the correct dissemination of the measurement standards throughout the region. The name of one such unit of weight is still used today: the carat, which is equal to 0.2 grams and is used for jewellery. The carat derives from carob seeds that have low variability in weight and were chosen to serve as standard measure for the lowest unit of weight. Low variability is one requirement for measurement standards.

The development of new measurement capabilities is an on-going process and closely related to technical innovations, social and commercial demands and scientific progress. Out of the numerous existing examples, the Industrial Revolution provides a good illustration of the impact of evolving developments as far as metrology is concerned. During the 18th century the Industrial Revolution brought about significant changes in manufacturing, mining, transportation,

and technology. The progress in precision measurements and the construction of machines allowed the mass production of interchangeable parts with small tolerances with regard to their dimensions. One effect of mass production was the increase in trade. International trade was growing also due to better and faster transport by steamships. Since the Industrial Revolution started in the United Kingdom manufacturers used the Imperial System of Units. Other countries used other systems. These different systems limited the interchange ability of parts and created technical barriers to trade in general. The need for an internationally accepted system of units in measurements became clear but it was still a long way to its realization.

7.1.3. Steps towards an international metrology system

The international metrology system is a by-product of the French Revolution with the political motivation to harmonize units all over France. The concept was to establish units of measurement based on constants of nature thus making measurement units available 'for all people, for all time'. The unit of length, the meter, was defined as 1/10,000,000 part of the distance between the equator and the pole of the earth. The unit of mass was defined by a cube of water with the content of 1/1,000 m^3 corresponding to 1 litre. For practical measurements artefacts made out of platinum were produced for the meter and the kilogram as the basis of the metric system established on 22 June 1799. The French Government invited British scientists already in 1790 to participate in the new definition of measurement units and the creation of an international system. But due to the prevailing political condition this did not happen. Nevertheless, the metric system was gradually accepted by other countries. This further led to the creation of the Meter Convention (*Convention du mètre*) signed in Paris by 17 States in 1875.

The Meter Convention founded the International Bureau of Weights and Measures in Sèvres near Paris and established its financial and management structure. Furthermore, the Meter Convention enabled governments to act in common accord on matters related to units of measurement by defining permanent organizational structures for member governments in the metrology field.[132]

During the years the Metric System was further developed and is now called *Système International d'Unités*, or the International System of Units, SI. The International Bureau cooperates with national metrology institutes, which have been established in many countries. In Germany such an institute was established in 1889 under the name of *Physikalisch-Technische Reichsanstalt* on the request of scientists and industrialists arguing that such an institute is necessary for enhancing the competitiveness of German products. A few years later the USA, the UK and other countries created their national institutes. Their main tasks are to realize and maintain the national units of measurement and to cooperate with regional and international metrology organizations to ensure the correct dissemination of the units and to create confidence in measurements.

132 Please refer to the official BIPM website for further information: www.bipm.org/en/convention.

The International System has gained unprecedented worldwide acceptance. Nevertheless, it is not the official (legal) system of units of all nations. In some countries traditional units are still in use such as the gallon, pound, inch or mile in the USA. Local units which are unique to individual rural market places are still in use in some African countries. Cereals are sold by volume on rural markets for local customers using containers the shape and volume of which might vary from market place to market place. In contrast to international trade local trade is not affected by the many standards prevalent in so many areas of the world. But in international trade the International System is predominant.

7.1.4. Metrology as part of the quality infrastructure

Exports to the EU and many other countries that have regulations in place are required to fulfil minimum quality standards. These requirements are formulated:

- either qualitatively, for example "The product shall not create any damage or danger when properly used";
- and/or for example quantitatively "The product must not contain more than x mg per kg of substance Y".

The EU created a system to check such regulations through tests and measurements carried out under the responsibility of the Member States but according to harmonized procedures which ensure the acceptance of the results by all Member States.

Besides and in addition to obligatory regulations voluntary standards also stipulate criteria for specific products, procedures or systems. In the case of food hundreds of ISO standards relate to quality in production, testing, transport, storage, labelling, nomenclature, and terminology. The ISO 22000[133] family of standards deals with food safety in particular, whereas the Hazard Analysis and Critical Control Points, HACCP, stipulates a system of seven principles which identifies, evaluates and controls significant hazards for food safety.[134] Measurements and tests are always required. All actors involved in the food chain from the farmer to the retail seller must be familiar with the requirements relevant for their job and must observe them to ensure quality and to avoid hazards. In order to achieve this objective a quality infrastructure needs to be in place which provides standards and regulations, access to test houses (laboratories), calibration laboratories, and certification and accreditation bodies. Moreover, these service providers should be acknowledged at the regional and international level by the relevant organizations. The reason is that international trade is of growing importance and relies heavily on conformity assessment procedures stating the compliance of products with agreed upon standards or compulsory regulations. The diagram below shows schematically the interaction of the different elements of a national quality infrastructure for the production chain of shrimps.

133 ISO 22000: 2004 Food Safety Management Systems – Requirements for any Organization in the Food Chain. International Organization for Standardization, 1, rue de Varembé, case postale 131, CH 1211 Geneva 20, Switzerland.

134 Strategies for Implementing HACCP in Small or Less Developed Businesses, 1999. World Health Organization, Food Safety Programme, Avenue Appia 20, 1212 Geneva 27, Switzerland.

7.1.4.1. National quality system

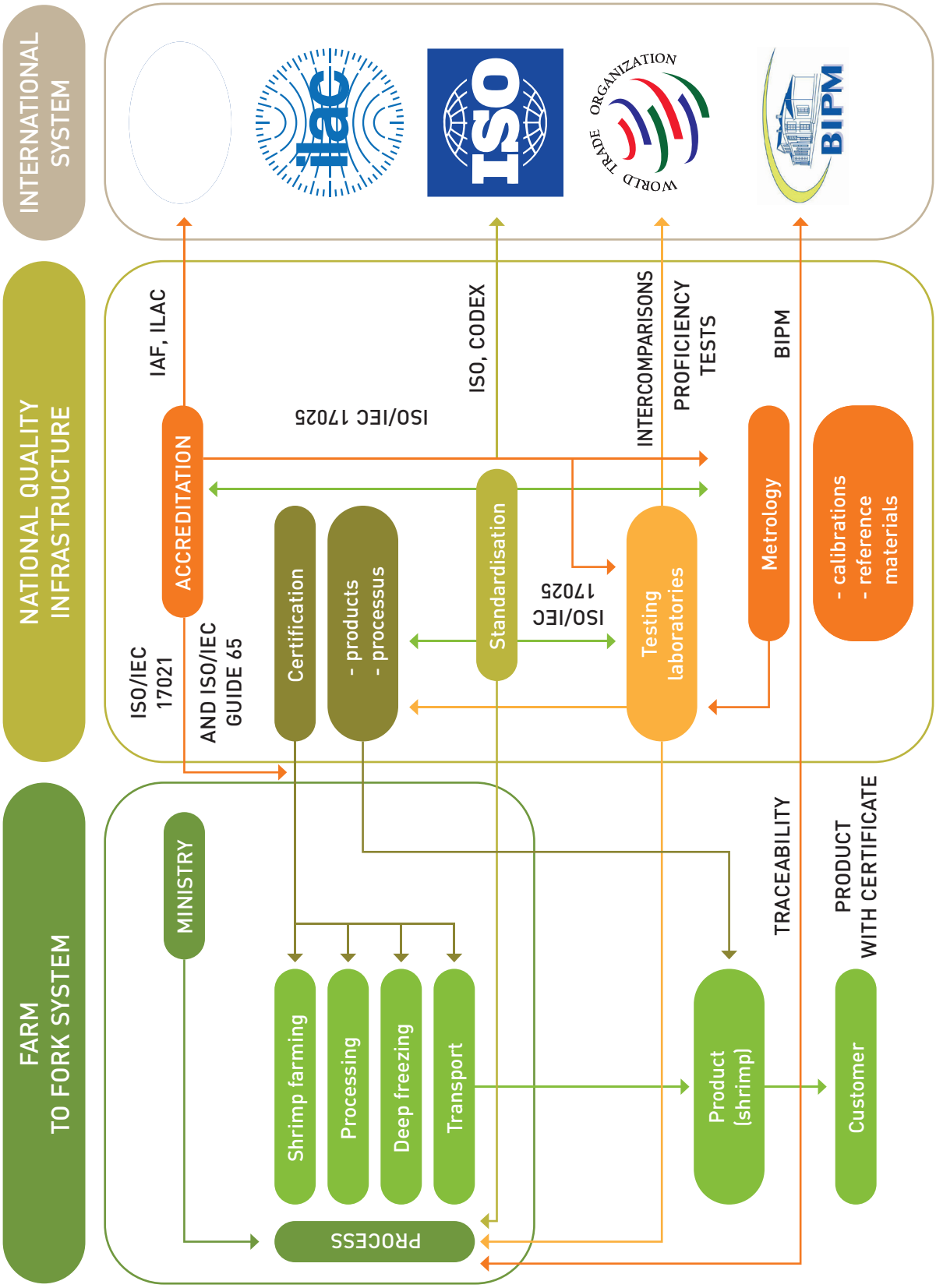


Figure 1 - Interaction of international organizations with bodies of the national quality infrastructure necessary for ensuring the quality of a product (e.g. shrimp)
Source: C. Sanetra and R.M. Marban, The answer to global quality challenge: A national quality infrastructure (available from PTB, presse@ptb.de).

As far as metrology is concerned, sophisticated systems have been developed at the international, regional and national level. These pursue the aim of mutual acceptance of measurement and calibration results thereby establishing confidence in the competence of laboratories and test houses with regard to their measurements. Details will be given in the next sections.

Another important activity is the metrological advice given to regulators and standardization bodies that, for example, fix tolerable limits for measuring instruments, for toxic substances, pesticide residues and the like. Such limits are given in Annex A of the WTO Agreement on Sanitary and Phytosanitary Measures (also referred to as the SPS Agreement) for pesticide residues in fruits or aflatoxins in nuts.[135] The problem here lies in the detection of such substances often being very difficult and depending on many parameters. Since it makes no sense to fix limits which cannot be measured or where different methods deliver different results, expert knowledge of metrologists should be taken into account. Exceeding tolerable limits will result in non-acceptance of shipments. Due to this fact the importance of correct and reliable measurements becomes evident. Economic losses can be very high as shown in the example of Box 1 below, and wrong decisions based on wrong measurements cannot be tolerated.

The EU ban on Ugandan fish exports

European countries detected high levels of bacterial contamination in fish products from Lake Victoria resulting in a ban on fish exports in 1997. The impact of this ban caused losses in export earnings of about 40 million US$ and 2000 job losses in fish factories and 32000 in fishing activities. Only after an internationally recognized laboratory established with EU support could guarantee compliance with EU Directives were exports to the EU allowed again.

Source: J.S.Wilson and V.O. Abiola (eds), Standards & Global Trade, A Voice for Africa, *The World Bank 2003.*

7.1.5. The different fields of metrology

Science, society, industry, commerce, citizens and governments: all rely on measurements and create demand for more, new and better measurement methods for their specific purposes. Metrology is therefore often subdivided into:

- Scientific metrology: this part of metrology deals with problems common to all metrological questions at a scientific level irrespective of the quantity itself. For instance, it touches on the general theoretical and practical problems related to units of measurement, the development of measurement standards and their realizations, the problem of errors in measurement and the metrological properties of measuring instruments.

135 The WTO Agreement Series – Sanitary and Phytosanitary Measures, www.wto.org/english/res_e/booksp_e/agrmntseries4_sps_e.pdf.

- Industrial metrology: this discipline focuses on measurements in production and quality control. Typical issues are the application of measurements for and during production and tests, management of measuring equipment and regular calibrations.
- Legal metrology: this term relates to mandatory technical requirements. Legal metrology services verify these requirements in order to guarantee correct measurements in areas of public interest, such as trade, health, environment or safety.
- Metrology in chemistry: this part of metrology is mentioned here because it is relatively new and of special importance for food safety and medical laboratories. Measurements in chemistry often serve to determine the amount of a specific component in a sample, for example lead (Pb) in blood or drinking water. Furthermore, chemical measurements are very often carried out under conditions which cannot be controlled and defined as in the case of physical measurements. Efforts are therefore needed to develop measurement standards, standard reference materials and standard measurement and calibration procedures and analytical methods. Not always can traceability to the SI be achieved.

7.2. QUANTITY AND UNITS

To measure a quantity (e.g., the weight of a sample) requires a unit (e.g., kilogram or gram) in order to receive a meaningful result. Nowadays, the International System of Units, in short SI, has been adopted by the Meter Convention, which is one of the oldest intergovernmental agreements.

The SI is the modern form of the Metric System. Its aim is to measure quantities in SI units as far as possible. The SI consists of seven base units and a number of derived units. The base units are:

- the meter (symbol m) for the quantity length;
- the kilogram (symbol kg) for the quantity mass;

- the second (symbol s) for the quantity time;
- the ampere (symbol A) for the electric current;
- the kelvin (symbol K) for the thermodynamic temperature;
- the mole (symbol mol) for the amount of substance; and
- the candela (symbol cd) for the luminous intensity.

The derived units originate from the base units and can be expressed as a product or a quotient with the proportionality factor one. Some examples:

- the quantity velocity expressed in SI base units: m/s;
- the quantity density expressed in SI base units: kg/m^3.

Some derived units have special names and symbols, for example the newton (symbol N) for the quantity force expressed in SI base units: kg m/s^2.

There are some units which do not belong to the SI, so-called off-system units, but which are in use world-wide and accepted for use together with SI units. Examples of such units are the day, the hour, the minute, the litre, the degree.

Examples of units used in special subject fields are the millimetre of mercury, mmHg, for the pressure in human body fluids, the nautical mile, the hectare.

Although the SI is applied worldwide there are also other systems of units used in certain countries.

The following rules apply for the expression and notation quantities and units:

Multiples and submultiples are strictly expressed as decimals. For example 1 kg = 1,000 g = 1,000,000 mg where the prefix kilo with the symbol k is used for the multiplying factor 1000 and the prefix milli with the symbol m is used for the multiplying factor 0.001.

Other prefixes and their symbols are given in Table 1 below.

Table 1: Prefixes and their symbols

Figure	Multiplying factor	Prefix / Symbol
1,000,000,000,000	10^{12}	T (e (giga)
1,000,000,000	10^{9}	M (méga)
1,000,000	10^{6}	k (kilo)
1,000	10^{3}	h (hecto)
100	10^{2}	da (deca)
10	10^{1}	d (déci)
0.1	10^{-1}	c (centi)
0.01	10^{-2}	m (milli)
0.001	10^{-3}	µ (micro)
0.00,001	10^{-6}	n (nano)
0.00,000,001	10^{-9}	p (pico)
0.00,000,000,001	10^{-12}	

Note:

The full stop is used as decimal sign in English speaking countries, whereas the comma is often used as decimal sign in other countries.

A space should be left between groups of three digits on either the right or left hand side of the decimal place. Commas should not be used as thousands separators.

In mathematical operations only unit symbols (m/s) should be used, not full unit names (meter/second).

It should be clear to which unit symbol a numerical value belongs and which mathematical operation applies to the value of a quantity.

Example: 20 cm x 12 cm not 20 x 12 cm.

7.2.1. Definition of units

As outlined above the SI is based on 7 base units and several derived units. Definitions of the base units have been changed several times as measurement possibilities and the demand for higher accuracy increased. In former times, the definitions were based on materialized prototypes which represented the units. Only the kilogram still belongs to this category. Nowadays, base units are defined with the help of physical constants so that they can be realized everywhere anytime. The advantage of this approach is that such definitions are not based on a single prototype which can be damaged or lost.

Definitions:

- **the kilogram** is equal to the mass of the international prototype of the kilogram;
- **the meter** is the length of the path travelled by light in a vacuum during a time interval of 1/299,792,458 of a second;
- **the second** is the duration of 9,192,631,770 periods of the radiation corresponding to the transition between the two hyperfine levels of the ground state of the caesium-133 atom;
- **the ampere** is that constant current which, if maintained in two parallel straight conductors of infinite length, of negligible circular cross-section, and placed 1 meter apart in vacuum, would produce between these conductors a force equal to 2 x 10^{-7} newton per meter of length;
- **the kelvin** is the fraction of 1/273.16 of the thermodynamic temperature of the triple point of water;
- **the mole** is the amount of substance of a system that contains as many elementary entities as there are atoms in 0.012 kg of carbon-12. When the mole is used, the elementary elements must be specified and may be atoms, molecules, ions, electrons or other particles, or specified groups of such particles;
- **the candela** is the luminous intensity in a given direction of a source that emits monochromatic radiation of a frequency 540 x 10^12 hertz and has a radiant intensity in that direction of 1/683 watts per steradian.

7.2.2. Realisation of units

The kilogram is realised by a cylinder made of an alloy for which the mass fraction of platinum is 90 % and the mass fraction of iridium is 10 %. The international prototype is kept at the BIPM. Copies of the international prototype are kept in many national metrology institutes.

Other base units are realized by an experiment. Instead of using a pendulum for the definition of a constant time interval, the realisation of the second is of much higher precision using a certain number of periods of a specified transition of a Caesium-133 atom. Although this sounds simple the realization requires a sophisticated physical set up. Commercially available atomic clocks allow to realize the second according to the definition. The same holds true for the realizations of other units.

7.3. DEFINITION OF MEASUREMENT STANDARDS AND THEIR HIERARCHY

Only those *VIM* based definitions which are relevant for this chapter are given here. Not all examples provided in the *VIM* are included and remarks from the author are added.

7.3.1. Definitions

Certified reference material: reference material, accompanied by documentation and issued by an authoritative body which provides one or more specified property values with associated uncertainties and traceabilities while using valid procedures.

Example: human serum with assigned quantity value for the concentration of cholesterol and associated measurement uncertainty stated in an accompanying certificate, used as a calibrator or measurement trueness control material.

'Documentation' is given in the form of a 'certificate' (see ISO Guide 31:2000).

Procedures for the production and certification of certified reference materials are given, for example in ISO Guide 34 and ISO Guide 35.

Intrinsic measurement standard: measurement standard based on an inherent and reproducible property of a phenomenon or substance.

Example: triple-point-of-water cell as an intrinsic measurement standard of thermodynamic temperature.

International measurement standard (etalon): measurement standard recognized by the signatories to an international agreement and intended to serve worldwide.

Example: the international prototype of the kilogram.

Remark: international organizations, other than the Meter Convention, may also define international standards, for example the World Health Organization.

Measurand: quantity intended to be measured.

The specification of a measurand requires knowledge of the kind of quantity, description of the state of the phenomenon, body, or substance carrying the quantity, including any relevant component, and the chemical entities involved.

Measuring interval, measuring range: set of values of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental measurement uncertainty, under defined conditions.

Measurement standard (etalon): realization of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference.

Remarks: on top of the hierarchy feature the measurement standards realized according to the definition of the unit. In the early days, there were only two prototypes: one for the kilogram and one for the meter, both kept at the BIPM. They served as international measurement standards to which all national standards were connected by comparison measurements. Nowadays, this is the case for the kilogram only.

National measurement standard: measurement standard recognized by the national authority to serve in a State or economy as the basis for assigning quantity values to other measurement standards for the kind of quantity concerned.

Primary measurement standard: measurement standard established using a primary reference measurement procedure, or created as an artefact, chosen by convention.

Secondary measurement standard: measurement standard established through calibration with respect to a primary measurement standard for a quantity of the same kind.

Reference material: material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

Reference materials with or without assigned quantity values can be used for measurement precision control whereas only reference materials with assigned quantity values can be used for calibration or measurement trueness control.

Reference measurement standard: measurement standard designated for the calibration of other measurement standards for quantities of a given kind in a given organization or at a given location.

Transfer measurement device: device used as an intermediary to compare measurement standards.

Travelling measurement standard: measurement standard, sometimes of special construction, intended for transport between different locations.

Example: portable battery-operated caesium-133 frequency measurement standard.

Working measurement standard: measurement standard that is used routinely to calibrate or verify measuring instruments or measuring systems.

7.3.2. The hierarchy of measurement standards

Figure 2 below shows schematically the hierarchy of measurement standards at the national level and an example for the realization of the quantity pressure on the right. Pressure is a derived unit N/m^2 (newton/m^2) with the special name pascal and the symbol Pa where N is the symbol for force with the special name newton (kg/[m s^2]).

National standards and their custodian should be defined by law. Usually, it is the task of the national metrology institute, NMI, to maintain the national standards and to calibrate reference standards, which are used by calibration laboratories, for example. Reference standards are used to calibrate working standards used by factories or others who maintain their own working standards. Working standards are used to calibrate or verify measuring equipment of the end user on the work bench or in the laboratory (among others). The accuracy decreases from top to bottom of the pyramid because errors and uncertainties influence every measurement.

A national measurement standard should be of the highest accuracy in the country. It has to be compared with an international standard, if there is one, or with a national standard of another country in order to check the metrological performance and to maintain traceability.

On the right side of the figure three types of standards are shown: two realize the quantity pressure according to the definition, the third one shows a comparator-type realization. A national standard must not necessarily be a primary standard. The result of each calibration has to be documented in all details such as the identification of the standard and the calibrated instrument, the ambient conditions, the calibration procedure, the date and the name of the operator, the result of the calibration as a function of the measuring range with the applicable uncertainties.

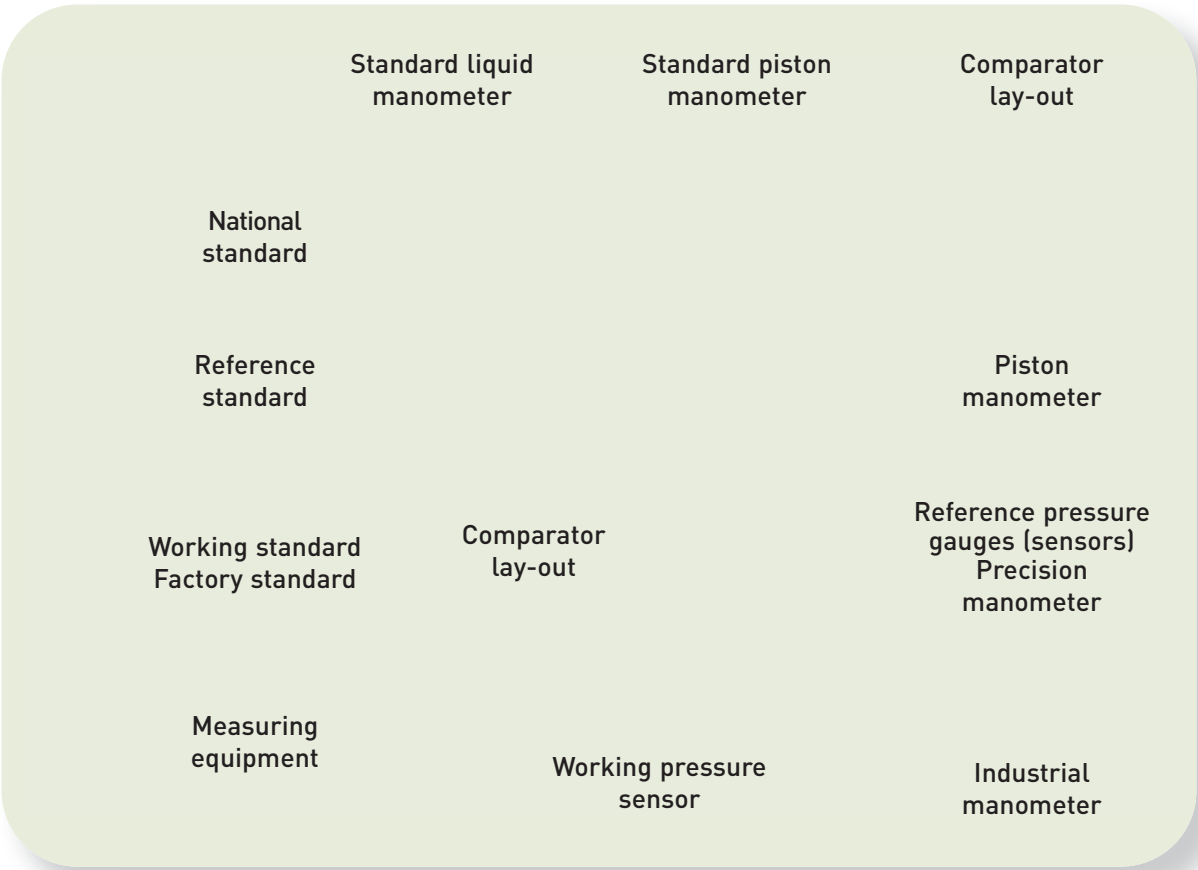


Figure 2 - Hierarchy of measuring equipment

7.4. THE NATIONAL TRACEABILITY CHAIN

The national traceability chain is a sequence of hierarchically organized measurement standards and calibrations aiming to relate a measurement result to a national measurement standard. An example is shown in figure 2: the pressure sensor, an industrial manometer used at the work bench, is calibrated by a working standard of higher accuracy. This working standard may belong to the company/factory that uses the industrial manometer to carry out in-house calibrations. If the company does not have its own working standards it must ask a calibration laboratory to carry out the calibration. In both cases the working standard has to be calibrated with the help of a reference standard of higher accuracy which in turn has to be calibrated using the national measurement standard. In this way, the measurement result obtained at the workbench can be related ('traced') to the national standard. It is possible that more than three calibration steps are necessary or advisable, depending on the difference of accuracies between the measuring instrument and the national measurement standard. The national measurement standard should be calibrated by an international standard or compared with other national measurement standards to ensure traceability to the SI.

Traceability in chemistry is more diverse than for physical quantities because the link to the SI cannot always be established. In this case measurements should be traceable to a reference material. If possible, the reference material should be certified with regard to the concentration of the analyte in a specified matrix or as a pure substance. Many NMIs, Designated Institutes and private companies produce and provide reference materials.

In any case the calibration must be documented and the measurement uncertainty calculated and stated in the calibration certificate. As an exception, the verification of measuring instruments by working standards may be expressed as within or without of fixed tolerable error limits.

7.4.1. The National Metrology Institute, NMI

Many countries maintain a specialized body acting as National Metrology Institute, although they may also be referred to as National Physical Laboratory (UK), National Institute of Standards and Technology (USA) or Bureau of Weights and Measures.

National Metrology Institutes are designated by national decisions (laws) which also define their tasks. They may differ from country to country but typically comprise the following tasks:

- keeping the national measurement standards and publishing best measurement capabilities;
- providing calibrations and verifications;
- supervising, supporting national calibration and verification services;
- representing the country in relation to NMIs of other countries and in matters of metrology with regional and international organizations;

- assuring traceability for the national system through cooperation in the international system;
- advising the Government on metrology issues;
- offering technical support to industry, regulators and others;
- checking types of measuring instruments for their use in regulated areas;
- carrying out research in metrology.

NMIs are usually public institutes financed by the Government. Their installation and operation require high financial resources and highly qualified personnel. It is therefore necessary to design the NMI in a way that reflects the demand of the country. The national standards should realize the units with the highest accuracy in the country in order to serve its calibration need. If the demand is low traceability could be provided through a foreign NMI for economic reasons. If specialized institutes exist they can be integrated into the national metrology system through official appointment to act as Designated Institutes. They assume typical tasks of an NMI in their special subject fields. The advantage of such an approach lies in existing resources being used to enlarge metrological capabilities and to avoid duplications. As the importance of metrology in non-traditional areas such as chemistry, medical and food laboratories is increasing, the number of Designated Institutes is growing.

A fairly new task of NMIs is the publication of their measurement and calibration capabilities. This information is not only of interest to their clients in the country but it is also of importance at the regional and international level (see Section 6.7.1.). The reason lies in the growing demand for conformity assessment in international trade. These assessments are widely based on tests and measurements and the acceptance of certificates depends on the trust in test and measurement results. This trust can be enhanced by demonstrating the competence in measurements and calibrations through accreditation. Accreditation is the confirmation by a third party that a laboratory is competent to carry out its tasks according to specified standards (see Figure 1 above). Part of the evaluation procedure verifies measurement and calibration capabilities through participation in comparison measurements. NMIs and Designated Institutes have the possibility to participate in comparison measurements and proficiency tests organized at regional and international level to demonstrate their competence.

7.4.2. Calibration laboratories

Usually, the NMI provides calibrations at the highest level in the country. This high level is neither needed nor advisable for most measurements. Therefore, specialized laboratories provide calibrations as a private business. In some countries these laboratories are organized and form a national calibration service. It is expected that calibration laboratories operate according to rules and requirements as fixed, for example, by the international standard ISO/IEC 17025.[136] There, management and technical requirements are specified which have to be fulfilled by calibration

136 *Le système international d'unités* (The International System of Units), ed. 1998, Bureau international des poids et mesures (BIPM).

laboratories to prove their competence. The accreditation of a laboratory by a national accreditation body is an official confirmation of its competence within the scope covered by the accreditation. Reassessments and inter-comparison measurements ensure that the competence is maintained. Otherwise the accreditation has to be modified or withdrawn. This procedure enhances the trust in calibration certificates.

In some countries laboratories need an official permission or approval to carry out tests or calibrations. This is also sometimes called accreditation without necessarily applying international standards as prerequisite.

7.4.3. Legal metrology

According to the definition of the International Organization of Legal Metrology, legal metrology is the *"part of metrology relating to activities which result from statutory requirements and concern measurement, units of measurement, measuring instruments and methods of measurement and which are performed by competent bodies".*

The main objective is to ensure correct measurements in areas of public interest and to provide traceability through traceable calibrated measurement standards for verifications of measuring instruments under legal control. One of these areas is trade, traditionally the starting point of legal metrology and regulated through laws often called 'Weights and Measures Act'.

Legislation may also cover areas like health, environment or safety. The scope of legal metrology may differ from country to country.

Tools used to ensure correct measurements usually consist of:

a. type tests of measuring instruments to check whether the design of the instrument ensures correct measurements and corresponds to the legal requirements;

b. type approval which is the official permission to use instruments of the tested type for legal metrology purposes;

c. initial and periodic verifications to check whether tolerable error limits and obligatory features are respected;

d. stamping and sealing of the instruments to prevent and detect manipulations.

The competent bodies responsible for legal metrology activities are usually called legal metrology services.

The preventive system described above has more and more been replaced by a repressive system in Europe shifting the responsibility from governmental authorities to manufacturers and users of measuring instruments. The observation of the legal regulations is checked by inspectors through surveillance and on spot visits.

7.5. MEASUREMENT ACCURACY, MEASUREMENT ERROR, MEASUREMENT UNCERTAINTY

A measurement can be characterized in different ways depending on the information of interest. The VIM defines more than 20 terms which describe specific aspects of measurements. Only three of them will be described here in more detail because of their general relevance.

7.5.1. Measurement accuracy

The closeness of agreement between a measured quantitative value and a true value of a measurand is called accuracy. Measurement accuracy is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error.

Accuracy classes of measuring instruments are used for their easy classification. Accuracy of a measuring instrument describes its ability to provide results close to a 'true' value represented by a standard. The accuracy may be expressed in percentage (%) of the measuring range or a fraction of a certain value. A voltmeter of class 1 means that the error of the indication of the instrument should not be larger than 1 % of the upper limit of the measuring range. If the measuring range is 0 to 100 V, one has to anticipate an error of 1 V for any measurement in the given measuring range. Measurements at the lower end of the measuring range will yield higher relative inaccuracies. For example the accuracy of a measurement of 5 V may be 20 % equal to 1 V. The user has to decide if this is sufficient for the application in question. If not, another measuring instrument or another measuring range has to be used.

Other definitions of accuracy classes exist for weighing instruments (class I for the highest, class IIII for the lowest accuracy) and weights (E1 for the highest, M3 for the lowest accuracies). Details are given in OIML Recommendations Nos. R 76-1[137] and R 111[138] respectively.

7.5.2. Measurement error

The measured quantity value minus a reference quantity value is called measurement error.

The concept of 'measurement error' is used when there is a single reference quantity value to refer to. This occurs if a calibration is made by means of a measurement standard with a measured quantity value having a negligible measurement uncertainty or if a conventional quantity value is given, in which case the measurement error is known.

The concept of 'permissible errors' is applied in legal metrology and other applications if it is sufficient to decide whether the measurement is within specified limits (permissible errors). The calculation of a measurement uncertainty is not required. Legal metrology inspectors use working standards with higher accuracy than the instruments to be verified. For instance, a measuring instrument (e.g., a manometer) of accuracy class 1, meaning the error is 1 % of the highest indication of the measuring instrument can be checked by an instrument of class 0.1 of the same measuring range to decide whether the permissible error of 1 % is respected or not. If the measuring instrument respects the permissible error limits it will be marked and/or certified as verified.

137 OIML R-76-1 Non-automatic weighing instruments. Part 1: Metrological and technical requirements – Tests, www.oiml.org/en/files/pdf_r/r076-1-e06.pdf.

138 OIML R 111-1 Weights of classes E1, E2, F1, F2, M1, M1-2, M2, M2-3 and M3. Part 1: Metrological and technical requirements, www.oiml.org/en/files/pdf_r/r111-1-e04.pdf.

Similar procedures are applied in other areas to check whether permissible or tolerable limits are respected, for example for checking the results of proficiency tests.

7.5.3. Measurement uncertainty

To weigh a sample and express the result as

$$m = 1.043 \text{ kg}$$

is sufficient for daily life purposes. However, it would not by sufficient if the same sample has to be measured at different places with different weighing instruments and when the results are used to determine whether they are acceptable within defined limits. These decisions are frequently required when regulations specify permissible limits which are checked independently by the producer and his customers. There are examples where some customers rejected a shipment which other customers accepted due to different measurement results. The decision on right or wrong can only be taken if sufficient information about the 'quality', the uncertainty, of the measurement is known.

The measurement uncertainty is *"a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used".* The estimation of the uncertainty of a measurement is therefore of high importance. The problem of how to determine and express uncertainty has been discussed extensively. In the end the concerned international organizations elaborated and published the *Guide to the expression of uncertainty in measurement, the GUM,*[139] which is now widely applied and accepted, for example by accreditation bodies. Every calibration certificate must contain information about the uncertainty as part of the result of the calibration. The following is an attempt to give a rough idea about the GUM's uncertainty concept.

Mathematically, the measurement quantity X is considered as a stochastic variable with a probability distribution. The result x of a measurement is an estimate of the expectation value E(X) with a standard uncertainty u(x) equal to the square root of the estimate of the variance V(X). In this example, expectation and variance can be obtained from repeated weighing by statistical processing. The expectation value is calculated as the mean value of n repeated measurements:

139 Guide to the Expression of Uncertainties in Measurement, GUM, available from: International Organization for Standardization, 1, rue de Varembé, CP 131, CH 1211 Geneva 20, Switzerland, www.iso.org/sites/JCGM/GUM-introduction.htm.

The standard uncertainty u is given by the positive square root of the experimental variance:

If the expectation value can be obtained by statistical processing of a series of measurements, the evaluation is of **Type A** according to the uncertainty concept of the GUM.

For **Type B**, expectation and variance are estimated by other methods. Since the probability distribution cannot be obtained by statistical processing of repeated measurements one has to select probability distributions based on other information. For example, even if repeated indications of a digital weighing instrument were all identical, the uncertainty of the measurement would not be zero. The reason for this lies in there being a range of input signals that give the same indication. If the smallest digit is 1 mg, the indication x will be the same in the range (x- ½) mg < x < (x+ ½) mg because of the resolution limits of the device that produces the digital indication. In this range the probability will be the same for all indications which can be described by a rectangular probability distribution with the variance:

$u^2 = (1/2 + 1/2)^2/12$, implying a standard uncertainty of $= 0.29$ mg.

In many cases a measurand is not measured directly but is determined from other quantities. For example, the electrical power P can be measured by the potential difference V at the terminals of a temperature-dependent resistor that has a resistance R0 at temperature t0 and a linear dependent temperature coefficient of resistance α. For the estimation of the uncertainty the temperature dependence of the resistor has to be taken into account:

$$P = V^2/R0\ [1+ \alpha\ (t - t0)]$$

where, t is the temperature of the resistor produced by the dissipation of power. The contributions of the quantities V, R0, α and t to the uncertainty have to be estimated and combined according to the law of propagation to obtain the combined standard uncertainty.

The uncertainty quoted in a measurement result is usually given as an expanded uncertainty, calculated by multiplying the combined standard uncertainty by a numerical coverage factor k determining an interval of confidence. The intended purpose is to provide an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand. For k = 2 this fraction is approximately 95 % and for k = 3 approximately 99 %. The coverage factor used is typically between 2 and 3.

The estimation of uncertainty may require information:

- associated with authoritative published quantity values;
- associated with the quantity value of a certified reference material;
- obtained from a calibration certificate;
- about drift;
- obtained from the accuracy class of a verified measuring instrument;
- obtained from limits deduced through personal experience;

All of which requires a profound knowledge of the measurement and statistics.

The estimation of the uncertainty requires the following steps:

- identification of all important components which contribute to the uncertainty (including components for which only best estimates are available);
- calculation of the standard uncertainty of each component of measurement uncertainty using either Type A or Type B evaluation;
- calculating the combined uncertainty by combining the individual uncertainties according to the law of propagation. This implies that for a sum or difference of components the combined uncertainty is the square root of the sum of the squared standard uncertainties of the components. For a product or quotient of components, the same "sum/difference" rule applies for the relative standard uncertainties of the components;
- calculating the expanded uncertainty by multiplying the combined uncertainty with the coverage factor k;
- expressing the measurement result in the form: $X = x \pm U$.

The uncertainty U should be given with no more than two significant digits and x should be rounded to the same number of digits.

Note

The estimation of uncertainty is a must for calibration professionals, for example responsible staff members of calibration and testing laboratories. It requires training and experience. Besides the GUM, which describes the statistical tools in detail and presents many examples, the EURACHEM/CITAC Guide will be of special interest for laboratories working in the field of food safety. More sources can be found in the Internet.

For internal checks of measuring devices used in daily routine work, methods are applied which are much simpler and less time-consuming. The aim is to check whether the equipment delivers results which are acceptable according to specified permissible limits. The method used should be documented in the standard operating procedure of the equipment concerned. It should follow either a published standard or the instructions of the manufacturer. Otherwise, the method has to be validated, *i.e.* it has to be demonstrated that the specified requirements are adequate for the intended use.

7.6. WHY AND HOW TO ENSURE CORRECT MEASUREMENTS?

Measuring equipment used to check compliance with regulations, standards or specified permissible limits shall be calibrated or verified. This requirement should already be taken into account for the procurement of equipment as well as other performance requirements specified in standards, regulations, international recommendations and Directives of the European Union. A careful selection of the equipment is a precondition for obtaining satisfactory results.

For an in-house measurement management system it is advisable to identify all instruments to:

- establish and maintain calibration/verification procedures;
- maintain calibration/verification records; and
- label calibrated or verified instruments.

The ISO 10012:2003 Standard on measurement management system – Requirements for measurement processes and measuring equipment[140] – provides a good guidance and further sources of information in the bibliography. General requirements are given in the international standard ISO 9001:2000, Quality Management Systems – Requirements[141] – under clause 7.6, which corresponds

140 ISO 10012:2003 Standard on measurement management system – Requirements for measurement processes and measuring equipment. International Organization for Standardization, 1, rue de Varembé, CP 131, CH 1211 Geneva 20, Switzerland.

141 ISO 9001:2000, Quality Management Systems – Requirements, International Organization for Standardization, 1, rue de Varembé, CP 131, CH 1211 Geneva 20, Switzerland.

to clause 8.3 of ISO 22000:2004 Food Safety Management Systems – Requirements for any Organization in the Food Chain:[142]

"7.6 Control of monitoring and measuring devices

The organization shall determine the monitoring and measurement to be undertaken and the monitoring and measuring devices needed to provide evidence of conformity of product to determined requirements".

Starting from the product or service the parameters to be measured and monitored must be identified. This might start with the incoming inspection of materials, samples, parts or components for which a measurement may be necessary.

Essential measurements during the working process are those necessary to ensure compliance with specifications. They have to be identified along with the measuring equipment and are subject to procedures of the above-mentioned standard. The measuring instruments used should have accuracy ten times or at least three times better than the tolerances or permissible limits to be checked.

"Where necessary to ensure valid results, measuring and test equipment shall:

a. *Be calibrated or verified at specific intervals, or prior to use, against measurement standards traceable to international or national measurement standards; where no such standards exist, the basis used for calibration or verification shall be recorded;*
b. *Be adjusted or re-adjusted as necessary;*
c. *Be identified to enable the calibration status to be determined;*
d. *Be safeguarded from adjustments that would invalidate the measuring results;*
e. *Be protected from damage or deterioration during handling, maintenance and storage."*

These requirements outline the responsibilities of the organization to ensure that its measuring equipment delivers valid results. If the organization does not have measuring standards for the calibration of measuring equipment it has to use the services of a calibration laboratory also for the re-calibration of its measuring standards.

Figure 3 outlines key factors to consider in choosing the right calibration laboratory. In case of chemical measurements the term 'quantity' should be replaced by 'analyte' (in specified matrices(s). Furthermore, traceability may not be possible to the SI but to certified or acknowledged reference materials.

142 ISO 22000: 2004 Food Safety Management Systems – Requirements for any Organization in the Food Chain. International Organization for Standardization, 1, rue de Varembé, CP 131, CH 1211 Geneva 20, Switzerland.

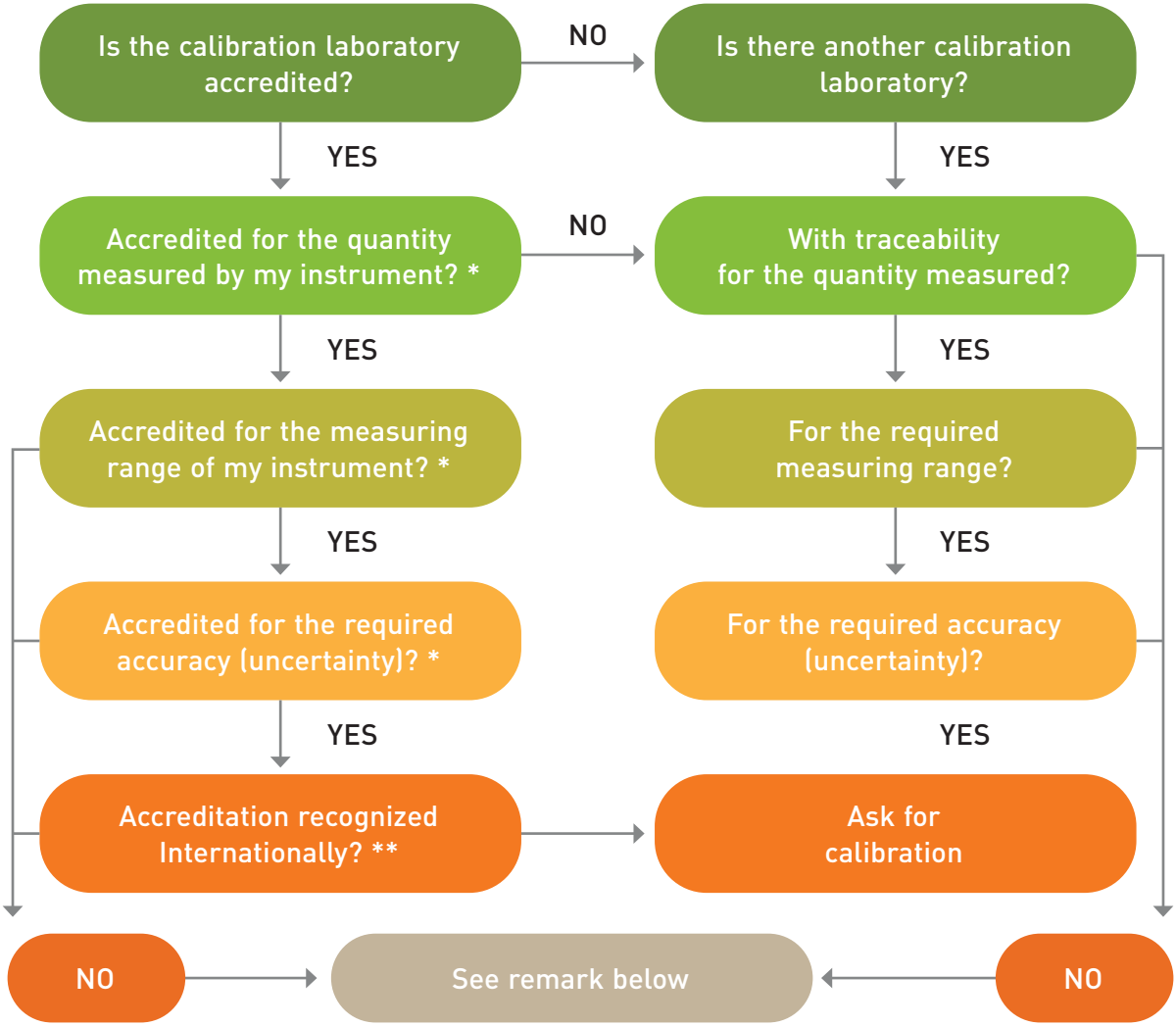


Figure 3 - Key factors to consider in choosing a calibration laboratory
** Laboratories are accredited for specified quantities, measuring ranges and uncertainties. All three elements should satisfy your requirements. The accreditation certificate should be checked to verify these three elements and to establish to which national measurement standard (certified reference material) the laboratory is traceable. The results and the scope of calibration (measuring range, uncertainty) must be mentioned in the calibration certificate.*
** If an internationally acceptable calibration certificate is required, a laboratory accredited by an accreditation body that has signed the ILAC Mutual Recognition Arrangement has to be chosen. This arrangement of the International Laboratory Accreditation Cooperation, also called ILAC Arrangement, entered into force on 31 January 2001.[143] Even if there is no such requirement, *laboratories accredited by an accreditation body signatory to the ILAC Arrangement should be preferred.*

Note

If no calibration laboratory can satisfy all requirements, the one fulfilling most requirements should be chosen.

143 International Laboratory Accreditation Cooperation, called ILAC Arrangement, entered into force on January 31, 2001, https://www.ilac.org/ilacarrangement.html. Le lien ne fonctionne pas

7.6.1. Calibration intervals

Calibration Intervals are usually recommended by the manufacturer of the measuring or test equipment, and should be observed. However, the performance of the instrument depends on its treatment and its use. In some situations, immediate recalibration is required, for instance when the measuring result obtained is doubtful or unexpected. Re-calibration is certainly necessary after overloading, improper electrical supply or other instances of mishandling. The user of the instrument is responsible for requesting recalibration in such cases. Waiting until the date of a recommended re-calibration would imply the risk of incorrect measurements. The calibration results should be used to build-up a history of the instrument and its long-time performance. The history will reveal information about drifts which can be used to adjust calibration in.

7.6.2. Internal quality control

Internal quality control is good laboratory practice, especially in analytical chemical laboratories. It requires a continuous, critical evaluation of the laboratory's own analytical methods and working routines. The control encompasses the analytical process starting with the sample entering the laboratory and ending with the analytical report. An important tool of the internal quality control is the use of control charts.

The basis of the internal quality programme is that the laboratory runs control samples together with the routine samples and documents the results. Control samples of known values should be used and selected specifically to check the upper and lower measuring range for the analyte in question. The results allow to check the daily work and provide evidence about the laboratory's quality also for the customer.

7.6.3. External quality control

External quality control is exercised to demonstrate the capabilities of a laboratory and to compare the results of the participating laboratories. To reach this objective a reference laboratory provides test samples which have to be analysed by the participating laboratories. If available, certified reference materials should be used to ensure traceability. The results are evaluated by the reference laboratory and objectively compared with other laboratories using the same methodologies for every parameter. The results are forwarded to the participating laboratories with a statement whether permissible limits have been met or not. In case of failure the concerned laboratory should take corrective actions. External Quality Assessment programmes are accepted around the world as invaluable tools to assess the performance of laboratories. Provider of test samples should be independent and not belong to companies with activities related to the analytical equipment to be checked. EQA programmes have created awareness and improvements among participants and proved to be a necessary exercise to check the competence of laboratories and to ensure compatibility of results of participating laboratories.

7.7. INTERNATIONAL AND REGIONAL ORGANISATIONS

In order to coordinate measurement activities at the international several institutions and organisations have been set up.

7.7.1. The Meter Convention (*La Convention du mètre*) and the BIPM

The Meter Convention is a diplomatic treaty signed in Paris in 1875 by representatives of seventeen governments to coordinate international metrology and the development of the metric system (now SI). At the current state (October 2012), 56 nations are members of the Convention. The Meter Convention established three main organs to develop and coordinate the international measurement system (please revert to Figure 4 for an illustration of the organizational structure of the Meter Convention's):

- The General Conference on Weights and Measures (*Conférence générale des poids et mesures* or CGPM) – the principal decision making organ of the Metre Convention meeting every four to six years;
- The International Committee for Weights and Measures (*Comité international des poids et mesures* or CIPM) – a technical advisory body to the CGPM consisting of eighteen prominent metrologists;
- The International Bureau of Weights and Measures (*Bureau international des poids et mesures* or BIPM) –the premises which host the laboratories, the secretariat and provides metrology services to the CGPM and the CIPM.

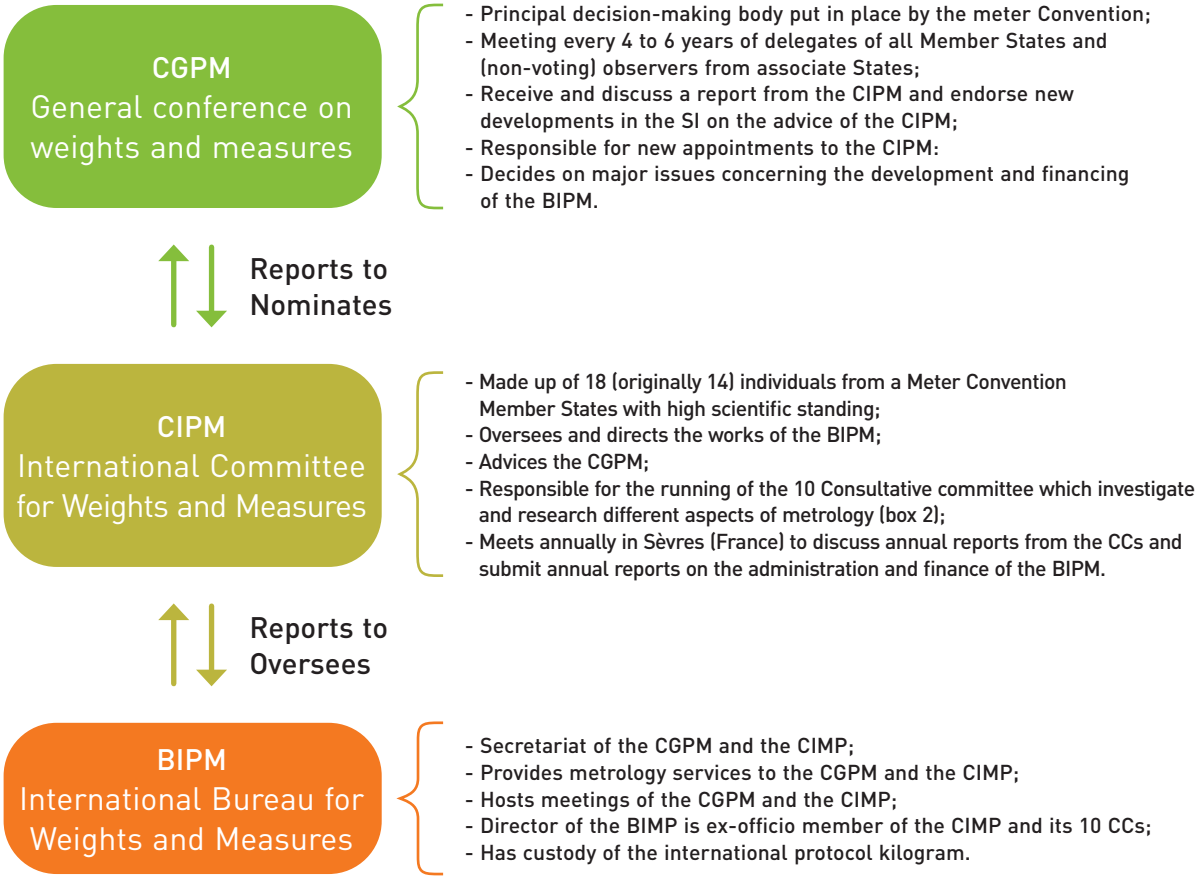


Figure 4 - Organisation structure of the Meter Convention

As Figure 4 above shows, the BIPM operates under the supervision of the International Committee of Weights and Measures, or CIPM, which itself answers to the General Conference of Weights and Measures, or CGPM.

The CGPM is the highest and principal decision making organ of the Meter Convention. It elects the 18 members of the CIPM and decides on the major issues with regards to the financing and administration of the BIPM. It brings together the representatives of all Metre Convention members once every four to six years to discuss the work accomplished based on CIPM reports, and examines, discusses and adopts resolutions of international scope.

The CIPM acts as a technical advisory committee to the CGPM. It oversees and directs the work of the BIPM and prepares proposals for the CGPM. The CIPM has created a set of Consultative Committees, at present ten bringing together the world´s experts in specified metrology fields. The first CC to be established was on electricity (CCE) in 1927, the most recent one on amount of substance (CCQM) established in 1993. This CC is of special interest for food safety because it deals with measurements and primary measurement methods for chemical analysis which are also applied to check the chemical composition of food and to detect unwanted substances such as pesticides. Box below lists all CCs of the CIPM.

CIPM CONSULTATIVE COMMITTEES (CCS)

CCAUV: Consultative Committee for Acoustics, Ultrasound and Vibration

CCEM: Consultative Committee for Electricity and Magnetism

CCL: Consultative Committee for Length

CCM: Consultative Committee for Mass and Related Quantities

CCPR: Consultative Committee for Photometry and Radiometry

CCQM: Consultative Committee for Amount of Substance – Metrology in Chemistry

CCRI: Consultative Committee for Ionizing Radiation

CCT: Consultative Committee for Thermometry

CCTF: Consultative Committee for Time and Frequency

CCU: Consultative Committee for Units

The BIPM is based in Sèvres, near Paris in France. It hosts the secretariats of the CGPM and the CIPM and also hosts the meetings of these two organizations. It also provides metrology services as described below.

The BIPM has an international staff of about 70 and a budget financed by the contributions of its member states. The scientific work of the BIPM centres on the following main topics:

- mass;
- time;
- electricity;
- ionizing radiation; and
- chemistry.

In each of these areas activities concentrate on:

- establishment and maintenance of reference standards having the best possible long-term stability, *i.e.* standards that change the reference value they realize as little as possible over time;
- organization of and participation in international comparisons (see Figure 5 and explanations given there) and carrying out calibrations;
- improving of reference standards, comparisons and measurement techniques.

These activities are essential in order to develop and maintain the best international reference standards, to use them for comparison measurements with national standards maintained by NMIs and to provide the best measurement techniques. This work requires also a close cooperation with NMIs which are active in the same field and in fields not directly covered by laboratory work of the BIPM such as length, force or acoustics. The reason for this is that sufficient NMIs can provide standards for the quantities in question and the international reference value is then fixed through CIPM key comparison measurements (see Section 7.1.2. and figure 5). Additional facilities at the BIPM would be too costly.

There are some Joint Committees of the BIPM with other international organizations created for particular tasks as described below:

- JCGM – Joint Committee for Guides in Metrology: examples for the work of the JCGM are the "Guide to the expression of uncertainty in measurement" (GUM) elaborated in cooperation with ISO, IEC, IFCC, IUPAC, IUPAP; OIML) and the "International vocabulary of basic and general terms in metrology" (VIM) elaborated in cooperation with IEC, IFCC, ISO, IUPAC, IUPAP and OIML;
- JCTLM – Joint Committee on Traceability in Laboratory Medicine: This Committee works on the establishment of international references of importance in Laboratory Medicine, together with the International Federation of Clinical Chemistry, IFCC.

7.7.1.1. Joining the Meter Convention

Countries can participate in the Meter Convention in two forms:

- full participation, in which case the respective country would be referred to as a Member State;
- associate participation, in which case the respective country would be referred to as an Associate.

In order to become a Member State, the government of the country needs to fulfil the following criteria:

- having diplomatic relations with France (the depository of the Treaty) and willing to pay their annual contribution to the BIPM;
- contact the Director of the BIPM (BIPM, Pavillon de Breteuil and F-92312 Sèvres Cedex. Fax: +33 1 45 34 86 70);
- inform the French Foreign Minister of its intent by letter delivered through its embassy in Paris;
- payment of the first annual contribution plus an entry fee equal to the first annual contribution is made directly to the BIPM.

In 1999 the CGPM created the category of Associate for those countries not in a position to become members (for example because their measurement capabilities are not advanced enough). In order to become an associate, a country needs to fulfil the following criteria:

- contact is first made with the Director of the BIPM (BIPM, Pavillon de Breteuil, and F-92312 Sèvres Cedex. Fax: +33 1 45 34 86 70);
- an application expressing the wish of the State or Economy to become an Associate of the CGPM is sent to the Director of the BIPM by the State's government (*i.e.* a ministry in charge of relations with intergovernmental organizations) or through its Embassy in Paris – or, for an Economy, by its official representative;
- the first payment of an annual subscription is made to the BIPM.

One of the principal purposes for a state to become an associate of the Meter Convention is to enable it to participate in the CIPM MRA, which is described below.

7.7.1.2. CIPM mutual recognition agreement

In order to facilitate the recognition of calibration results and to enhance the confidence in calibration certificates issued by NMIs at international level, the so-called CIPM Mutual Recognition Arrangement has been established in 1999 as an addition to the Meter Convention. It pursues the following objectives:

- to establish the degree of equivalence of national measurement standards maintained by NMIs;
- to provide for the mutual recognition of calibration and measurement certificates issued by NMIs; and
- thereby to provide governments and other parties with a secure technical foundation for wider agreements related to international trade, commerce and regulatory affairs which rely on traceable measurements.

In order to be able to join the CIPM MRA, an NMI has to demonstrate its capabilities in the following fields:

- calibration and measurement capabilities: a critical review is carried out by senior experts (a so called peer review) of the declared calibration and measurement capabilities to check whether they are realistic and trustworthy. These experts are composed of BIPM staff and members of NMIs of the relevant Regional Metrology Organisation. The CMC for a physical quantity in a specified measuring range is that with the smallest uncertainty realized by the NMI or DI in question;
- international comparison measurements: results of successful participation in international comparisons of measurement standards must be available, details and explanations are given in Figure 5;
- operation of a quality management system: a peer review of the quality system is carried out to check the management of the laboratory, the qualification of staff, the laboratory environment, and so on.

The CIPM MRA can be signed by Directors of NMIs of members and of Associate Members if the above mentioned conditions are fulfilled. According to the CIPM MRA, signature also requires the prior approval of the same body that has the authority to approve the application to become an Associate.

Although only one NMI per country can sign the CIPM MRA, so called Designated Institutes, DIs, that hold recognized national standards in the country may also participate in the CIPM MRA and are subject to processes mentioned above with regard to the national standards they maintain.

The implementation of the CIPM MRA is supported by Regional Metrology Organizations, RMOs (see Section 7.3) and the Joint Committee of RMOs and the BIPM, JCRB. The terms of reference of the JCRB are defined in Appendix E of the CIPM MRA, which charges the Joint Committee with:

a. coordinating the activities among the RMOs in establishing confidence for the recognition of calibration and measurement certificates, according to the terms of the CIPM Mutual Recognition Arrangement;
b. making policy suggestions to the RMOs and to the CIPM on the operation of the CIPM MRA;

c. analysing the application by each RMO of the criteria of the CIPM MRA;

d. analysing and entering into Appendix C the proposals of each RMO in respect of the calibration and measurement capabilities of their member NMIs and reporting to the CIPM;

e. facilitating appropriate inter-regional supplementary comparisons;

f. writing an annual report on the activities of the Joint Committee to the CIPM and to the signatories of the CIPM MRA.

More details can be obtained on the BIPM Website under:
www.bipm.org/utils/en/pdf/jcrb_contact_details.pdf.

The efforts necessary to create and demonstrate the required competence in measurement and to ensure traceability through participation in comparison measurements is shown in Figure 5 below.

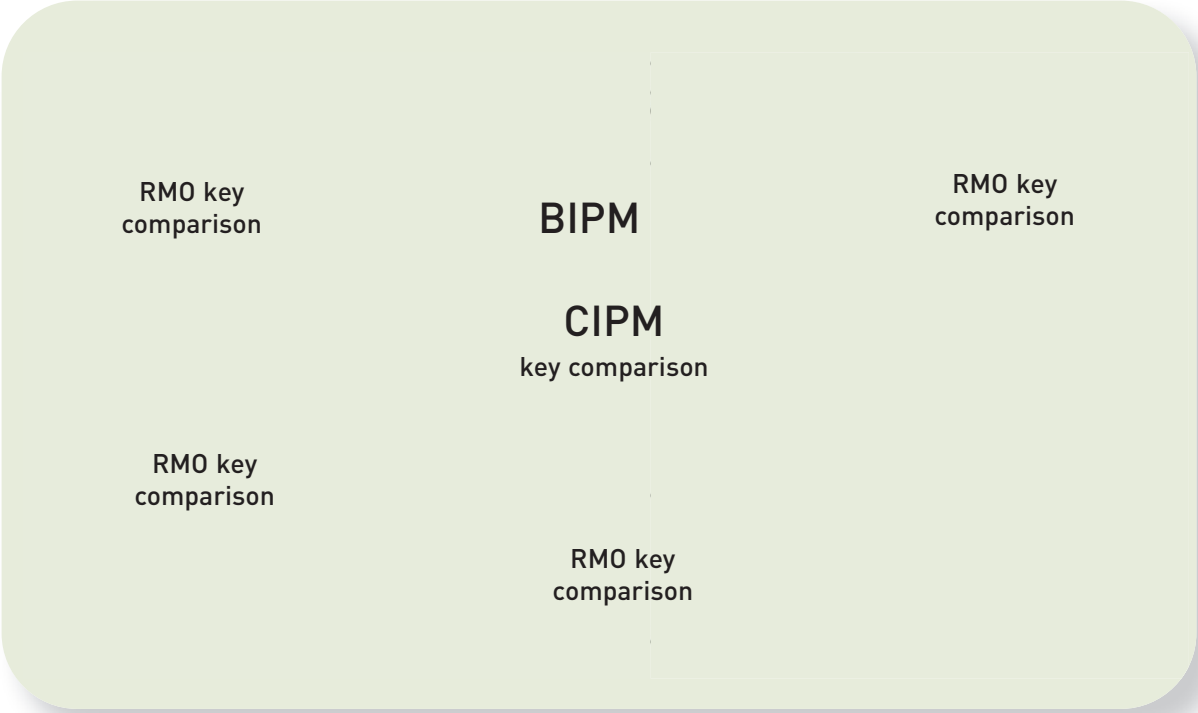


Figure 5 - Key comparisons scheme

	National metrology institute (NMI) participating in CIPM key comparisons
	NMI participating in CIPM key comparisons and in regional metrology organization (RMO) key comparisons
	NMI participating in RMO key comparisons
	NMI participating in ongoing BIPM key comparisons
	NMI participating in a bilateral key comparison
	International organization signatory to the MRA

The CIPM delegates the task of organizing comparisons within the CIPM MRA to its Consultative Committees. Each CC chooses the key quantities (for example 'cadmium and lead in natural water' chosen by the CCQM or 'diameter' chosen by the CCL), provides the samples to be measured by the participants (for example a certified reference material in the case of cadmium and lead in natural water or an artefact such as a proofing ring in the case of diameter), defines the measurement methods, collects, evaluates and approves the results before publication in the Key Comparison Data Base. Each CC is composed of the world's most competent laboratories and some or all CC members of the CC in question participate in the key comparison, which is then generally referred to as a 'CCxx key comparison' to specify the field xx of expertise (in the examples above CCQM respectively CCL).

The BIPM itself carries out comparisons in the field of mass for example because it maintains the international Kilogram prototype. These are usually bilateral comparisons with the national Kilogram standards of NMIs. Such BIPM key comparisons and the CC key comparisons form the CIPM key comparisons.

Key comparisons are essentially of two types:

- CIPM key comparisons, of international scope, are carried out by those participants having the highest level of skills (reaching the lowest uncertainties) in the measurement involved, and are restricted to laboratories of member states of the Meter Convention. The CIPM key comparisons deliver "the reference value", the best realization with the lowest uncertainty for the chosen key quantity;
- RMO key comparisons, of regional scope, are organized at the scale of a region (though they may include additional participants from other regions) and are open to laboratories of associates as well as member states of the Meter Convention. These key comparisons deliver results of participants that are not qualified for CIPM key comparisons or could not participate in CIPM key comparisons for other reasons. But there are always some NMIs that have also participated in CIPM key comparisons to provide the link to the reference value delivered through CIPM key comparisons. The RMO key comparisons deliver complementary information about the measurement capabilities of the region without changing the reference value. The RMOs collaborate closely with the BIPM with regard to RMO key comparisons and the review of CMCs from members of the region.

The NMI may be substituted by the competent DI if a quantity is concerned for which the DI maintains the national standard.

The text of the CIPM MRA is supplemented by the following appendices:

- Appendix A: List of participating NMIs and DIs (from 51 member states and 36 associates) and of 4 international organizations: International Atomic Energy Agency (IAEA), Vienna; Institute for Reference Materials and Measurements (IRMM), Geel; World Meteorological Organization (WMO), Geneva and the European Space Agency (ESA), Paris;
- Appendix B: Results of key comparisons (about 1 000);
- Appendix C: Calibration and measurement capabilities, CMCs, of NMIs and DIs (more than 20 000);
- Appendix D: List of key comparisons.

This information is available to the public on the BIPM Website: www.bipm.org/en/cipm-mra.

All registered and published CMCs (Appendix C) have undergone an evaluation by NMI experts (peers) under the supervision of the regional metrology organization and coordinated internationally by the JCRB.

7.7.1.3. Activities related to food safety

Metrology in chemistry is of increasing importance for food safety and constitutes a new challenge for BIPM and CCQA activities. The problem is that chemical measurements are more complex and take place under conditions which very often cannot be controlled and differ in terms of composition. For instance, it makes a difference to determine cadmium in water or cadmium in meat. Often the primary objective of chemical metrology is to determine the amount of components of interest, for example the amount of cadmium in water, not the total composition of the sample. For the time being many chemical measurements are traceable to an agreed upon standard, a reference material or a reference method to ensure comparability of results. A direct link to the SI is however not always possible. The CCQM works on the following food topics:

- nutritional constituents;
- contaminants;
- genetically modified organisms (GMO); and
- other.

Furthermore the CCQM has carried out the following CCQM Key Comparisons to ensure comparable results in the field of:

- pesticide residues;
- antibiotics in meat;
- growth hormones in meat;
- vitamins and minerals;
- drinking water;
- GMO.

These examples show that the scope of work follows the demands of science, governments, and all in need of reliable and trustworthy measurements.

7.7.2. The International Organisation of Legal Metrology – OIML

Since the Meter Convention and their working organs focus on measurement units and standards, the need for an international organization working on requirements for measuring instruments was felt already at the beginning of the 20th century. But it took many years before the International Organization of Legal Metrology, OIML as an intergovernmental treaty organization could be established in 1955. The objective of OIML is to promote the global harmonization of legal requirements for measuring instruments and measuring procedures. To reach this objective

the OIML works closely with numerous international and regional institutions having activities linked to metrology, standardization and related fields. The work of the OIML aims at promoting credible measurement practices.

The organizational structure is as follows:

- The International Conference, or IC, which takes place every four years, is composed of OIML Member State delegations and observers from Corresponding Members and international and regional institutions in liaison. It defines the general policy and budgetary lines and sanctions the budged allocation and the International Recommendations as the highest organ of the OIML. The IC elects the CIML President and two Vice Presidents.
- As the steering committee of the OIML, the International Committee of Legal Metrology, or CIML, under the leadership of its President meets annually to review the Organization's technical progress and administrative operations. It prepares and implements the IC decisions, supervises the work of the Technical Committees and the International Bureau of Legal Metrology (BIML). The Committee is composed of one appointed representative from each OIML Member State.
- Drafts of International Recommendations are elaborated by Technical Committees and Subcommittees, the composition of which includes representatives from Member States, international standardization and technical organizations, manufacturers' associations and regional regulatory bodies. Under the coordination of a secretariat, experts establish drafts of International Recommendations and technical guidelines for measuring instruments and testing procedures for measuring instruments subject to legal controls.
- The Presidential Council (PC) acts as an advisory group for the CIML President. The PC is composed of the CIML President and the two Vice-Presidents, and a limited number of CIML Members, appointed by the CIML President. The PC generally meets twice a year on the request of the CIML President to discuss and advice on issues raised by the President.
- The International Bureau of Legal Metrology, or BIML, is based in Paris with a staff of 10 persons. It is the Secretariat and Headquarters of the OIML and as such undertakes the following activities:
  - coordination of the technical work undertaken by OIML Technical Committees and Subcommittees;
  - organization of OIML Conferences and CIML Meetings;
  - management of the finances of OIML Organization.

  Furthermore, the BIML implemented and maintains the OIML Certificate System and the Mutual Acceptance Arrangement. The BIML publishes OIML Recommendations, Documents, Vocabularies, Guides, Expert Reports and the Bulletin, and maintains the organization's Website (www.oiml.org). Liaisons are also maintained with International, Regional and National bodies in order to promote legal metrology globally.

Through its institutional structure, the OIML has developed a worldwide technical structure that provides its Members with metrological guidelines for the elaboration of national and regional requirements concerning the manufacture and use of measuring instruments for legal metrology applications. The OIML develops model regulations, International Recommendations, which provide Members with an internationally agreed-upon basis for the establishment of national legislation on various categories of measuring instruments. Given the increasing national implementation of OIML guidelines, more and more manufacturers are referring to OIML International Recommendations to ensure that their products meet international specifications for metrological performance and testing, the use of the SI System and the concept of traceability to the SI. OIML members are morally obliged to transpose International Recommendations into national regulations in case they want to regulate areas covered by International Recommendations.

The main elements of an International Recommendation are:

- scope, application and terminology;
- metrological requirements;
- technical requirements;
- methods and equipment for testing and verifying conformity to requirements;
- test report format.

7.7.2.1. OIML membership

Countries may participate in the OIML activities in two forms:

- Member States which participate actively in technical activities;
- Corresponding Members which join the OIML and its organs as observers.

In order to become an OIML member state the country has first to ratify the Convention (the international treaty establishing the OIML). Such ratification by the government of the country or economy in question is transmitted through diplomatic channels to the French Government (trustee of the OIML Convention) which then registers the accession and informs both other OIML Member States and the BIML once these formalities have been completed. The detailed procedure of accession, also as Corresponding Member, is available from the BIML, and the OIML Convention may be downloaded from the OIML Website.

The benefits of membership consist in the possibility to influence the work of the organization and to provide input for International Recommendations and other documents prepared by the organization. Corresponding Members have the possibility to follow-up the work but without active contributions and no right to vote.

7.7.2.2. The OIML mutual acceptance arrangement

In 1991, the OIML introduced a system that allows manufacturers to issue OIML Certificates of Conformity for types of measuring instruments after all the tests, evaluations and examinations specified in the relevant OIML Recommendation have been carried out and after compliance with the requirements of this Recommendation has been demonstrated. OIML Certificates are accompanied by an OIML Test

Report following the Test Report Format which is part of the relevant International Recommendation. Acceptance of these Certificates by national legal metrology services is voluntary. By using OIML Certificates a duplication of tests can be avoided.

To further increase confidence in OIML Certificates of Conformity, the OIML established the OIML Mutual Acceptance Arrangement. Under the MAA, which is a framework document, individual Declarations of Mutual Confidence (DoMCs) will be signed for categories of instruments for which the OIML Certificate of Conformity can be issued. By signing these DoMCs, participants (either issuing authorities making out the test reports or authorities accepting the test reports) will declare confidence in the test results issued by other participants. Participants will be of two kinds:

- those who issue Test Reports (they will provide evidence of competence, impartiality and quality);
- those who do not issue Test Reports but accept and make use of them.

Further information is provided under "Framework for a Mutual Acceptance Arrangement on OIML Type Evaluations", www.oiml.org/en/files/pdf_b/b010-amended-2012-e11.pdf.

The aim of the MAA is for its participants to accept and utilize MAA Evaluation Reports validated by an OIML MAA Certificate of Conformity. For manufacturers, it avoids duplications of type approvals in different countries. For legal metrology authorities it avoids the investment in test equipment and qualified staff for carrying out their own type tests.

7.7.3. Regional metrology organizations

In addition to the international organizations described in Sections 6.7.1. and 6.7.2. above, three types of regional metrology organizations exist which differ with regard to their member bodies:

a. National Metrology Institutes and Designated Institutes;

b. Legal Metrology Services;

c. Combination of a) and b).

7.7.3.1. RMOs with NMI membership

Type A RMOs are essential elements for the BIPM with regard to the evaluation of the CMCs of their member NMIs and DIs and the coordination and organization of regional comparison measurements in the frame of the CIPM MRA. In addition and depending on the specific needs their activities may include:

- cooperation in metrology research;
- facilitating traceability to primary realizations of the SI;
- cooperation in the development of the metrological infrastructure in member countries, organizing comparison measurements and providing traceable calibrations in the region;
- joint training, exchange of experience and consultation;

- cooperation with the BIPM and the JCRB;
- sharing of technical capabilities and facilities.

Type A RMOs

- **EURAMET e.V.**, EA with 37 Members or Associates of European NMIs and an Institute of the European Union, the Institute for Reference Materials and Measurements, IRMM (more details can be obtained under www.euramet.org);
- **Asia Pacific Metrology Programme**, APMP, with 23 Full Member and 6 Associate Member NMIs (more details can be obtained under www.apmpweb.org).

Le lien n

7.7.3.2. RMOs with legal metrology service membership

Type B RMOs cooperate typically in areas concerning:

- regulations of special interest for the region;
- training;
- exchange of experience;
- facilitation of intra-regional trade;
- sharing verification equipment and providing verifications across borders.

Type B RMOs

- **Asia Pacific Legal Metrology Forum**, APLMF, (more information can be obtained under www.aplmf.org);
- **European Cooperation in Legal Metrology**, WELMEC (more information can be obtained under www.welmec.org).

7.7.3.3. RMOs with both NMIs and legal metrology service membership

Type C RMOs combine functions of both Type A and Type B RMOs as described above.

Type C RMOs

- **Intra-Africa Metrology System,** Afrimets, with six sub-regional organizations (www.afrimets.org);
- **Euro-Asian Cooperation of National Metrological Institutions,** COOMET (www.coomet.org);
- ***Sistema Interamericano de Metrología*** (Inter American Metrology System), SIM, with five sub-regions (www.sim-metrologia.org.br).

Le lien ne fonctionne pas

7.7.4. Interaction with other international organisations

Metrology is of fundamental importance and impinges increasingly on every human activity. It is therefore obvious that cooperation with other relevant non-metrological organisations exists.

7.7.4.1. Scientific organisation

On the scientific level the International Union of Pure and Applied Physics, or IUPAP, and the International Union of Pure and Applied Chemistry, or IUPAC, cooperate with organs of the Meter Convention mainly in the field of nomenclature, symbols, units, measurement uncertainty, physical constants and reference materials.

7.7.4.2. Standardization organisations

Requirements concerning measuring instruments, their calibration, tolerable limits, units, traceability and so on are laid down in numerous standards and regulations. These requirements are part of the ISO families on Quality Management Systems (ISO 9000), on Environmental Management Systems (ISO 14000), and on Food Safety Management Systems (ISO 22000), just to mention some examples. The ISO/IEC Standard 17025 General Requirements for the Competence of Testing and Calibration Laboratories specifies the conditions under which such laboratories should operate.

7.7.4.3. Accreditation organisations

The ISO/IEC Standard 17025 General Requirements for the Competence of Testing and Calibration Laboratories is used by accreditation bodies as a basis for the evaluation of laboratories to check their competence if they ask for accreditation. Accreditation is third-party recognition of a laboratory's technical competence, quality system and impartiality.

The accreditation of calibration laboratories requires traceability of their measurement standards, the calculation of the measurement uncertainty and the operation of a quality management system. Accreditation is therefore a tool to implement the concepts of traceability and the calculation of measurement uncertainty with the aim to enhance the confidence in the competence of accredited calibration laboratories.

On the international level, the International Laboratory Accreditation Cooperation, ILAC, cooperates with various laboratory regional accreditation schemes. Through the evaluation of the participating bodies, the international acceptance of test and calibration data is enhanced. In 2003, the ILAC Mutual Recognition Arrangement, ILAC MRA, was established and signed by more than 60 members (for details see: http://www.ilac.org). This ILAC MRA is another example of how to make best use of services provided according to international requirements or standards without undue repetition of measurements and tests. Due to a close cooperation of the concerned international organizations (for example the Meter Convention, OIML, ISO, IEC, IFCC, ILAC) there are no contradictions in the concepts of the MRAs and the technical requirements.

7.8. CONCLUDING COMMENT

The concept of assuring uniform and reliable measurements through traceable calibrations and inter comparisons on the national, regional and international level is well established. It requires a close cooperation of the international, regional and national organizations concerned. New developments will change the daily life and will challenge the responsible organizations to react. What started in 1875 as an international activity with a few countries participating has grown in the meantime with regard to members and tasks. But there are still a lot of problems to be solved and a lot of counties need to be integrated into the international measurement system so that they can benefit from its achievements.

7.9. ANNEX

A.1. Further information

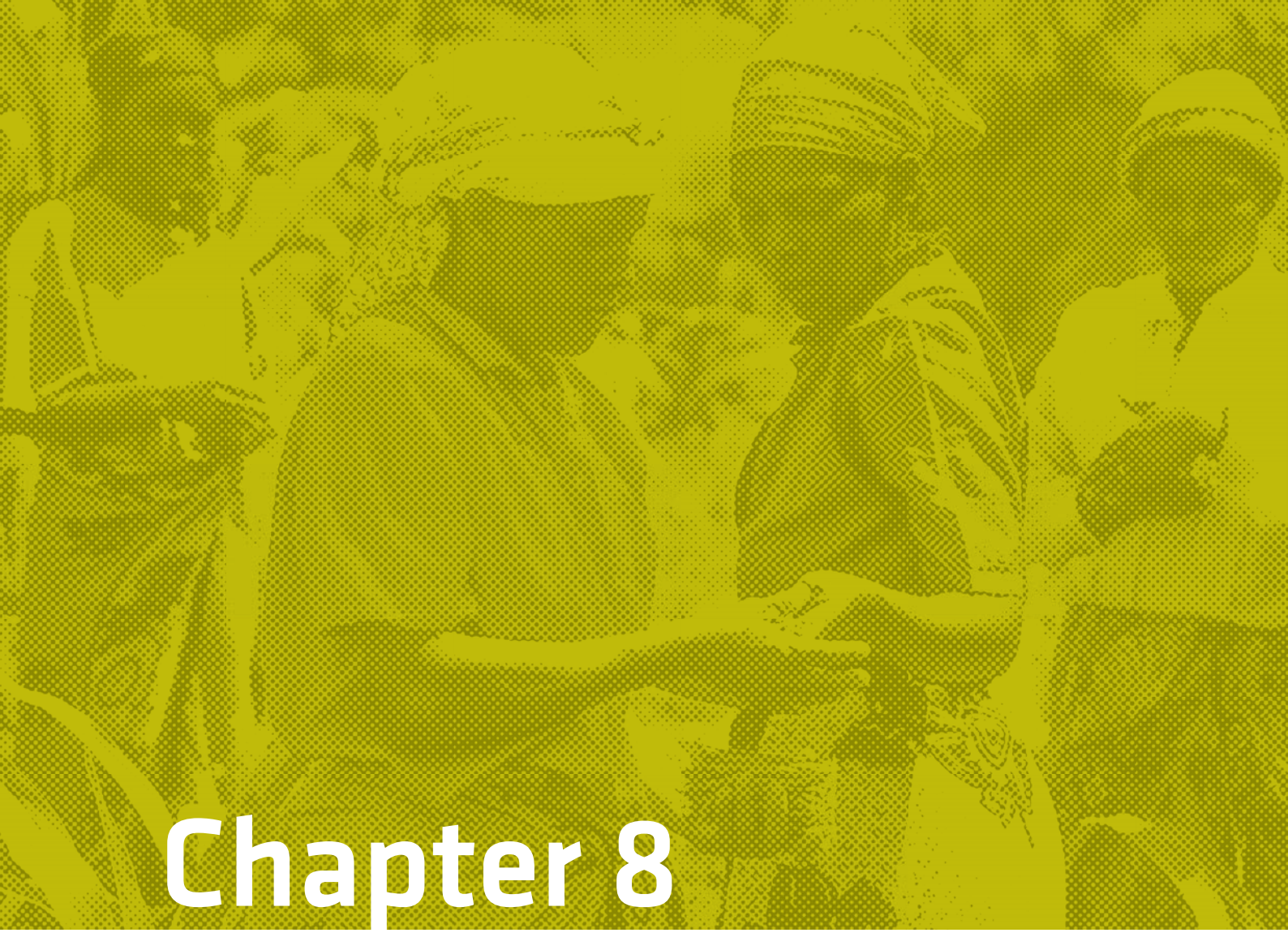
Besides the references given above here are some hints for further information:

- The International Trade Centre has published Guides specifically for Small and Medium-Sized Entrepreneurs from Developing Countries. Some of them focus on Quality and Export and on problems in relation to Standards, Metrology, Certification and Accreditation, which are also of interest in connection with topics of this chapter. These are in particular the following:
  - "Road Map for Quality, ITC Guidelines for the review of the standardization, quality management, accreditation and metrology (SQAM) infrastructure at national level", 2004;
  - "Export Quality Management, A Guide for Small and Medium-sized Exporters", 2nd ed, 2011;
  - "Influencing and Meeting International Standards, Challenges for Developing Countries", 2003.

All available from International Trade Centre UNCTAD/WTO, ITC Palais des Nations, CH 1211 Geneva 10, Switzerland, www.intracen.org.

- A comprehensive overview on metrology with a lot of examples is contained in Metrology – In Short, 3rd ed., 2008, prepared by Euramet and available from: www.euramet.org/index.php?id=objectives Le lien ne fonctionne pas

The Web sites of the organizations mentioned have been widely been for the compilation of this chapter.



Chapter 8

Method validation

8.1. GENERAL INTRODUCTION

8.1.1. Context

This chapter provides guidance on the validation of methods for chemical analysis and for microbiological examination of food and feedstuffs. It is aimed at helping laboratory analysts and managers in Competent Authorities to validate methods used for the official control of food and feedstuffs.

Laboratory analysis provides important information regarding the compliance of foods with SPS measures, and there are numerous reasons why foods are analysed.

For example, samples are **analysed for official purposes** to ensure compliance with maximum residue limits for **contaminants** or compositional requirements laid down in EU Regulations and Directives. Analyses are also carried out to ensure that **additives** used in food are approved for use in that type of food and that where limits are set for the amount of an additive that may be used, those limits are not exceeded. Analysis is also used to detect undeclared or unapproved additives or usage of additives. Another area where analysis can play a major role is in the detection of **ingredients of food** that are derived from **genetically modified raw materials**.

Microbiological examination of food is carried out to ensure compliance with microbiological criteria, such as those laid down in EU Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. However, it is not only in the official control of food that chemical analysis and microbiological examination are important. **Food manufacturers have a responsibility** to ensure that food sold to consumers complies with all legal requirements and is safe. They are also required to ensure that products are not labelled or presented in such a way as to mislead consumers. Again access to reliable and accurate analyses is essential.

Furthermore the price of some commodities such as oilseeds, cereals etc., is frequently related to parameters such as oil and protein content. Small differences in the measured oil or protein content can make a significant difference to the value of a particular consignment. For this reason methods used to determine these parameters, need to be both accurate and precise. The determination of parameters such as fat, protein, and moisture content are method dependent, so it is important that the different parties in any transaction agree on the method to be used.

In all these cases it is important that the laboratory performs its analysis in a way which gives results which are as accurate as possible and it is essential that anyone sending samples for analysis can trust the results. Therefore the laboratory analysis must also be seen to be valid and accurate, which gives rise to the need for audits and accreditation, and for data on validation of methods to be available for scrutiny.

8.1.2. Method validation principles

Method validation is the **process of confirming**, by assessing method performance criteria, **that the analytical procedure employed for a specific analysis is suitable for its intended use**. Analytical methods need to be validated or revalidated before their introduction into use, or whenever the conditions for which the methods have been validated change.

Accreditation of an analytical method requires that it is assessed as suitable in relation to the requirements for use of the method and the general requirements for validation are described in DS/EN/ISO/IEC/17025.[144]

General principles are also described in documents such as:

- The NMKL Procedure No.4 on the validation of chemical analytical methods.[145]
- IUPAC Technical report, 2002 (Harmonized guidelines for single-laboratory validation of methods of analysis.[146]
- Method validation and quality control procedures for pesticide residue analysis in food and feed (EC Doc. No. SANCO/12495/2011).[147]
- Commission decision 2002/657/EC "Implementing Council DIRECTIVE 96/23/EC Concerning the Performance of Analytical Methods and the interpretation of results".[148]

Parameters that are generally considered most important for validation of analytical methods are specificity, selectivity, precision, trueness, extraction recovery, calibration curve, linearity, range, detection limit, quantification limit and ruggedness.

The extent of validation should be related to the requirements and scope of the method and what is analytically possible. Prior to validation of a method a protocol is prepared in which the scope of the method and its validation criteria are defined. For example, if the method is to be used for qualitative trace residue analysis, there is no need to examine and validate its linearity over the full dynamic range of the equipment or if maximum residue limits are to be controlled with the method, limit of detection does not need to be validated. This protocol may contain items such as:

- relevant sample types;
- chemical structure of the analytes;
- legal limits, requirements for detection limits (specification of the applicants needs in connection with the testing);
- concentration levels (standard curve and lower and upper levels of tested concentrations);
- possible interferences;
- studies of the binding of the analytes to matrix;
- stability study of analytes;
- detailed description of experiments that will be conducted to perform validation.

144 DS/EN/ISO/IEC/17025, 2nd ed., General requirements for the competence of testing and calibration laboratories (2005).

145 NMKL Procedure No. 4. Validation of chemical methods (2009).

146 M. Thompson, S.L.R. Ellison and R. Wood, "Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report)", *Pure Appl. Chem.*, vol. 74, No. 5, 835-855 (2002).

147 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No. SANCO/12495/2011.

148 Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002).

The protocol could be succeeded by the following steps:

- verify relevant performance characteristics of equipment;
- check availability of qualifying materials, for example, standards and reagents;
- perform pre-validation experiments;
- adjust method parameters or/and acceptance criteria if necessary;
- perform full internal (and external) validation experiments;
- develop SOPs for executing the method in the routine analysis;
- define type and frequency of system suitability tests;
- QC checks for the routine analysis;
- document validation experiments and results in the validation report.

8.2. PRINCIPLES OF ANALYSIS OF FOODS

8.2.1. Validity and reliability

To obtain usable results we need tests which are both scientifically valid and reliable. Validity refers to the extent to which a measurement is well-founded and corresponds accurately to the real world; *i.e.* does it measure what you want it to measure. Reliability refers to the consistency of a measurement systems, such that a measure is said to have a high reliability if it produces consistent results under consistent conditions.

8.2.2. Basic principles of analysis

To ensure an acceptable level of validity and reliability, the following basic principles should be observed by any laboratory undertaking chemical analyses or microbiological examinations of food:

- analytical measurements should be made using methods and equipment which can be shown to be fit for their intended purpose;
- staff carrying out chemical analyses or microbiological examination of samples should be both qualified and competent;
- there should be a regular independent assessment of the technical performance of a laboratory via assessment from an accreditation body and participation in relevant proficiency tests;
- analytical measurements made in one laboratory should be consistent with those made in another laboratory;
- for analyses such as the determination of fat, protein or moisture content and where it is necessary to compare results, laboratories should agree on the method to be used. This is also the case for laboratories undertaking the microbiological examination of samples;
- organisations making analytical measurements should have well defined quality control and quality assurance procedures.

In the EU to ensure that these conditions are met, Article 12 of Regulation (EC) No. 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules requires that 'Competent Authorities' may only designate laboratories that operate and are assessed and accredited in accordance with the following European standards:

a. EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories';
b. EN ISO/IEC 17011 on 'General requirements for accreditation bodies accrediting conformity assessment bodies'.

Accreditation is therefore a legal requirement for laboratories carrying out chemical analysis or microbiological examination of samples as part of the official control of food and feedstuffs to be accredited. It is also increasingly the case that laboratories undertaking analysis of food and feed for purposes other than official control are also required to be accredited.

One of the main advantages for a laboratory of accreditation is that it provides an indication to the laboratory's customers, that the laboratory is technically competent in those analyses that are included in their scope of accreditation. The results reported by an accredited laboratory are generally accepted without question, minimizing the need for re-testing. Within the EU test certificates issued by an official laboratory in one Member state are accepted by the Competent Authority in another Member state, thus allowing for the free movement of goods.

ISO 17025 covers both management and technical requirements for accredited testing laboratories. The technical requirements are covered in Section 5 of the standard and include a number of requirements related to the validation of methods. However, other documents are also relevant. A full list of relevant references quoted in this document is provided in Annex 1.

8.2.3. The need for method validation

8.2.3.1. Reliability of results

If the results obtained from chemical analyses or microbiological examinations are unreliable, then such results have little value and the test might as well have not been carried out.

When samples are submitted to a laboratory for analysis, the person submitting the sample expects to be able to trust the results that are reported and usually only challenges them when they are obviously out of line with expected results or in the case of a dispute.

The laboratory and its staff have a clear responsibility to justify the customer's trust by providing correct analytical results that can be clearly shown to be fit for purpose.

8.2.3.2. Fitness for purpose

Chemical analysis or microbiological examination of samples is normally carried out for a particular purpose and to provide analytical data that can be used to confirm compliance with a particular standard or as part of an investigation to solve a problem.

Tests carried out must be both appropriate and fit for purpose.

Method validation enables chemists or microbiologists to demonstrate that a method is 'fit for purpose'.

Not only must tests be appropriate and fit for purpose but the final report must present the results of analysis in such a way that the customer can readily understand it and draw appropriate conclusions.

For an analytical result to be fit for its intended purpose it must be sufficiently reliable that any decision based on it can be taken with confidence. For this reason the method performance must be validated and the uncertainty on the result, at a given level of confidence, estimated. It is more and more the case that when results of analysis are used to confirm compliance with legal standards, the uncertainty of the result is taken into account.

Regardless of how good a method is and how skilfully it is used, an analytical problem can only be solved by the analysis of samples that have been taken correctly and are relevant to the problem. They must also be truly representative of the bulk of material from which they are taken. In other words, the results of analysis can only ever be as good as the sample that is submitted to the laboratory.

8.2.3.3. When should methods be validated?

A method should be validated when it is necessary to confirm that its performance characteristics are adequate and relevant to the purpose of the analysis to be carried out. Method validation is required when:

- new methods are developed;
- an established method is revised to incorporate improvements in methodology or when its scope is extended to cover additional types of sample;
- the results obtained from routine analytical quality control indicate that there are changes in the performance of an established method over time;
- an established method is used in a different laboratory, or by different analysts or with different instrumentation;
- to demonstrate the equivalence between two methods, e.g. a new method and a standard method.

The extent of validation or revalidation required will depend on the nature of the changes made in reapplying a method to different laboratories, instrumentation, operators, and the circumstances in which the method is going to be used. Some degree of validation is always appropriate even where a laboratory is using methods published by bodies such as CEN, ISO, National Standards bodies, AOAC etc., or other recognized sources.

At the very least, any laboratory using a method that has been collaboratively studied and for which performance characteristics such as recovery, repeatability and reproducibility are available, must be able to demonstrate that they can use the method in such a way that they can meet the performance requirements.

The extent of validation required by individual laboratories is normally significantly less when the laboratory chooses to use a method that has been validated by collaborative study such as those published CEN, ISO, National Standards bodies, AOAC etc.

8.3. REQUIREMENT FOR METHOD VALIDATION AND ISO 17025

The validation process is described by ISO 17025. Clause 5.4 of ISO 17025 specifies the requirements for 'Test and calibration methods and method validation'.

Clause 5.4.1 specifies that the laboratory must use appropriate methods and procedures for all tests and/or calibrations within its scope.

International, regional or national standards or other recognized specifications that contain sufficient and concise information on how to perform the tests and/or calibrations do not need to be supplemented or re-written as internal procedures if these standards are written in such a way that they can be used as published by the laboratory staff. In some cases it may be necessary to provide additional documentation for optional steps in the method or additional details to clarify certain aspects of the method.

Clause 5.4.2 concerning the selection of methods requires that the laboratory uses test and/or calibration methods, including methods for sampling, which meet the needs of the customer and which are appropriate for the tests and/or calibrations it undertakes.

There are different methods which can be chosen for testing of a particular parameter. ISO 17025 also states that methods published in international, regional or national standards shall preferably be used. The laboratory must ensure that it uses the latest valid edition of a standard unless it is not appropriate or possible to do so. When necessary, the standard must be supplemented with additional details to ensure consistent application.

When the customer does not specify the method to be used, the laboratory is required to select appropriate methods. As far as is possible, the methods selected are those that have been published either in international, regional or national standards, or by reputable technical organizations, or in relevant scientific texts or journals, or as specified by the manufacturer of the equipment.

Laboratory-developed methods or methods adopted by the laboratory may also be used if they are appropriate for the intended use and if they are validated. These may include:

- non-standard methods;
- laboratory-designed/developed methods;
- standard methods used outside their intended scope;
- amplifications and modifications of standard methods.

The laboratory must inform the customer regarding the method chosen. The laboratory must also confirm that it can properly operate standard methods before introducing the tests or calibrations. Where a published standard method is revised, the laboratory must also carry out further validation to confirm that it is still competent and can produce valid results by use of the revised method. The laboratory must inform the customer when the method proposed by the customer is considered to be inappropriate or out of date.

Clause 5.4.3 which covers laboratory-developed methods requires that the introduction of test and calibration methods developed by the laboratory for its own use shall be a planned activity and must be assigned to qualified personnel equipped with adequate resources.

Clause 5.4.4 which covers Non-standard methods requires that when it is necessary to use methods not covered by standard methods, these must be subject to agreement with the customer and must include a clear specification of the customer's requirements and the purpose of the test and/or calibration. The method developed must have been validated appropriately before use.

Therefore, laboratories must ensure that the methods they apply have been subjected to a proper validation process. This applies to the introduction of new test methods to the laboratory, as well as the periodic re-validation of existing tests.

8.4. VALIDATION APPROACH

8.4.1. Selection of approach to validation

Validation is required to confirm that method chosen is fit for its intended use. The validation must therefore be as extensive as is necessary to meet the needs of the given application or field of application.

The laboratory must record the results obtained and the procedure used for the validation. This information must be included in a validation report that is available for each method included in the laboratory's scope of accreditation. The report must include a statement confirming that the method is fit for the intended use.

The techniques used for the determination of the performance of a method should be one of, or a combination of, the following:

- calibration using reference standards or reference materials;
- comparison of results achieved with other methods;
- inter-laboratory comparisons;
- systematic assessment of the factors influencing the result;

Validation of a method should include an assessment of the uncertainty of the results obtained when using the method. This assessment should be based on a scientific understanding of the theoretical principles of the method and practical experience.

If changes are made to validated non-standard methods, the influence of such changes should be documented and, if appropriate, a new validation should be carried out. The extent of any revalidation will depend on the extent of changes to the method.

Clause 5.4.5.3 of ISO 17025 requires that the range and accuracy of the values obtainable from validated methods (e.g. the uncertainty of the results, detection limit, selectivity of the method, linearity, limit of repeatability and/or reproducibility, robustness against external influences and/or cross-sensitivity against interference from the matrix of the sample/test object), as assessed for the intended use, shall be relevant to the customers' needs.

8.4.2. Method validation by collaborative study

It is normally the case that where a method is being developed that will be applied in many laboratories, method validation will be carried out by means of a collaborative study involving a group of laboratories.

There are a number of protocols covering validation of methods by collaborative study. One example is given by ***AOAC – Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis***.

However, this is not always a suitable option for industrial laboratories. The application for which the method is required may be very specific and the number of laboratories likely to be interested in collaboration to develop the method may be extremely limited. Those that might be interested could be competitors. Where it is inconvenient or impossible for a laboratory to validate a method by collaborative study a number of issues need to be addressed.

- Can laboratories validate methods on their own, and if so, how?
- Will methods validated in this way be recognized by other laboratories?
- What sort of recognition can be expected for in-house methods used in a regulatory environment?

Developing methods without the benefit of collaboration with other laboratories inevitably reduces the amount of validation data that can be gathered. The main drawback is that information regarding inter-laboratory comparability is very restricted. If necessary, it may be possible to get some idea of the comparability of analytical results for a particular method with those obtained by other laboratories by either using the method to analyse certified reference materials; or by comparing the results obtained using the method under development with those obtained using a corresponding validated method.

8.4.3. Degree of validation required

Bearing in mind the purpose of the analysis, the laboratory must decide which method performance parameters need to be determined in order to validate the method and demonstrate that it is fit for its intended purpose.

In deciding on the degree of validation required a number of factors need to be taken into account. Validation requirements may be specified in guidelines covering

particular types of testing. These guidelines should be followed where available and appropriate.

Official recognition of a method may require validation by means of a collaborative study. Regulatory requirements may require a particular method to be followed to the letter even though the laboratory considers it to be unsound or inaccurate. Additional validation will be needed to confirm the satisfactory performance of individual analysts.

The degree of validation must be related to the analytical requirement. Having identified the analytical requirements of a method, the laboratory can identify the performance characteristics that need to be validated.

For methods that are to be used to provide a qualitative or quantitative result, the following performance characteristics need to be included in method validation:

- confirmation of identity;
- selectivity/specificity;
- limit of detection;
- limit of quantification.

Where the method is to be used for analysis of samples in which the analyte is present in more than one form, or there is a requirement to determine extractable, free or total analyte, method validation should include steps for confirmation of identity and recovery.

Where the method is used to determine specific analytes such as metallic contaminants, mycotoxins etc., at low levels, *i.e.* mg/kg, µg/kg etc., method validation should include the following steps:

- confirmation of identity;
- limit of detection;
- limit of quantification;
- working and linear ranges.

For any quantitative method it is necessary to determine how accurate the method is and its precision. Particularly for those methods that are used in official control, it necessary to determine the uncertainty of the method and the way in which the uncertainty is expressed. Validation of these types of method must therefore also include:

- recovery;
- accuracy/trueness;
- repeatability precision;
- reproducibility precision.

If the results obtained need to be compared with results obtained in other laboratories, validation must include an assessment of:

- ruggedness/robustness;
- reproducibility precision.

Similarly if the results obtained by use of the method will be used to show compliance with a commercial specification, validation must include an assessment of:

- accuracy;
- reproducibility precision.

8.5. METHOD PERFORMANCE PARAMETERS

8.5.1. Confirmation of identity and selectivity/specificity

The selectivity (or specificity) of a test is **an important feature of the test** to be determined through validation.

Selectivity (or specificity) is defined as the ability of a method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test. Specificity is defined by the AOAC as 'The ability of a method to measure only what it is intended to measure.'

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) define 'Specificity' as "*the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix etc.*".

Specificity and selectivity

Specificity and selectivity are often used interchangeably. Specificity, in general, is the ability of a method to measure[149] only what it is intended to measure and where response is only produced by the analyte. This characteristic is often a function of the measuring principle and the function of the analyte under study.

A key consideration of specificity is whether the method is able to differentiate a compound quantitatively from homologues, analogues, or metabolic products of the residue of interest under the experimental conditions employed.

Selectivity refers to the ability of a method to determine accurately and specifically the analyte of interest **in the presence of other components** in a sample matrix under the stated conditions of the test, meaning ability to distinguish response from the analyte and response from interfering substances.

Since very few methods respond to only one analyte, the term selectivity is usually more appropriate than specificity. A selective method must provide for the identification of the compound being measured. Likewise for screening methods it is important that the method is selective, thereby reducing the number of false positives. Suitable identification tests should be able to discriminate between compounds of closely related structures that are likely to be present. For chromatographic procedures,

149 Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002).

representative chromatograms should be used to demonstrate selectivity and individual components should be appropriately labelled. For critical separations, selectivity can be demonstrated by the resolution of the two components that elute closest to each other.

Samples with relevant interfering substances and blank samples with and without the analytes should be analysed in order to determine the degree of interference.

It should be examined whether:

1. interfering substances can lead to false-positive results;
2. identification of the analyte is inhibited by interfering substances leading to false negative results;
3. quantification of analyte is influenced by interfering substances.

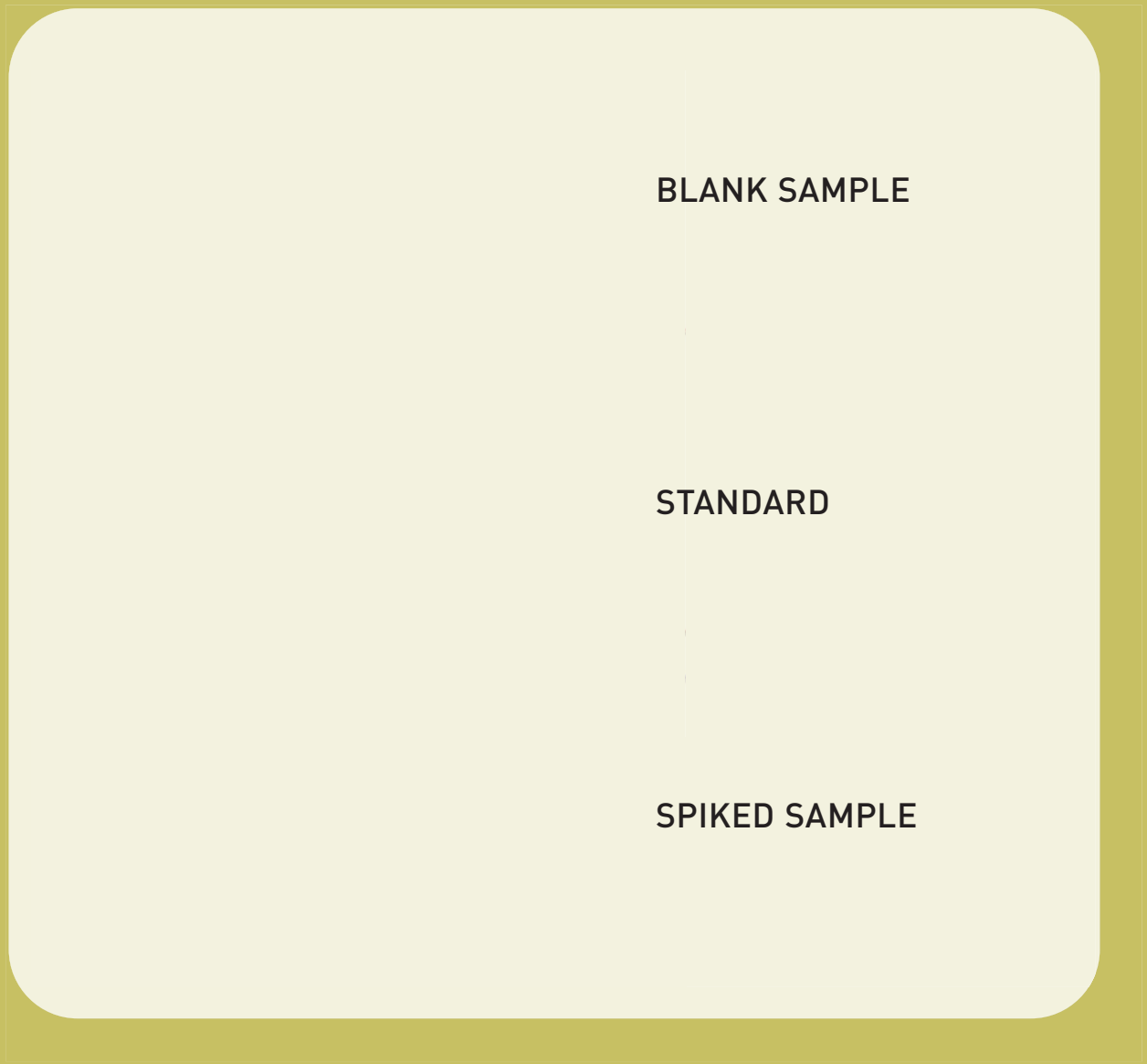


Figure 1 - Analysing selectivity in chromatography: Co-chromatography

Three samples are analysed: a) blank sample, b) standard and c) fortified sample.

The concentration of standard should be adjusted so that the height of the two peaks are approximately the same. In this example **the selectivity of the method is probably not suitable because of interfering peak**.

In general analytical methods include a measurement stage which may or may not be preceded by an isolation stage. Confirmation of identity is established when it can be demonstrated that the signal produced at the measurement stage, or other measured property, which has been attributed to the analyte, is only due to the analyte and not from the presence of something chemically or physically similar or arising as a coincidence.

Whether or not other compounds interfere with the measurement of the analyte will depend on the effectiveness of the isolation stage and the selectivity/specificity of the measurement stage. Selectivity and specificity are measures which assess the reliability of measurements in the presence of interferences.

For those analyses where the measurement stage is non-specific, it may be possible to state that certain analytes do not interfere, but a series of experiments in which potentially interfering substances are deliberately added, must have been carried out to confirm that this is the case. It is far more difficult to state that nothing interferes since there is always the possibility that some sample matrices may include substances that have not previously been recognized as interfering with the analysis. There will be other cases where chemical interferences can be identified for a particular method but in reality the chances of encountering them in the samples normally analysed by the method will be more or less zero. The analyst has to decide at what point it is reasonable to stop looking for interferences. Selectivity and specificity must also be taken into account when validating qualitative methods of analysis as well as for quantitative methods.

In some cases, interfering substances are present which cannot be separated from the analyte of interest. In other cases the analyst may not be aware of the presence of interfering substances. In either case, those interferences will have a number of effects. Depending on how the identity of the analyte is established interferences may:

- inhibit confirmation, for example by distorting the signal arising from the analyte.
- have the effect of apparently enhancing the concentration of the analyte by contributing to the signal attributed to the analyte, (or conversely suppressing the concentration of the analyte if they contribute a negative signal).

The selectivity of a method is usually investigated by studying its ability to measure the analyte of interest in test portions to which specific interferences have been deliberately introduced.

Where it is unclear whether or not interferences are already present, the selectivity of the method under development can be investigated further by comparing results obtained from the method being developed, with those obtained using other independent methods or techniques.

An example of the approach the issue of specificity is given in the box below.

Example of specificity measures

A peak in a chromatographic trace may be identified as being due to the analyte of interest on the basis that a reference material containing the analyte generates a signal at the same point on the chromatogram. But, is the signal due to the analyte or to something else which coincidentally co-elutes? It could be either or both. Identification of the analyte by this means only is unreliable and some form of supporting evidence is necessary. For example, the chromatography could be repeated using a column of different polarity, to see whether the signal and the signal generated by the reference materials still appear at the same time. Where a peak is due to more than one compound, a different polarity column may be a good way of separating the compounds. If available, gas chromatography with mass spectrometric detection could be used to confirm the identity of the analyte or show a mixture to be present. Some detectors can monitor peak purity.

UV/Visible diode-array detectors and mass-spectrometers acquire spectra on-line throughout the entire chromatogram. The spectra acquired during the elution of a peak are normalized and overlaid for graphical presentation. If the normalized spectra are different, the peak consists of at least two compounds.

Examples of pure and impure HPLC peaks are shown in Figure 2. While the chromatographic signal indicates no impurities in either peak, the spectral evaluation identifies the peak on the left as impure. The level of impurities that can be detected with this method depends on the spectral difference, on the detector's performance and on the software algorithm. Under ideal conditions, peak impurities of 0.05 to 0.1 % can be detected.

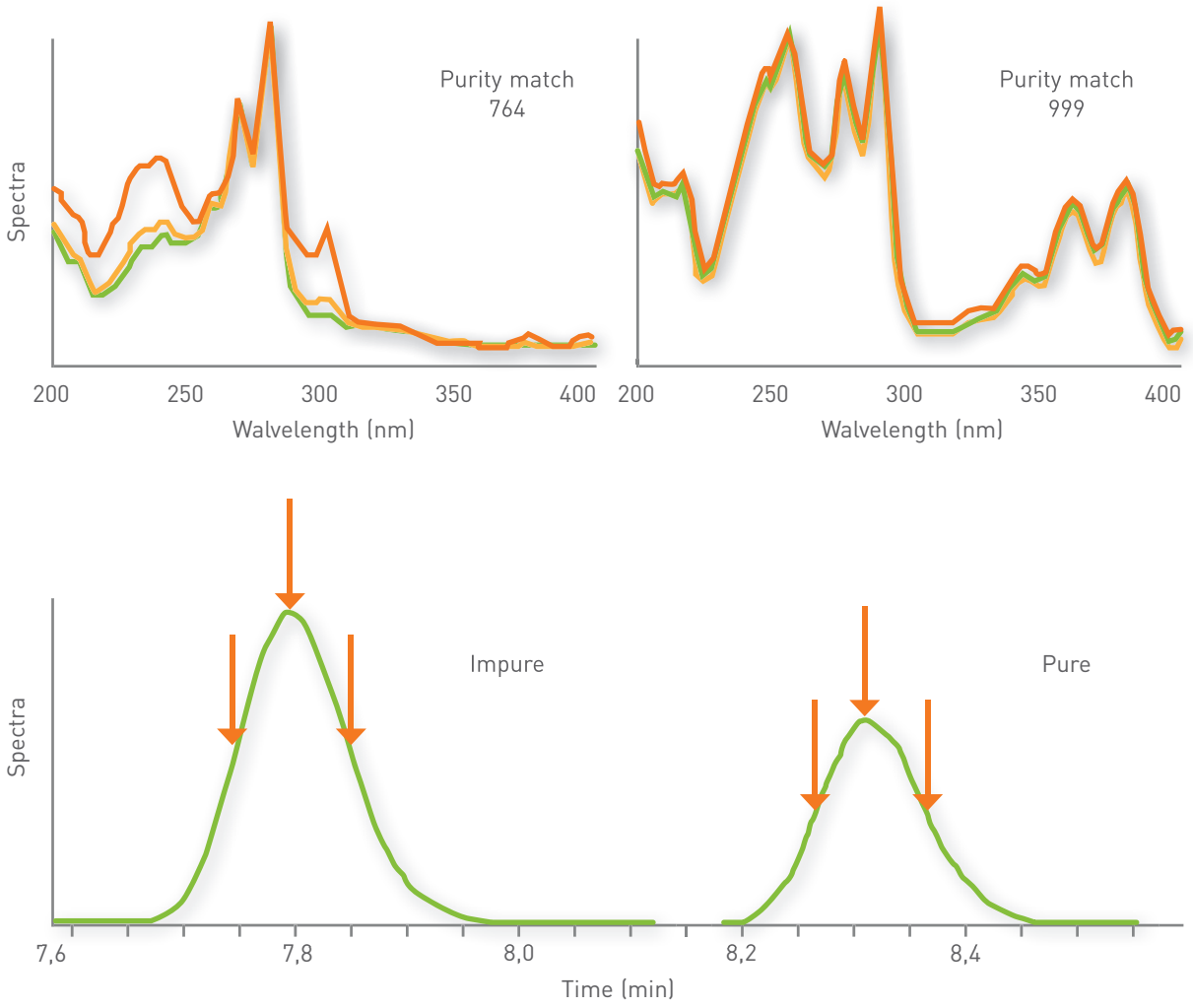
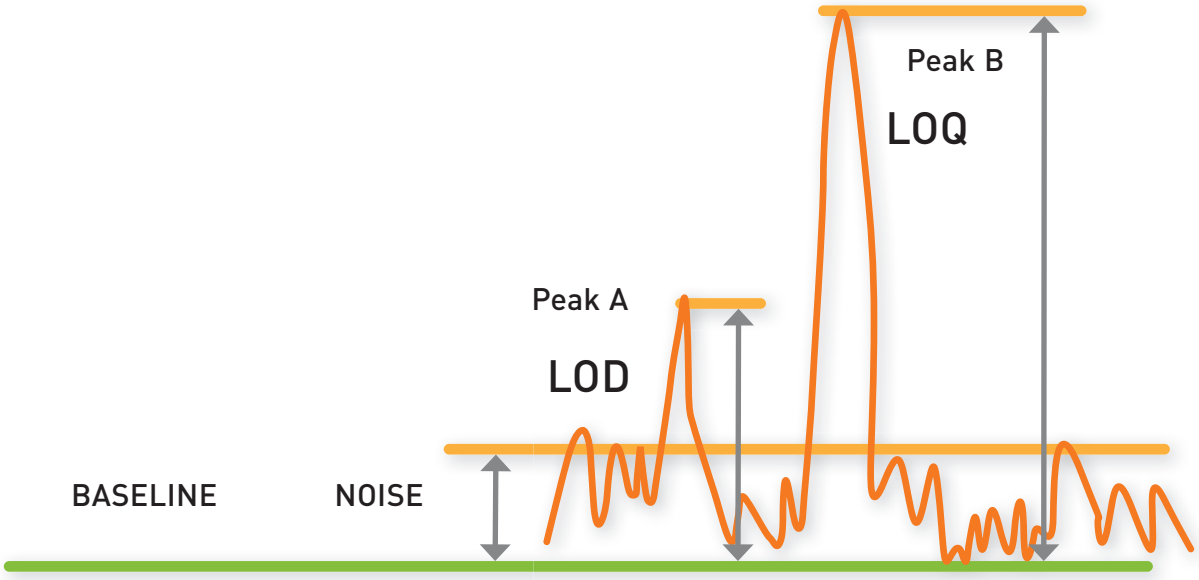


Figure 2 - Examples of pure and impure HPLC peaks. The chromatographic signal does not indicate any impurity in either peak. Spectral evaluation, however, ***identifies the peak on the left as impure.***

Another aspect of selectivity which must be considered is where an analyte may exist in the sample in more than one form such as:

- bound or unbound;
- inorganic or organometallic;
- different oxidation states.

8.5.2. Limit of Detection (LOD)



Where measurements are made at low analyte levels, e.g. in trace analysis, it is important to know what is the lowest concentration of the analyte that can be confidently detected by the method.

The limit of detection is the point at which a measured value is larger than the uncertainty associated with it.

Other definitions of the limit of detection include:

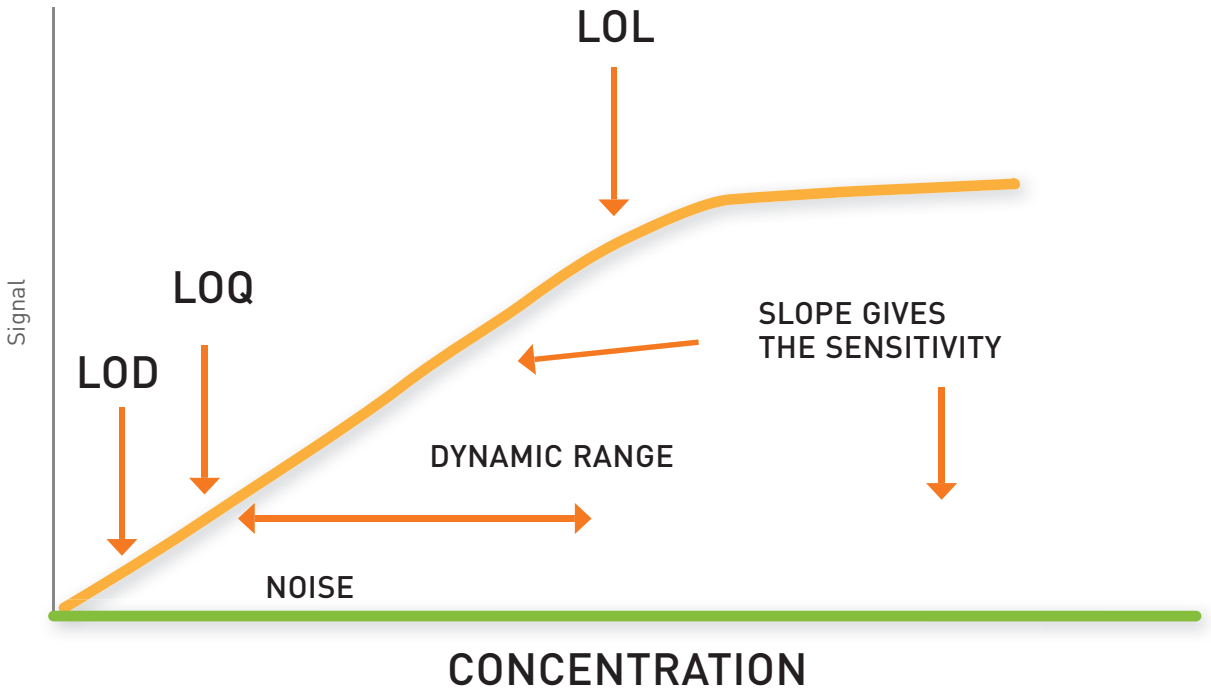
- the lowest content that can be measured with reasonable statistical certainty – AOAC;
- the lowest concentration of analyte in a sample that can be detected, but not necessarily quantified under the stated conditions of the test – NATA.

In chromatography the detection limit is the injected amount that results in a peak with a height at least twice or three times as high as the baseline noise level.

For validation purposes it is normally sufficient to provide an indication of the level at which detection becomes problematic. For this purpose the 'blank + 3s' approach is usually sufficient.

Both the mean and the standard deviation of the sample blank are likely to be dependent on the matrix of the sample blank. The limit of detection will therefore be matrix dependent.

8.5.3. Limit of Quantitation (LOQ)



The AOAC defines the limit of quantitation as *"[The content] equal to or greater than the lowest concentration point on the calibration curve".*

It is also known as the Limit of Reporting.

NATA defines the limit of quantitation as *"The lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test".*

The 'limit of quantitation' (LOQ) is strictly the lowest concentration of analyte that can be determined with an acceptable level of repeatability precision and trueness.

It may also be defined as the analyte concentration corresponding to the sample blank value plus 5, 6 or 10 standard deviations of the blank mean.

It is also sometimes known as 'limit of determination'.

For chromatographic methods, the limit of quantitation is the minimum injected amount that gives precise measurements, typically requiring peak heights 10 to 20 times higher than baseline noise.

8.5.4. Working and linear ranges

For any quantitative method, it is necessary to determine the range of analyte concentrations over which the method may be applied.

At the lower end of the concentration range the limiting factors are the values of the limits of detection and/or quantitation.

At the upper end of the concentration range limitations in the relationship between the instruments response and analyte concentration may arise dependent upon the instruments measurement system. Many detection systems e.g. electron capture detectors, have limitations in their detection capacity and at higher concentrations of analyte, the detectors response plateaus.

Within the working range of an analytical method, there is normally a range where the instruments response versus the analyte concentration, is linear. The working range and the extent of any linear range within the working range are normally established as a part of the method validation.

Calibration and linearity

A calibration curve is a graph in which concentration is plotted along the *x*-axis and analytical response is plotted along the *y*-axis. The line connecting the points represents the calibration curve. The calibration curve study is generally performed by preparing standard solutions, from 0 to 150 % or 50 to 150 % of the concentration likely to be encountered (typical the MRL-value).[150] A minimum number of five-six concentration levels are required to allow detection of curvature in the plotted data.

Testing for linearity will be performed to give assurance that the method is valid for its intended use throughout the specified ranges. If the method is used for analysis at low concentrations, the measurements should further include additional levels in this area. The following conditions must be assessed for the calibration curve and linearity:

- graphical representation (linearity / nonlinearity) displaying individual determinations and the average values;
- curve formula and determination coefficient;
- assessment and plot of residuals;
- linearity testing.

The linearity is evaluated mathematically by calculation of a regression line with the method of least squares.[151] This is normally done automatically after analysis of the calibrants or these calculations can be performed in electronic spread sheets.

150 M. Thompson, S.L.R. Ellison and R. Wood. Harmonised guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Appl. Chem., vol. 74, No. 5, 835-855 (2002).

151 M. Mulholland and D.B. Hibbert. *J. Chromatogr.*, vol.762, No. 73, 1997.

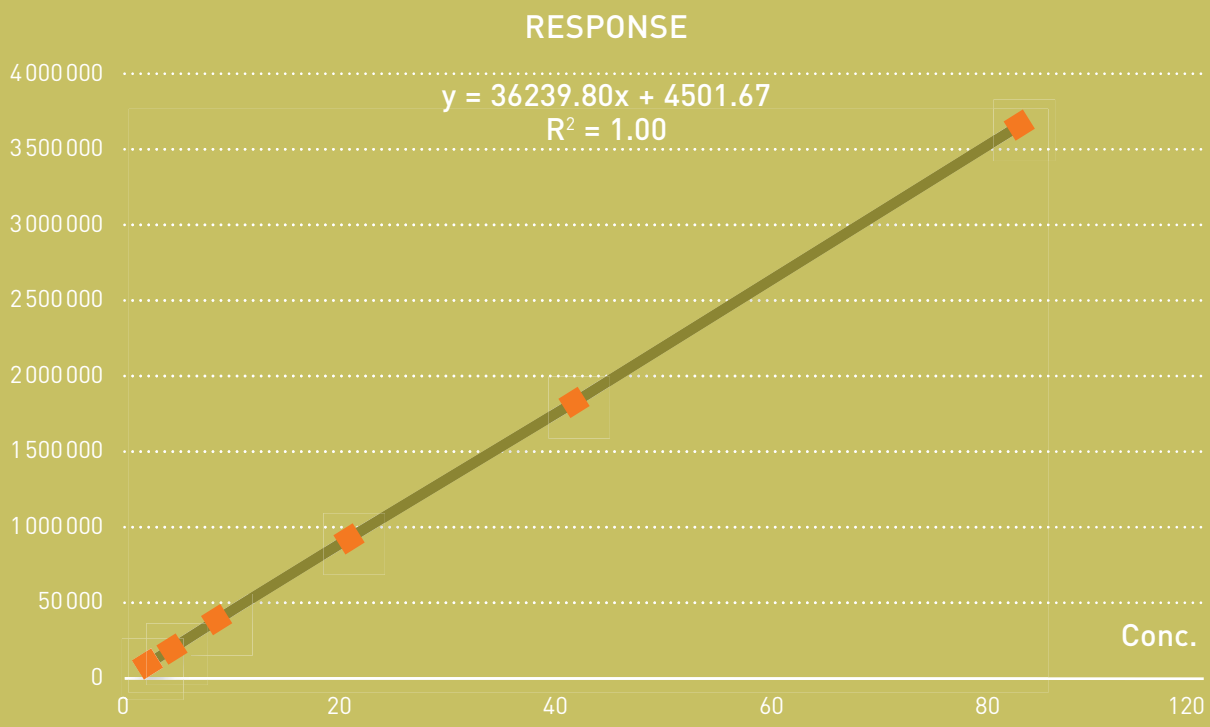


Figure 3 - Drawing a calibration curve
(with its equation and the value of the coefficient of determination r^2)

Assessment of linearity is done by a linearity test or from the graphical representation and plot of residuals. The residuals tends to grow as concentration grows (B) is quite common in analytical calibration, and it is a pattern suggestion that calibration data are best treated by weighed regression. If the residuals are showing a systematic trend, e.g. negative at low concentrations, positive at high concentrations (C) a different curve fit is suggested.

The residual plot (**A**) shows a satisfactory distribution of residuals.

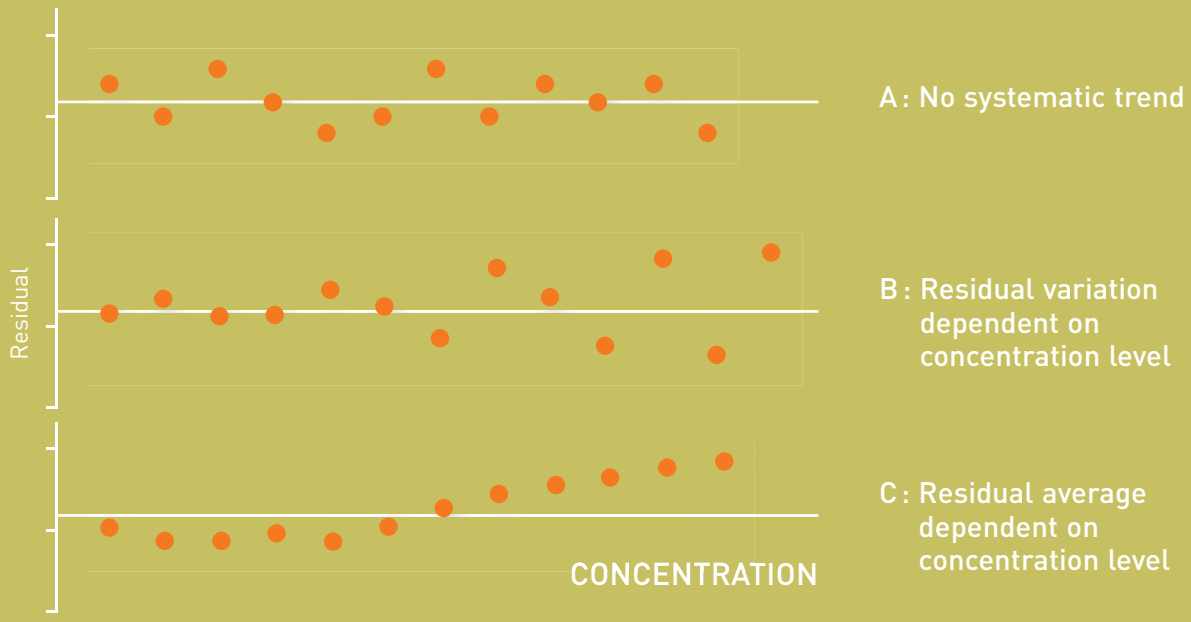
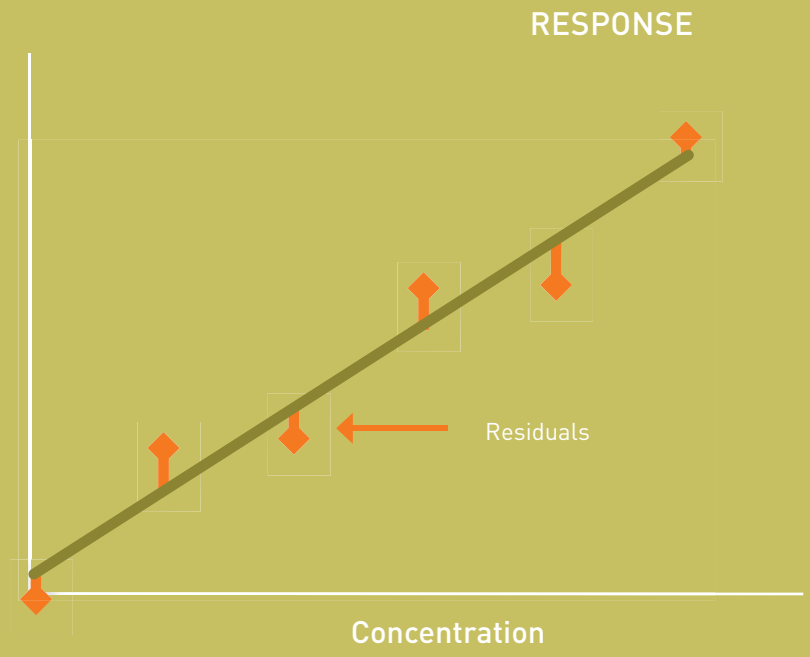


Figure 4 - Drawing of residues

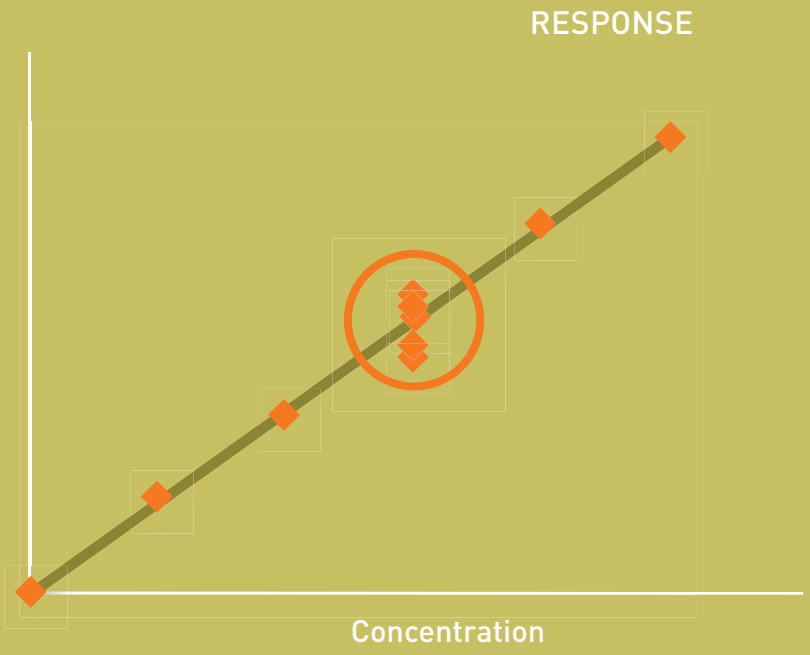
Linearity can be evaluated as recommended by Tiley.[152]

152 P.F. Tiley. The misuse of correlation coefficients. Chemistry in Britain 162-163 (1985).

Testing is based on the ratio of the two variances, s_1 variance for the adjustment error (distance of data point to the curve) and $s_2$variance of the y's (precision within the concentration level).s_2 could either be calculated from n repeated determinations carried out at the same concentration, preferable near the middle of the calibration curve, or with the same number of repetitions for all concentration points (shown here in green plot):



$$s_1^2 = \frac{1}{n-2} \sum (y - \hat{y})^2$$



$$s_2^2 = \frac{1}{n-1} \sum (y - \bar{y})^2$$

The values $s1^2$ and $s2^2$ are stochastically independent, and is F-distributed with n-2 and n-1 degrees of freedom under the zero hypothesis: H0: *The correlation between x and y is linear.*

See in Annex an example of such a linearity test proposed by Tiley.

If linearity is proved, the subsequent routine calibration can be simplified (e.g. by using 2-point calibration). Notice that if such a simplified calibration will be used, the validation should be based on this reduced calibration method, selected for routine use. If it is decided not to include calibration in each series, criteria for acceptance of specified parameters that can vary from assay series to assay series should be described. In some cases linearity cannot be demonstrated even after any transformation. When using immunoassays normally a sigmoid curve is used and only a part of the used concentration range is linear.

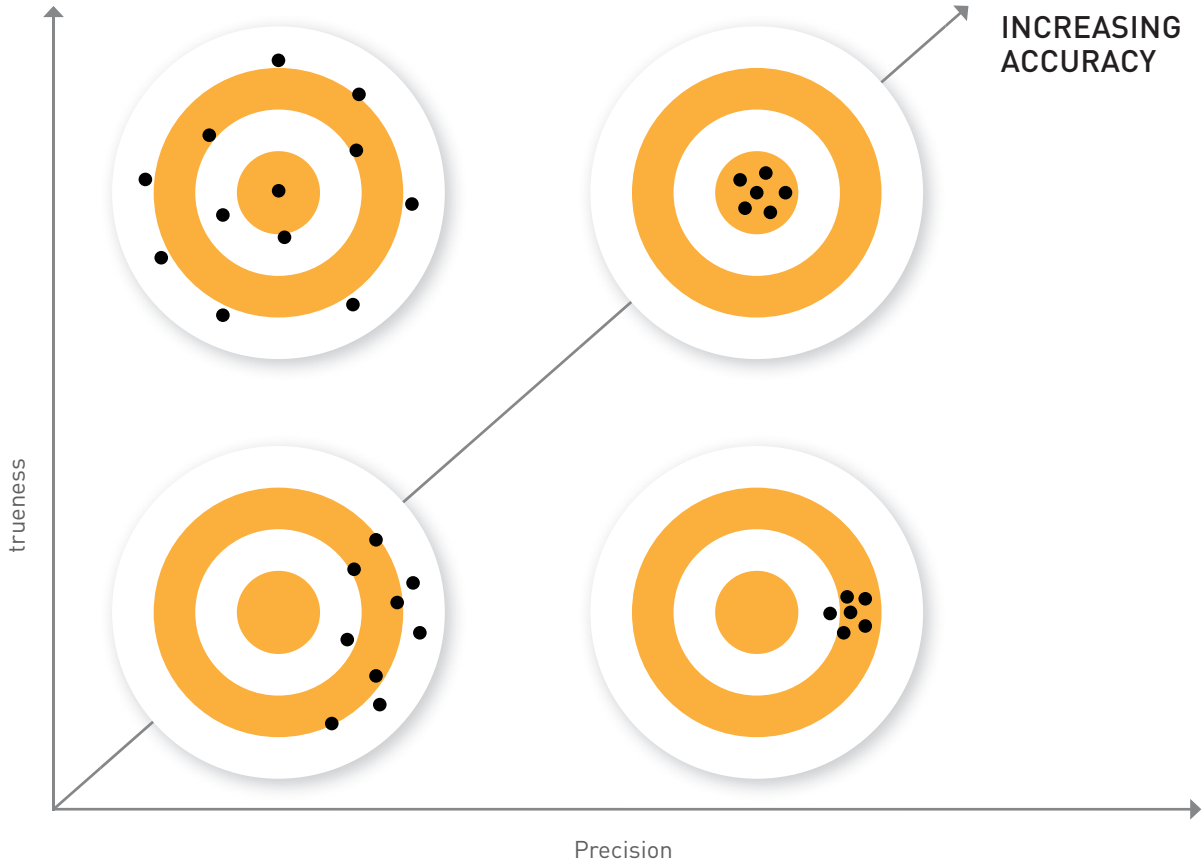
Linearity is therefore not "a must" but it makes calculations simpler.

Normally the linearity of any calibration should be checked using standards with at least 10 different concentration values.

Evaluation of the working and linear ranges is also useful for deciding on what degree of calibration is required for a particular method, on a daily basis. Certainly during the initial stages of method validation, it is advisable to investigate the variance across the working range. However, in many cases and within the linear range, one calibration point may be sufficient, to establish the slope of the calibration line. Elsewhere in the working range, multi-point (preferably 6+) calibration will be necessary. The relationship of instrument response to concentration does not have to be perfectly linear for a method to be effective but where a curved relationship between instrument response and concentration exists, the curve should be repeatable from day to day.

It is important to remember that the working and linear range may be different for different matrices depending on the effect of any interferences that may be present in different sample matrices.

8.5.5. Accuracy



Accuracy is defined as the closeness of agreement between a test result and the accepted reference value.

Accuracy expresses the closeness of a result to a true value

In method validation the likely accuracy of results is quantified by assessing both systematic and random effects on results. Accuracy is, therefore, normally studied as two components: 'trueness' and 'precision'.

8.5.6. Trueness

8.5.6.1. Means of assessing trueness

The 'trueness' (of a method) is a measure of how close the mean of a set of results (produced by the method) is to the true value. Trueness is normally expressed in terms of bias.

In practice the assessment of trueness relies on comparison of mean results from a method with known values *i.e.*, trueness is assessed against a reference value (*i.e.* true value or conventional true value). Two basic techniques are available:

- checking against reference values for a characterised material;
- another characterized method.

Reference values are ideally traceable to international standards. Certified reference materials (CRM) are generally accepted as providing traceable values; the reference value is then the certified value of the CRM. Reference values, certified or otherwise, may be absolute or conventional *i.e.*, generally agreed upon for a particular purpose.

To check trueness using a reference material, the mean and standard deviation of a series of replicate tests is determined and the values obtained, compared with the characterized value for the reference material.

The ideal reference material is a certified, natural matrix reference material. Ideally the matrix of the reference material should be similar to the samples of interest. Unfortunately, in many cases appropriate certified reference materials are either not available or their availability is limited. In such cases alternative methods for the preparation of suitable reference materials, need to be used. These include:

- materials prepared by spiking typical materials with pure certified reference materials or other materials of suitable purity and stability;
- typical, well-characterized materials checked in-house for stability and retained for in-house QC.

Validation of a method needs to be appropriate to the intended use of the method, so the reference material selected must be appropriate to the use.

For regulatory work, a relevant certified material should be used wherever possible. Ideally, matrix matched, certified reference materials should be used.

For methods used for long term in-house work, a stable in-house material or certified reference material should be used.

For short-term or non-critical work, a prepared standard or spike is often sufficient.

To check against an alternative method, compare results from the two methods for the same sample or samples. The sample(s) may be CRMs, in-house standards, or simply typical samples. There are advantages to using CRMs, since these have known stability and homogeneity, and because they have certified values for analyte concentrations, the results obtained from their use, provide an indication of any bias associated with the method, with respect to international standards.

The main disadvantages of CRMs are that they are expensive and they may not be representative of typical samples.

It is important to remember that it may be necessary to repeat a trueness check in those cases where the originally validated method is used for the analysis of samples that have radically different matrices or analyte concentration levels from those used in the original validation.

8.5.6.2. Interpreting bias measurements

There are two principle components of bias to be addressed in method validation. These are bias due to the method and bias due to the laboratory. The method bias arises from systematic errors inherent to the method whichever laboratory uses it. Laboratory bias arises from additional systematic errors peculiar to the laboratory and its interpretation of the method. In isolation, a laboratory can only estimate the combined bias.

For most purposes the acceptability of bias should be decided on the basis of overall bias measured against appropriate materials or reference methods, taking into account the precision of the method, any uncertainties in reference material values, and the accuracy required by the end use of the reported results. The use of statistical significance tests is recommended.

8.5.7. Precision

Precision is a measure of **how close results are to one another**, and is usually expressed by measures such as standard deviation, which describe the spread of results.

Precision is usually specified in terms of standard deviation or relative standard deviation (RSD%) or coefficient of variation (CV%)[153].The latter two are most used because they are relatively constant over a considerable concentration range that ideally covers the level of interest.

The measured standard deviation can be subdivided into **2 categories**:

1. Repeatability
2. Reproducibility

Another method for the expression of accuracy is 'measurement uncertainty'. It may also be defined as "the closeness of agreement between independent test results obtained under stipulated conditions.' The two most common measures of precision are 'repeatability' and 'reproducibility', but others are available.

8.5.7.1. Repeatability

Repeatability (r) is a measure of the variability to be expected when a method is performed by a single analyst on one piece of equipment over a short period of time, *i.e.* the sort of variability to be expected between results obtained when a sample is analysed in duplicate.

153 ISO 3534-1. Statistics – Vocabulary and symbols – Part 1: Probability and general statistical terms (1993).

Repeatability will be obtained when one operator using one piece of equipment over a relatively short time period carries out the analysis. Repeatability can help in determining the sample preparation procedure, the number of replicate samples to be prepared, and the number of injections required for each sample in the final method setting. Within-laboratory reproducibility expresses within-laboratory variations and reproducibility represents the precision obtained between laboratories with the objective to verify that the method will provide the same results in different laboratories. In accordance with the guidelines on single-laboratory validation between-laboratory variation does not need to be part of the validation[154] and then it is important to pay attention to estimation of laboratory bias (or trueness).

Repeatability must be determined with min. 6 degrees of freedom e.g. one series of 7 samples, 2 series of 4 samples, 3 series of 3 samples etc. For some areas there may be other requirements for the number of degrees of freedom:

- Analytical methods for determination of **veterinary drug residues** used in accordance with Directive 96/23/EC control,[155] for determination of repeatability and within-laboratory reproducibility a minimum of three test series are analysed, each with a minimum of 6 replicates; [156]
- For analytical methods used **for pesticide control** at least 4 degrees of freedom must be obtained (e.g. one series of five samples, two series of 3 samples etc.)[157] for determination of repeatability.

8.5.7.2. Reproductibility

Reproducibility (R) is often defined as the difference between test results, obtained with the same method on identical test material **within a single laboratory or in different laboratories** (with different operators using different equipment).

If a sample is to be analysed by a number of laboratories for comparative purposes then a more meaningful precision measure to use is "reproducibility". In many cases, some in-between measure is the most useful. For example precision measured between different analysts, over extended timescales, **within a single laboratory**. This is sometimes known as "intermediate precision", but the exact conditions should be stated. Precision is usually expressed in terms of standard deviation or relative standard deviation.

154 M. Thompson, S.L.R. Ellison and R. Wood. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Appl. Chem., vol. 74, No. 5, 835-855 (2002).

155 Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products.

156 Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002).

157 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed. Document N° SANCO/12495/2011.

Within-laboratory reproducibility (in a single laboratory)

Within-laboratory reproducibility determined from an adequate number of determinations measured during at least three different series on different days. As mentioned above analytical methods for determination of veterinary drug residues for Directive 96/23/EC control each test series should include a minimum of 6 replicates (identical or different matrices).

The formula for calculating within-laboratory reproducibility is:

$s_{iR} = s_r + s_L$ (ISO 3534-1)[158]

where:

s_r = standard deviation of repeatability

s_L = between days standard deviation

Repeatability and within-laboratory reproducibility should be determined for **at least three concentration levels**. The lowest concentration level in the measuring range should be included. Several methods for calculations can be used.[159/160]

In Annex examples are given on calculation of S_r and S_{iR} with use of excel spread sheet.

When duplicate analysis in n series the formulas for S_r^2 and S_L^2 can be simplified to:

$$s_r^2 = \frac{\Sigma (y_{i1} - y_{i2})^2}{2n}$$

Where y_i is the result on series i, and

$$s_L^2 = \left[\frac{n \times \Sigma (\bar{y}_i)^2 - (\Sigma \bar{y}_i)^2}{n \times (n - 1)} \right] - \frac{s_r^2}{2}$$

158 ISO 3534-1. Statistics – Vocabulary and symbols – Part 1: Probability and general statistical terms (1993).

159 Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002).

160 ISO 3534-1. Statistics – Vocabulary and symbols – Part 1: Probability and general statistical terms (1993).

Example 1: Chloramphenicol in fish tissue has been analysed by adding 0.3 µg/kg to six samples of fish and analysed. This has been repeated on two other days. An ANOVA calculation is used with excel spread sheet and the data analysis option as described in the Annex.

	Day 1	Day 2	Day 3
Conc. 1 (µg/kg)	0.36	0.28	0.28
Conc. 2 (µg/kg)	0.31	0.33	0.29
Conc. 3 (µg/kg)	0.36	0.33	0.35
Conc. 4 (µg/kg)	0.32	0.33	0.30
Conc. 5 (µg/kg)	0.34	0.30	0.30
Conc. 6 (µg/kg)	0.28	0.33	0.29

S_r = 0.026 µg/kg ~ 8.3 % (CV%)

S_{iR} = 0.029 µg/kg ~ 9.0 % (CV%)

Example 2:

Deoxynivelanol in wheat flour has been analysed by adding 50 µg/kg to samples of wheat flour and analysed in **duplicate**. This has been repeated on five other days.

A simplified setup is when analysing samples in duplicate on different days. Results are given in the table below and S_r and S_{iR} are calculated as described in the Annex.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Conc. 1 (µg/kg)	49.0	56.0	62.5	44.0	50.2	52.2
Conc. 2 (µg/kg)	60.0	64.6	55.5	45.5	44.1	63.0

S_r = 5.8 µg/kg ~ 10.7 %

S_{iR} = 7.7 µg/kg ~ 14.2 %

Reproductibility

Reproductibility sR is determinined from replicate analysis of e.g. reference material in at least two laboratories. This means that for a single-laboratory validation this laboratory variation will not be estiated:

$s_R^2 = s_r^2 + s_L^2$

Examples of upper limit for reproducibility standard deviation:

Where s_L is standard deviation between laboratories and s_R is the repeatability standard deviation.

Acceptance criteria for precision depend very much on the types of analysis. For pharmacuetical quality control, precision of better than 1% RDS is early attained, while for biological samples the precision is more like 16% at the detection limit and 10% at higher concentration levels. For environmental and food samples, the reproductibility is very much dependent on the sample matrix, the level of the analyte, and on the analytical method, being in the range of 2% to more than 20% RSD.

Table 1 indicates the recommended upper limit for reproductibility standard deviation according to several guidelines (5, 13). The within-laboratory reproductibility should be below these values. For low concentrations (<100 µg/kg) higher standard deviation can be accepted but in these cases, techniques that can reduce the spread should be studied (e.g. use of internal standard or use of matrix-matched calibration standards) and should be used if they reduce the variance significantly.

Concentration	The coefficient of variation
<100 µg/kg	23 % *
<100 µg/kg	23 % *
<500 µg/kg	18 %
1,000 µg/kg (=1 mg/kg)	18 %
16 %	
16 %	

(*) *For concentrations lower than 100 µg/kg Horwitz equation gives unacceptable high values. Recommended upper reproducibility standard deviation is 22-23 %.*[161/162]

161 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed. Document N° SANCO/12495/2011.

162 Eurachem Guide. The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. Version 1.0 (1998).

Both repeatability and reproducibility are generally dependent on analyte concentration, and so should be determined at a number of concentrations and if relevant, the relationship between precision and analyte concentration should be established. Relative standard deviation may be a more useful measure in this case because concentration has been factored out and so it is largely constant over the range of interest provided this is not too great.

For pesticides the recommended upper limit is 25% (reproducibility) and 20% (within-laboratory reproducibility).[163]

8.5.7.3. Qualitative analysis

Qualitative analysis is effectively a yes/no measurement at a given threshold of analyte concentration. For qualitative methods precision cannot be expressed as a standard deviation or relative standard deviation, but may be expressed as true and false positive (and negative) rates. These rates should be determined at a number of concentrations, below, at and above the threshold level.

Data from a confirmatory method comparison should be used if such an appropriate method is available. If such a method is not available fortified and unfortified blank samples can be analysed instead:

- % false positives = false positives X 100/total known negatives;
- % false negatives = false negatives X 100/total known positives.

8.5.7.4. Declaration of precision characteristics

The following boxes give two examples of declarations of repeatability and reproducibility, taken from published standards.

163 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed. Document N° SANCO/12495/2011.

REPEATABILITY AND REPRODUCIBILITY OF ALKALINE PHOSPHATASE ACTIVITY TEST IN MILK AND MILK BASED DRINKS

Repeatability

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in no more than 5% of cases be greater than the values for r given in Table 1.

Table 1: Repeatability limit, r values

Product	Alkaline phosphatase activity level mU/l				
	20	40	100	350	500
Cow milk	-	21.50	22.10	89.60	93.30
Sheep milk	10.43	16.26	33.67	96.82	99.76
Goat milk	8.63	7.98	26.20	42.83	28.56

Reproductibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, will in no more than 5% of cases be greater than the values for R given in Table 2.

Table 2: *Reproducibility Limit, r values*

Product	Alkaline phosphatase activity level mU/l				
	20	40	100	350	500
Cow milk	-	31.80	51.00	136.40	211.10
Sheep milk	16.63	20.34	46.63	170.24	233.10
Goat milk	10.69	20.55	28.71	127.89	87.51

Source: EN ISO 11816 – 1 – 2006 Determination of Alkaline Phosphatase Activity – Fluorimetric method – Milk and Milk Based Drinks

DETERMINATION OF FAT CONTENT IN MILK PRODUCTS AND MILK-BASED FOODS BY THE WEIBULL – BERNTROP GRAVIMETRIC METHOD

Interlaboratory test

The values for repeatability and reproducibility are expressed at the 95% probability level and were derived from the results of an inter-laboratory trial carried out on infant foods in accordance with ISO 5725.

Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in no more than 5% of cases be greater than the following values:

- for products having a fat content of more than 5% (mass fraction): 0.2 g of fat per 100 g of product;
- for products having a fat content of 5% (mass fraction) or less: 0.1 g of fat per 100 g of product;
- for liquid products: 0.05 g of fat per 100 g of product.

Reproductibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in no more than 5% of cases be greater than the following values:

- for products having a fat content of more than 5% (mass fraction): 0.4 g of fat per 100 g of product;
- for products having a fat content of 5% (mass fraction) or less: 0.2 g of fat per 100 g of product;
- for liquid products: 0.1 g of fat per 100 g of product.

Source: BS ISO 8262-1:2005 Milk products and milk-based foods. Determination of fat content by the Weibull – Berntrop gravimetric method (Reference method) – Part 1: Infant foods

8.5.8. Sensitivity

Sensitivity may be defined as the change in the response of a measuring instrument divided by the corresponding change in the stimulus. This is effectively the gradient of the response curve, *i.e.* the change in instrument response which corresponds to a change in analyte concentration.

Where the response has been established as linear with respect to concentration, *i.e.* within the linear range of the method, and the intercept of the response curve has been determined, sensitivity is a useful parameter to calculate and use in formulae for quantitation.

8.5.9. Ruggedness (or robustness)

The ruggedness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Assessment of ruggedness provides an indication of the method's reliability during normal usage.

Ruggedness

Ruggedness is the ability of a method to remain unaffected by small changes in operational parameters and provides an indication of its reliability during normal usage. It is recommended to determine ruggedness during method development and optimization since factors with a significant influence on performance could be relevant for changing the method.

A measure of an effective analytical method is how well its performance stands up to less than perfect implementation. In any method there will be certain stages which, if not carried out sufficiently carefully, will have a severe effect on method performance and may even result in the method not working at all. These stages should be identified, usually as part of method development, and if possible, their influence on method performance evaluated using 'ruggedness tests'.

This involves **making deliberate variations to the method, and investigating the subsequent effect on performance**. It is then possible to identify the variables in the method which have the most significant effect and ensure that, when using the method, they are closely controlled. Where there is a need to improve the method further, improvements can probably be made by concentrating on those parts of the method known to be critical.

Ruggedness is normally evaluated during method development, typically by the originating laboratory, before collaborating with other laboratories.

Determining ruggedness

For determining the ruggedness of a method a number of parameters, such as extraction time, mobile-phase pH, mobile-phase composition, injection volume, source of column lots and/or suppliers, temperature, detection wavelength, and the flow rate, are varied within a realistic range and the quantitative influence of the variables is determined. A factorial design (e.g. a Youden design[164]) is a nice design to test different parameters and their influence on the test result; or the factors can be evaluated one factor at a time. It is recommended to analyse each factor setup in triplicate.

Ion suppression or ion enhancement is a known phenomenon when using LC-MS (/MS) and this matrix effect should be known and investigated, especially when LC-MS is used. In this context matrix effect is defined as the effect of the purified sample matrix on the quantitative measurement result.

The matrix effect may be determined by injecting the same amount of analyte in the solvent with and without purified sample matrix.

There are no requirements that matrix effects are absent, but if significant, the influence on trueness and precision should be known as well as the influence of matrix type.

8.5.10. Recovery

Analytical methods do not always measure all of the analyte of interest present in the sample. The analyte may be present in a variety of forms in samples not all of which are of interest to the analyst. In many cases a method is deliberately designed to determine only a particular form of the analyte.

In some cases a method cannot be used to determine all of the analyte present in a sample matrix and this may be as a result of an inherent problem in the method. Either way, it is necessary to assess the efficiency of the method in detecting all of the analyte present.

Because it is not usually known how much of a particular analyte is present in a test portion it is difficult to be certain how successful the method has been at extracting it from the matrix. One way to determine the efficiency of extraction is to spike test portions with the analyte at various concentrations, then extract the fortified test portions and measure the analyte concentration.

The inherent problem with this is that analyte introduced in such a way will probably not be held as strongly as that which is naturally present in the test portion matrix and so the technique will give an unrealistically high value for the method recovery. It is however the most common way of determining recovery efficiency and it is recognized as an acceptable way of determining method recovery. It must be remembered that falsely high values for recovery may be obtained.

164 W.J. Youden and E.H. Steiner, *Statistical Manual of the AOAC–Association of Official Analytical Chemists*. AOAC-1, Washington, DC, 1975.

Alternatively it may be possible to carry out recovery studies on reference materials, if suitable materials are available. Provided these have been produced by characterization of natural materials rather than by characterization of synthetic materials into which the analyte has been spiked, than the recovery values obtained should accurately represent the recoveries obtained by extraction of real test portions.

8.6. TOOLS OF VALIDATION

8.6.1. Blanks

Use of various types of blanks enable assessment of how much of the measured signal is attributable to the analyte and how much to other causes.

8.6.1.1. Reagent blanks

Reagents used during the analytical process (including solvents used for extraction or dissolution) are each analysed in isolation without any sample to determine whether they contribute to the measurement signal. The measurement signal arising from the analyte can then be corrected accordingly. Care should be taken to ensure that reagent blanks are sufficiently low before such blank values are used for correction of results.

8.6.1.2. Sample blanks

These are essentially matrices with no analyte. They are difficult to obtain but when such materials are available, they provide a realistic estimate of interferences that would be encountered in the analysis of test sample.

8.6.2. Samples/test materials

Test materials taken from real samples are useful because of the information they yield on interferences etc. which could be realistically encountered in day-to-day work. If the true analyte content of a test material is accurately known it can be used as a way of assessing the accuracy of the method. However the true analyte content is usually difficult to determine unless there is the possibility of using other methods which are known to show negligible bias.

8.6.3. Fortified materials/solutions

These are materials or solutions which have been fortified with the analyte(s) of interest. The fortification is usually made by addition of a known quantity of the analyte to the sample. These materials or solutions may already contain the analyte of interest so care is needed lest fortification inadvertently leads to levels outside of the range of applicability of the method.

Fortification with a known amount of analyte enables the increase in response to the analyte to be measured and calculated in terms of the amount added (assuming 100 % recovery), even though the absolute amounts of analyte present before

and after the fortification are not known. Note that most methods of fortification add the analyte in such a way that it will not be as closely bound to the sample matrix as it would be if it was present naturally. Therefore, recovery determinations obtained by fortification can be expected to be unrealistically high.

8.6.4. Spiked materials

These are similar to fortified materials and to some extent the terms are interchangeable. "Spiking" is the addition of a substance to the sample. However it does not necessarily have to be restricted to the analyte of interest. It could include anything added to the sample in order to gauge the effect of the addition. For example the sample could be spiked with varying amounts of a particular interference in order to judge at what concentration of the interfering, determination of the analyte was adversely affected. The nature of the spike obviously needs to be identified.

8.6.5. Incurred materials

These are materials in which the analyte of interest may be essentially foreign, but it has been introduced to the bulk at some point, prior to the material being sampled. The analyte is thus more closely bound in the matrix than it would be had it been added by spiking. The analyte value will depend on the amounts of analyte in contact with the material, the rates of take-up and loss by the matrix and any other losses through metabolism. The value of incurred sample for calibration purposes depends on how well the analyte value can be characterized.

The following are examples of incurred materials:

- herbicides in flour from cereal sprayed with herbicides during its growth;
- growth promoters in meat derived from animals fed with feeds containing the promoters.

8.6.6. Independently characterised materials

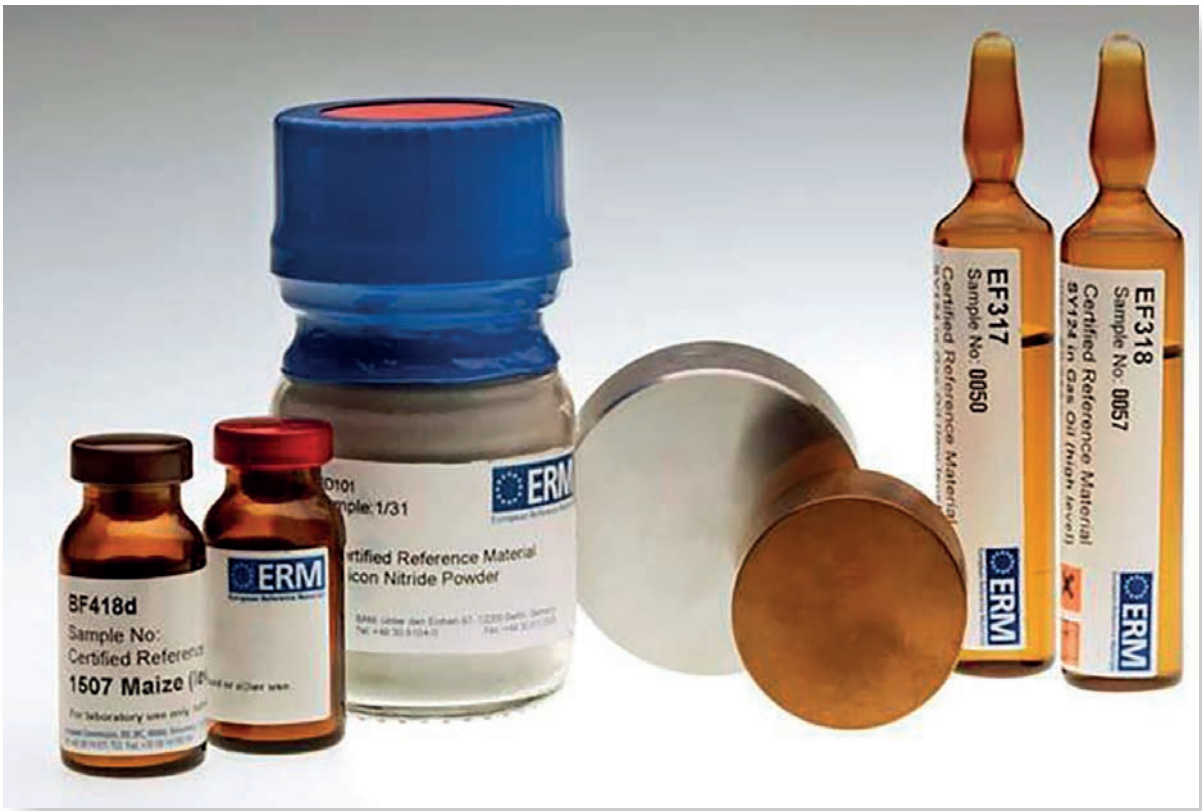
It is difficult to determine the bias of a method without knowing the true analyte content of the test material. If a material has been characterized by other means, for example, by a method which is known to have negligible bias, then it can be used as a reference material, a comparison can be made and the bias of the method under examination assessed.

8.6.7. Measurement standards

These are traditionally thought of as solutions of single substances but in practice can be anything in which a particular parameter or property has been characterized to the extent it can be used for reference or calibration purposes. The term standard includes items in which a range of physical parameters may be calibrated (*e.g.* a calibrated thermometer). Strictly, these are physical standards.

8.6.8. Reference materials and certified reference materials

A reference material is a material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.



Reference materials can be virtually any material used as a basis for reference, and could include laboratory reagents of known purity, industrial chemicals, or other artefacts. The property or analyte of interest needs to be stable and homogenous but the material does not need to have the high degree of characterization, traceability and certification normally associated with certified reference materials.

A Certified reference material is a reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure, which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

The characterization of the parameter of interest in a certified reference material is generally more strictly controlled than for a reference material, and in addition the characterized value is certified with a stated uncertainty by a recognized institution.

Characterization is normally done using several different methods, so that as far as possible, any bias in the characterization is reduced or even eliminated.

8.6.9. Replication

If used correctly, replicate analysis gives the analyst more information on the underlying statistics behind a particular measurement. Experiments involving replicate analysis should be designed to take into account all of the variations in operational conditions which can be expected during routine use of the method. The aim should be to determine typical variability and not minimum variability.

8.7. USING VALIDATED METHODS

When using methods developed elsewhere within the laboratory, a published method, or even a standard or regulatory method, there are two issues which need to be considered.

Is the existing validation data adequate for the required purpose or is further validation necessary?

If the existing validation data is adequate, then is the laboratory able to achieve the level of performance claimed possible in the method?

In other words:

- Is the analyst sufficiently competent?
- Are the available equipment and facilities adequate?

Generally, standard methods have been validated by some form of collaborative study and the standardization bodies which produce them frequently have statistical experts to help ensure that validation studies are correctly designed, performed and evaluated. It is dangerous to assume that just because a method is published as a National or International standard that you can take for granted that its published validation data will be fully applicable for the sample matrix or analyte concentration of all samples to which a particular laboratory may apply the method.

It is often assumed that standard methods can be used straight off the shelf and the published performance data achieved straight away by whoever uses the method. This is not a safe assumption.

Even those who are familiar or expert in the particular types of analysis covered by the method will need to practice before becoming fully proficient. When using validated methods (or for that matter any methods) the following rules are recommended to ensure that acceptable performance is achieved:

- The analyst should make themselves completely familiar with a new method before using it for the first time.
- Ideally the method will first be demonstrated to the analyst by someone already expert in its use.
- The analyst should then use it under initially close supervision, working with reference materials or practice samples.
- The level of supervision will be stepped down until the analyst is considered to be sufficiently competent to 'go solo'.
- Competence might be established by assessing the analyst's ability to achieve the levels of performance stated in the method, such as repeatability and limit of detection etc.

This is typical of the way someone might be trained to use a new method and laboratory training procedures will frequently be designed in this way with objective measures in place to test competence at intervals during the training.

The analyst should have read through the method and familiarized themselves with the theory behind the measurement, mentally rehearsing the various stages, identifying points where breaks can be taken, and parts of the process where the analyst is committed to continuous work.

Furthermore where reagents need to be prepared, the analyst will need to consider how stable they are once prepared and whether they need to be prepared in advance. A classic pitfall is to spend several hours preparing a number of samples and then finding the preparation of the reagent needed for the next stage of the work involves a complicated synthesis. Meanwhile the samples themselves are degrading.

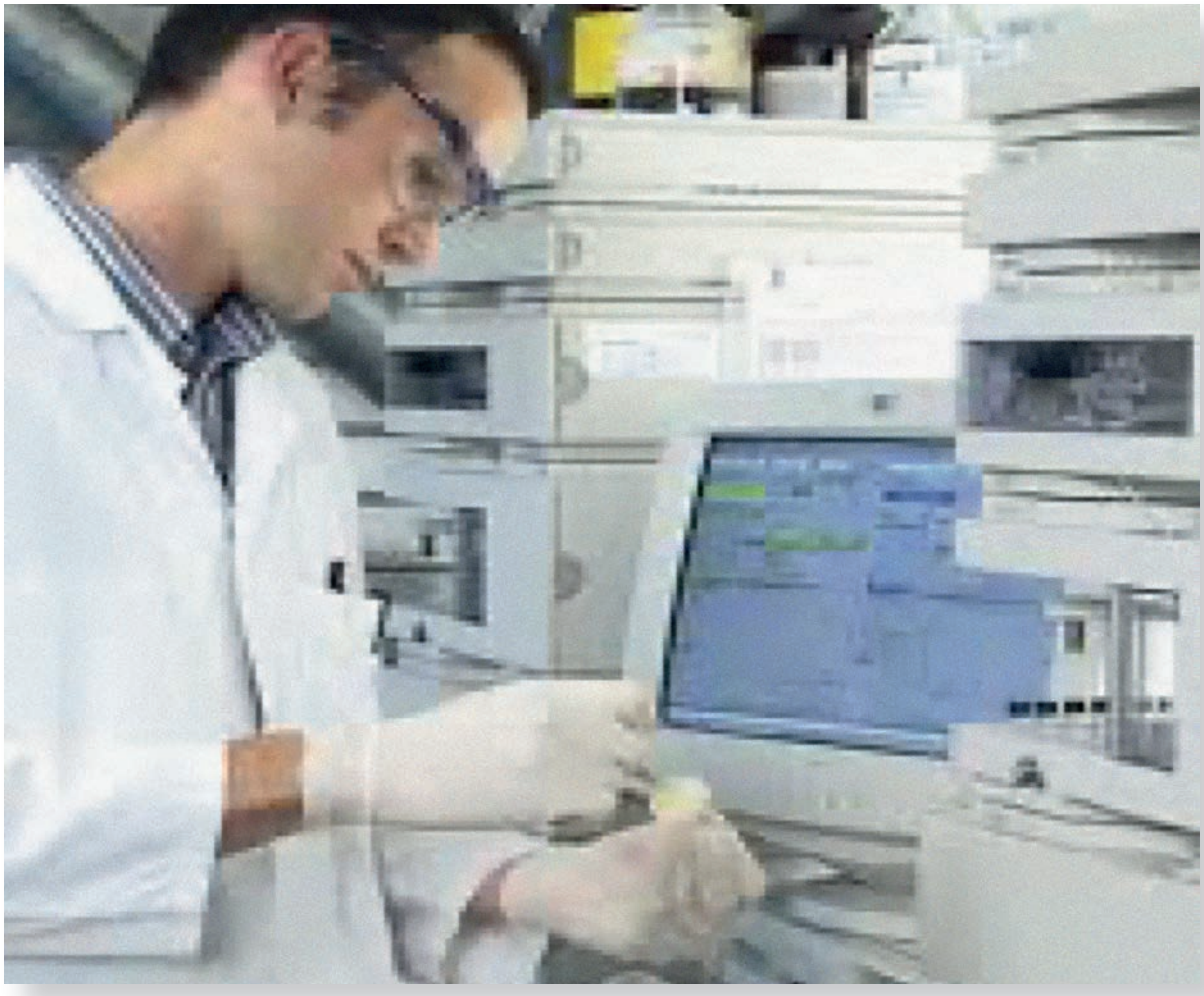
An assessment needs to be made of how many samples can be conveniently handled at a time. It is better to analyse a few samples well than to try to analyse a large number and have to repeat most of them.

The analyst should make sure everything needed for the method is available before work is started. This involves gathering together the right sort of equipment, reagents and standards (with any attendant preparation), perhaps reserving space in fume-cupboards etc.

If it is necessary to adapt or change someone else's validated method then appropriate revalidation will be necessary. Depending on their nature, the changes may well render the original validation data irrelevant.

8.8. USING VALIDATION DATA TO DESIGN ANALYTICAL QUALITY CONTROL

8.8.1. Analytical quality control



'Quality control' and 'quality assurance' are terms that are frequently used interchangeably. In practice quality assurance in analysis relates to the overall measures taken by the laboratory to ensure and regulate quality, whereas quality control describes the individual measures which are taken to monitor and control particular analytical procedures.

Method validation gives an idea of a method's performance capabilities and limitations which may be experienced in routine use while the method is in control. In routine use, specific controls need to be applied to the method to verify that it remains in control, *i.e.* it is performing in the way expected. During the validation stage the method is largely applied to samples of known content. Once the method is in routine use it is used for samples of unknown content.

It is common practice in many laboratories to continue analysing samples of known content alongside samples that are being routinely analysed and where the analyte content is not necessarily known. In this way the analyst can evaluate whether the variety of results obtained truly reflects the diversity of samples analysed or whether unexpected and unwanted changes are occurring in the method performance. It is good practice for these known samples to be analysed with every batch of samples as part of the quality control process.

The types and number of checks carried out will depend on the nature, criticality and frequency of the analysis, batch size, degree of automation, and test difficulty and also on the lessons learnt during method development and validation.

Quality control can take a variety of forms, both within the laboratory (internal) and between the laboratory and other laboratories (external), using the same or similar methods of analysis.

8.8.2. Internal QC

Internal quality control includes the use of some of the above validation tools (blanks, chemical calibrators, spiked samples, blind samples, replicate analyses and QC samples) to routinely monitor some of the performance parameters of the test.

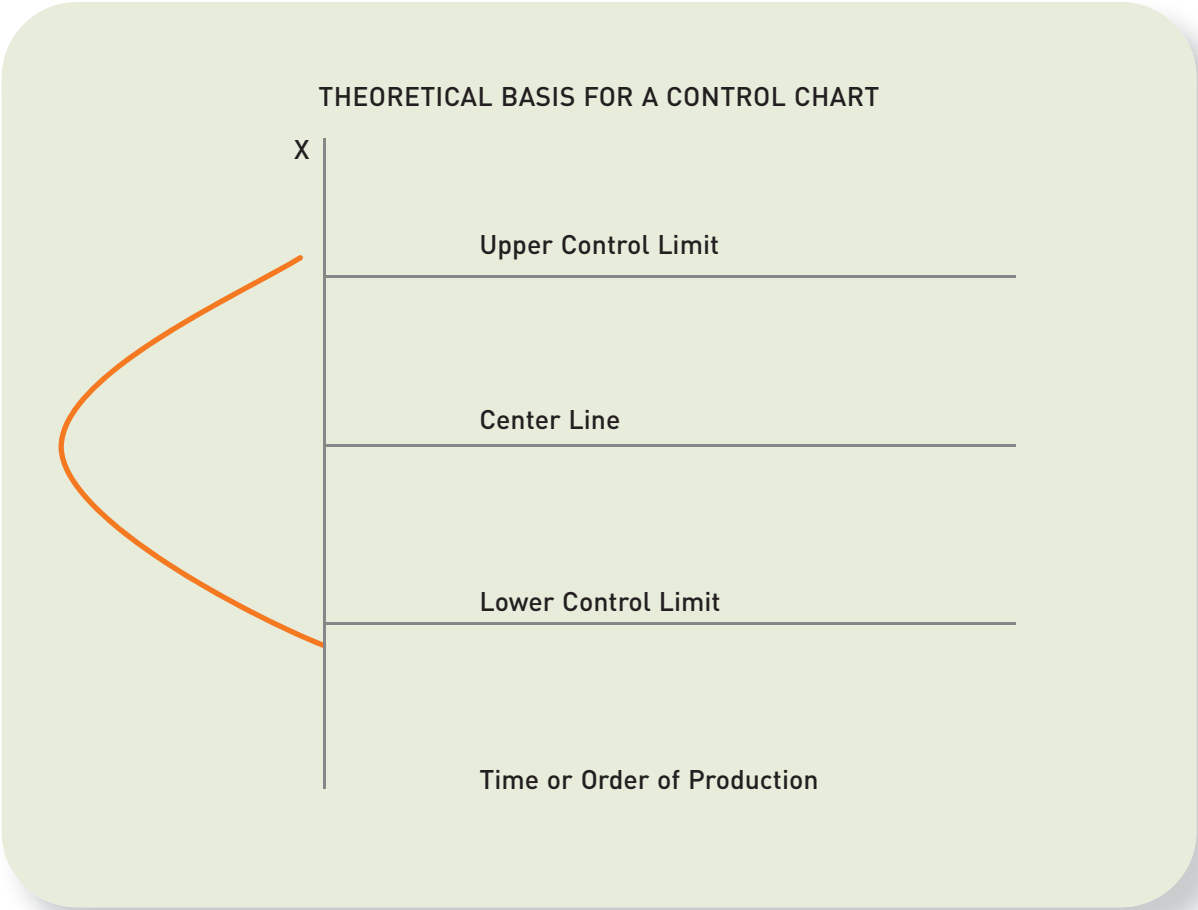
The QC procedures adopted must be sufficient to ensure the validity of the results. Different sorts of quality control may be used to monitor different types of variation within the process. QC samples, analysed at intervals in the analytical batch will indicate drift in the system.

Use of various types of blank will indicate the various sources of contributions to the instrument signal besides those that are from the analyte. They enable the analyst to ensure that calculations made for the analyte can be suitably corrected to remove any contributions to the response which are not attributable to the analyte.

Duplicate analyses provide a means of checking the repeatability of the method. QC samples are typical samples which over a given period of time are sufficiently stable and homogeneous to give the same result (subject to random variation in the performance of the analytical method) and available in sufficient quantities as to be available for repetitive analysis. They provide a means of checking for changes in the precision of an analytical procedure, which could adversely affect the result. Replicates can be adjacent in a batch (to check repeatability) or placed randomly (to check for drift).

Blind analysis is effectively a form of repeat analysis and provides a means of checking precision. It consists of replicated test portions placed in the analytical batch, possibly by the laboratory supervisor, and is referred to as blind analysis because the analyst is not normally aware of the identity of the test portions or that they are replicates. In this way the analyst has no preconceived ideas as to how the particular results obtained in a batch of analyses, should be related.

Standards and chemical calibrators placed at intervals in an analytical batch enable checks to be made to confirm that the response of the analytical process to the analyte is stable.



The use of control charts is recommended, particularly for monitoring results from QC control samples. These charts are often referred to as Shewart control charts. Over a period the random variation in performance of the analytical method can be monitored by monitoring the analysed value of the QC sample, usually by plotting it on the control chart. Limits may be set for the values on the chart (conventionally 'warning limits' are set at ±2σ (±2s) about the mean value, and 'action limits' are set at ±3σ (±3s) about the mean value.

Provided the plotted QC values conform to certain rules pertaining to the set limits, the QC is considered to be satisfactory. As long as the QC sample value is acceptable it is likely that results from samples in the same batch as the QC sample can also be regarded as acceptable.

In order to set realistic limits on the control chart, the initial calculations of mean and standard deviation must reflect the way the method is actually intended to be used on a day-to-day basis. These initial calculations of mean and standard deviation should be derived in such a way that all possible variations in operating conditions: different analysts; variations in laboratory temperature etc., are taken into account. If this is not done, then the standard deviation will be unrealistically small, resulting in limits being set on the chart, which cannot possibly be complied with in normal use.

The acceptability of the value obtained with the QC sample should be verified as early as practicable in the analytical run so that in the event of a problem as little effort as possible has been wasted in generating unreliable results for the analysis of the samples themselves. Where the results from QC samples indicate that the results for a particular batch of analysis are unreliable, all results for that batch must be discarded and the analysis of all samples from that batch, repeated.

It is the responsibility of the laboratory management to set and justify an appropriate level of quality control, based on risk assessment, taking into account the reliability of the method, the criticality of the work, and the feasibility of repeating the analysis if it doesn't work correctly first time.

It is widely accepted that for routine analysis, a level of internal QC of 5 % has been identified as reasonable, *i.e.*, 1 in every 20 samples analysed should be a QC sample. However, for routine methods with high sample throughput that during method validation have been shown to be rugged, a lower level of QC may be acceptable. For more complex procedures, a level of 20 % is not unusual and on occasions even 50 % may be required.

For analyses performed infrequently, a full system validation should be performed on each occasion. This may typically involve the use of a reference material containing a certified or known concentration of analyte, followed by replicate analyses of the sample and spiked sample (a sample to which a known amount of the analyte has been deliberately added). Those analyses undertaken more frequently should be subject to systematic QC procedures incorporating the use of control charts and check samples.

8.8.3. External QC

Perhaps the most common example external quality control is proficiency testing (also known as external quality assessment). Regular participation in proficiency testing schemes provides a recognised way for a laboratory to monitor its performance against both its own requirements and the norm of peer laboratories at national and international levels.

Proficiency testing helps to highlight reproducibility performance between laboratories and systematic errors *i.e.* bias. It can also be used to determine repeatability but this can also be checked more cost effectively using internal controls.

Accreditation bodies recognise the benefit of these schemes and strongly encourage laboratories to participate in proficiency testing as an integral part of their quality assurance protocols. It is important to monitor proficiency testing results as a means of checking quality assurance and take action as necessary. In certain instances, accreditation bodies may specify participation in a particular proficiency testing scheme as a requirement of accreditation.

The value of proficiency testing is of course only as good as the schemes themselves. Very often there may not be a scheme available which is relevant to the types of analysis that the laboratory wishes to check, especially if it is working in isolation.

Just as testing laboratories are required to be accredited to ISO 17025, the providers of proficiency testing services are required to be accredited to ISO 17043 – Conformity assessment – General requirements for proficiency testing.

8.8.4. Implications of validation data for calculation of results and reporting

It is important that the analyst is able to translate the data, generated during analysis of samples using the validated method, into answers which directly relate to providing the analytical results appropriate to the needs of the client.

The performance characteristics established during the validation process help to do this. Precision data for repeatability and reproducibility can be used to establish whether differences found when analysing samples are significant. Quality controls based on the validation data can be used to confirm that the method is in control and producing meaningful results. Estimation of the measurement uncertainty, associated with the method performance, enables expression of the result as a range of values in which the true value for the measurement can be said to lie with an accepted level of confidence. It is important that the analyst has access to validation data which can be used to support the validity of the results. This information should be available to be passed to the customer, on request.

Issues such as method validation, variability and measurement uncertainty need to be treated carefully in certain circumstances, such as for example, in legal or forensic contexts. It may be better to be open about the existence of uncertainty attached to measurements and be prepared to justify decisions made in the light of knowing that uncertainty.

Care also needs to be taken when trying to use an analytical result with its accompanying uncertainty to try to decide whether or not the original consignment from which the sample has been taken complies with a specification or limit. Sampling decisions may impact on the results obtained and such issues are not the responsibility of the analyst. However the analyst may be required to provide technical advice to assist in the decision making process.

When reporting results, the analyst must decide whether to correct for any biases which may have been detected or to report results uncorrected but acknowledge the existence of the bias.

Care should also be taken when reporting results as ‘not detected’. On its own this statement is meaningless. It is much better to report the result as less than the value for the limit of detection.

8.9. DOCUMENTATION OF VALIDATED METHODS

8.9.1. Need for documentation

Once the validation process is complete it is important to document the procedures so that the method can be clearly and unambiguously implemented. There are a number of reasons for this.

The various assessments of the method made during the validation process assume that, in use, the method will be used in the same way each time. If it is not, then the actual performance of the method will not correspond to the performance predicted by the validation data. Therefore, the method must be documented in such a way as to minimize the possibility of accidentally introducing variations to the method that adversely affect its performance. Proper documentation of methods is required for auditing and evaluation purposes and may also be required for contractual or regulatory purposes. Appropriate documentation of the method will help to ensure consistent application of the method from one occasion to the next.

8.9.2. Contents of validation documentation

The contents of a typical documented procedure are shown in the box below.

Typical documented procedure

Written procedures should contain at least the following information:

- appropriate identification;
- scope;
- description of the type of item to be tested or calibrated;
- parameters or quantities and ranges to be determined;
- apparatus and equipment, including technical performance requirements;
- reference standards and reference materials required;
- environmental conditions required and any stabilization period needed;
- description of the procedure, including:
  - affixing of identification marks, handling, transporting, storing and preparation of items,
  - checks to be made before the work is started,
  - checks that the equipment is working properly and, where required, calibration and adjustment of the equipment before each use,
  - the method of recording the observations and results,
  - any safety measures to be observed;
- criteria and/or requirements for approval/rejection;
- data to be recorded and method of analysis and presentation;
- the uncertainty or the procedure for estimating uncertainty.

8.9.3. Documents control

Documented methods form an important part of a laboratory's quality system and should be subject to an appropriate degree of document control. The purpose of this is to ensure that only methods and procedures which have been authorised as fit for use are actually used. Therefore as part of the documentation process, methods should carry information which enables the user to judge whether the method has been authorised for use and whether it is complete.

Other information should be available regarding the version number and date of the method; the author; how many copies of the method exist; and any copying restrictions.

From time to time methods may require updating. The technology on which the analytical procedure is based may for example have been improved, necessitating amendments to the documentation. Document control enables the smooth withdrawal of obsolete methods and issue of revised methods. Changes should be made only by those so authorised. This may be controlled in word processing where the relevant files may have widespread 'read-only' access and very limited 'write' access.

More details of the content and protocols for method documentation are shown in the Annex.

8.10. REVIEW AND EXTENSION OF VALIDATED METHODS

It is important to remember that analytical methodologies applied by a laboratory should be in a constant state of development. Methods should be periodically reviewed to ensure that they remain valid in the light of current scientific knowledge. Furthermore, the laboratory may wish to extend the scope of its accreditation to accommodate new or variant methods.

8.10.1. Review of methods

In reviewing existing methods a list of all required changes and updates should be produced. These should be classified as minor changes, major changes or quality improvements as follows:

- Minor changes; these would not substantially affect the methodology.
- Quality improvements; these would include changes to methodology that are likely to improve the quality of the results.
- Major changes; could be one of four types:
  - changes to enumeration methods
  - changes to presence/absence methods
  - changes to identification tests
  - new methods leading to an extension of the scope of accreditation

All staff working with the methods should be notified and appraised of any changes. A documentary record should be kept of changes to methods and should include the date they were introduced and the date and the way staff were informed. Training records should be updated as necessary and any further evidence of competency should be documented and retained.

Relevant customers and the accreditation body may also need to be informed of changes to methods that could have an impact on test results. Participation in recognized proficiency testing schemes can provide valuable information that can be used by individual laboratories for validation of their revised methods.

8.10.2. Extension of the scope of accreditation

Some changes to methods may be considered as extensions to a laboratory's scope of accreditation. This depends on the significance of the change. For example, changes in identification (confirmatory) tests where one test replaces another or is adapted, and changes to reporting arrangements, are significant changes. In such instances the accreditation body should be provided with evidence of retraining of staff, to demonstrate laboratories can perform the test, as well as IQC (spike) results.

However, on the other hand, new methods that a laboratory wants to add to its scope of accreditation that are based on published validated methods would not normally need full validation. However, laboratories would be expected to validate methods where this has not already been done. They may also be expected to demonstrate satisfactory performance in their laboratory. The following information should be submitted to the accreditation body to demonstrate that the laboratory is capable of performing the test and producing accurate results:

- details of the method;
- IQA records, based on in-house IQA scheme as described in the Quality Manual;
- copies of training records, including records for the assessment of competence, of all staff likely to use the method, based on own in-house training criteria as described in own Quality Manual;
- evidence of satisfactory performance in at least one EQA distribution (if an EQA scheme exists for the method);
- some example reports (if any changes affect the reporting of results).

8.11. VALIDATION OF MICROBIOLOGICAL TEST METHODS

8.11.1. Approach to validation of microbiological methods

Unlike the situation that exists for the validation of methods for chemical analysis of samples, very few certified reference materials exist for validation of methods for microbiological examination of samples. Furthermore the laboratory must validate existing standard methods whenever they are applied to sample types that are not specified in the original standard procedure.

A special approach is therefore required, and the validation of microbiological test methods should reflect actual test conditions. This may be achieved by using naturally contaminated products or products spiked with a predetermined level of contaminating organisms. For validation of microbiological methods, food products should be used that are naturally contaminated with organisms that may compete with the test organism.

The analyst should be aware that the addition of contaminating organisms to a matrix only mimics in a superficial way the presence of the naturally occurring organisms. However, it is often the best and only solution available. The extent of validation necessary will depend on the method and the application.

Within the laboratory's safety guidelines, up to 5 different strains should be tested. Two individuals should carry out the tests at the same time, and both should recover the test organism. As a minimum the strain maintained for IQA should be used. Laboratories should also use "wild" strains if available.

For quantitative microbiological test methods, the specificity, sensitivity, relative trueness, positive deviation, negative deviation, repeatability, reproducibility and the limit of determination within a defined variability should be considered and, if necessary, quantitatively determined in assays. The differences due to the matrices must be taken into account when testing different types of samples. The results should be evaluated with appropriate statistical methods.

Laboratories should retain validation data on commercial test systems (kits) used in the laboratory. These validation data may be obtained through collaborative testing and from validation data submitted by the manufacturers and subjected to third party evaluation *(e.g.* AOAC). If the validation data are not available or not wholly applicable, the laboratory shall be responsible for completing the validation of the method.

If a modified version of a method is required to meet the same specification as the original method, then comparisons should be carried out using replicates to ensure that this is the case. Experimental design and analysis of results must be statistically valid.

Even when validation is complete, the user will still need to verify on a regular basis that the documented performance can be met, *e.g.* by the use of spiked samples or reference materials incorporating relevant matrices.

The specific approach will depend on the type of tests, and in the following paragraphs, test for enumeration, presence/absence and identification are considered.

8.11.2. Enumeration methods

Reproducibility, repeatability and limits of detection of a new enumeration method should be established to demonstrate that the new method performs satisfactorily.

Assessment of the performance of a method should include tolerance and uncertainty of measurement, as well as reproducibility, repeatability and limits of detection.

- Reproducibility of the test should be assessed by at least 2 individuals per laboratory, working in parallel from the same homogenate at the same time,

and using the same batch of media. Assessment of the counts in at least 5 positive samples (spiked or natural, from a range of different food types) should be performed by each individual to establish whether or not the results are reproducible within the laboratory. Results should be within log10 0.5 of each other.

- Repeatability of the test should be assessed by one individual repeating the test on at least 5 replicates from the same homogenate of a single representative sample. Results should be within log10 0.5 of each other.
- Limits of detection of the test should be assessed by preparing a suspension of a test organism, and testing 5 10-fold dilutions (containing low numbers of organisms) in at least 5 food types. It is recommended that one reference organism, preferably a control strain used for IQA, should be used. Limits of detection can be determined as the lowest number of organisms that can be recovered in the sample size.

8.11.3. Presence/absence methods

Qualitative microbiological test methods, such as where the result is expressed in terms of detected / not detected and confirmation and identification procedures, should be validated by determining, if appropriate, the specificity, relative trueness, positive deviation, negative deviation, matrix effect, reproducibility and limit of detection.

In particular reproducibility and limits of detection of a new presence/absence method should be established to demonstrate that the new method performs satisfactorily. It is recommended that different food types (e.g., raw, dry etc.), representative of the range of products likely to be processed routinely for a particular organism, should be tested.

- Reproducibility of the test should be assessed by at least 2 individuals per laboratory, working in parallel on duplicate homogenates (A and B) at the same time, and using the same batch of media. Sample A should be tested by the new proposed method and produce a negative result (negative control). Sample B should be 'spiked' with the test organism, at about 25 to 50 cfu per 25 gram of food product. The target organism should be recovered from sample B by both individuals.
- Limits of detection of the method should be assessed by preparing a suspension of known numbers of a test organism and testing in 3 10-fold dilutions (to include low numbers of organisms). Different food types should be used. This should be performed in duplicate either by the same or different individuals, on the same or on separate occasions.

8.11.4. Identification tests

Reproducibility of a new identification test using both known positive and negative controls should be established to demonstrate that the new method performs satisfactorily. The test organisms should be those used for IQA.

Reproducibility of the test should be determined by its performance, at least once, by every member of staff who will be carrying it out on sample isolates. The correct result and interpretation should be obtained on each occasion. The date and results should be recorded in their training records.

8.11.5. Validation of alternative microbiological methods

EU Regulation No. 2073/2005 on microbiological criteria for foodstuffs requires that the reference methods for the microbiological examination of foods are those that that are published by ISO and/or CEN.

A reference method is the appropriate AOAC, FDA/BAM or USDA reference culture procedure that is applicable to the analyte and sample type that the method is intended to detect. Other internationally recognized methods may also be appropriate reference methods and will be considered on a case-by-case basis.

However, food business operators are permitted to use alternative, rapid methods for their routine microbiological examination of food, provide that it can be shown that an alternative method produces results that are equivalent to those obtained by the recognised reference methods of examination. The following guidelines are applicable to the validation of alternative methods for the microbiological examination of food.

- AOAC Guidelines for Validation of Qualitative and Quantitative Food Microbiological Official Methods of Analysis
- EN ISO 16140 – Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods.

EN ISO 16140 includes the following definition of an alternative method as a "method of analysis that demonstrates or estimates, for a given category of products, the same analyte as is measured using the corresponding reference method". The method can be proprietary or non-commercial, and does not need to cover an entire analysis procedure, *i.e.* from the preparation of samples to the test report.

The alternative method exhibits attributes should be appropriate to the users' needs, for example in terms of:

- speed of analysis and/or response;
- ease of execution and/or automation;
- analytical properties (precision, accuracy, limit of detection etc.);
- miniaturization;

The term 'alternative' is used to refer to the entire 'test procedure and reaction system'. This term includes all ingredients whether material or otherwise, required for implementing the method.

In the AOAC protocol, an alternative method is defined as "Method of analysis that demonstrates or estimates, for a given category of products, the same analyte as is measured by using the corresponding reference method". The method can be proprietary or non-commercial and does not need to cover an entire analysis procedure, that is, from the preparation of samples to the test results. Under the AOAC protocol, there are 3 options for the validation of alternative methods.

These are:

- Official Methods SM Program (OMA)
- Peer-Verified Methods SM Program (PVM)
- Performance Tested Methods SM Program (PTM)

OMA methods are validated by an inter-laboratory Collaborative Study in which experienced, competent analysts work independently in different laboratories, using a specific method to analyse replicated test samples for a particular analyte. The method to be collaboratively studied may be subjected to ruggedness testing prior to the Methods Comparison or Collaborative Study to determine its behaviour under various in-house operating conditions. More details are available in the AOAC Protocol.

8.12. ANNEXES

A.1. Definitions and references

Definitions

Accuracy: Closeness of agreement between a test result and the accepted reference value. It is determined by determining trueness and precision.[165]

Alpha error (α) means the probability that the tested sample is compliant, even though a non-compliant measurement has been obtained (risk that an innocent person goes to prison).[166]

Analyte: The substance that has to be detected, identified and/or quantified by application of the analytical method.[167]

Beta error (ß) means the probability that the tested sample is truly non-compliant, even though a compliant measurement has been obtained (risk that a guilty is released).[168]

Bias: The difference between the expectation of the test result and an accepted reference value.[169]

Calibration curve: A function which reflects the correlation between the content of an analyte in a sample, and the resulting measurement response.[170]

Decision limit, CCα: The limit at and above which it can be concluded with an error probability of α that a sample is non-compliant.[171]

165 ISO 3534-1. Statistics – Vocabulary and symbols – Part 1: Probability and general statistical terms (1993).
166 Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002).
167 *Ibid.*
168 *Ibid.*
169 ISO 3534-1. Statistics – Vocabulary and symbols – Part 1: Probability and general statistical terms (1993).
170 NMKL Procedure No. 4. Validation of chemical methods (2009).
171 Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002).

Degrees of freedom: Number of independent determinations of a given statistical size that can be implemented on the basis of a given dataset.

Detection capability, CCß: The smallest content of the substance that may be detected, identified and / -or quantified in a sample with an error probability of ß.[172]

Limit of detection: The smallest amount or concentration of analyte in the test sample that can be reliably distinguished from zero.[173]

Limit of quantification: The lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test.[174]

Linearity: The ability of the method to obtain test results proportional to the concentration of analyte.[175]

Measurement uncertainty: A parameter associated with the measurement result which characterizes the dispersion values that are reasonably attributable to the analyte.[176]

Measuring range: The range in which the method can be regarded as validated and which gives and acceptable trueness and precision.[177]

Precision: Closeness of agreement between independent test results obtained under stipulated conditions.[178]

Recovery: Percentage of the true concentration of a substance recovered during the analytical procedure.[179]

Repeatability: Precision under repeatability conditions.[180]

Repeatability conditions: Conditions where independent test results obtained with the same method on identical samples in the same laboratory by the same operator using the same equipment within short time interval.[181]

Reproducibility: Precision under reproducibility conditions.[182]

Reproducibility conditions: Conditions where test results are obtained with the same method on identical samples in different laboratories with different operators using different equipment.[183]

---

172 *Ibid.*

173 M. Thompson, S.L.R. Ellison and R. Wood, "Harmonised guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report)", *Pure Appl. Chem.*, vol. 74, No. 5, 2002, pp. 835-855.

174 Eurachem Guide, The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics, Version 1.0, 1998.

175 *Ibid.*

176 Eurachem, Terminology in Analytical Measurement – Introduction to VIM 3, 1st ed., 2011.

177 NMKL Procedure No. 4. Validation of chemical methods, 2009.

178 ISO 3534-1, *Statistics – Vocabulary and symbols* – Part 1: *Probability and general statistical terms*, 1993.

179 Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002.

180 ISO 3534-1, *Statistics – Vocabulary and symbols* – Part 1: *Probability and general statistical terms*, 1993.

181 *Ibid.*

182 *Ibid.*

183 *Ibid.*

Ruggedness: The resistance to change in the results produced by an analytical method when minor deviations are made from experimental conditions described in the procedure.[184]

Selectivity: The degree to which a method can quantify the analyte accurately in the presence of interferences.[185]

Specificity: The ability of a method to measure only what it is intended to measure.

Trueness: Closeness of agreement between the average value obtained from a large series of test results and an accepted reference value.[186]

Within-laboratory reproducibility: Within laboratory variation: different days, different analysts, different equipment etc.[187]

A.2. Calibration curve

The calibration curve should consist of minimum 5-6 concentration points and it is recommended to analyse each point in duplicate. Calibration data of C-vitamin with the concentration range 2.5 to 100 µg vitamin C/ml is shown below. Each level is analysed in duplicate giving a total of two repetitions.

µg/mL	Response
2,5	92023
2,5	91892
5	187248
5	186126
10	357074
10	355749
25	915327
25	917891
50	1807727
50	1853189
100	3604581
100	3637516

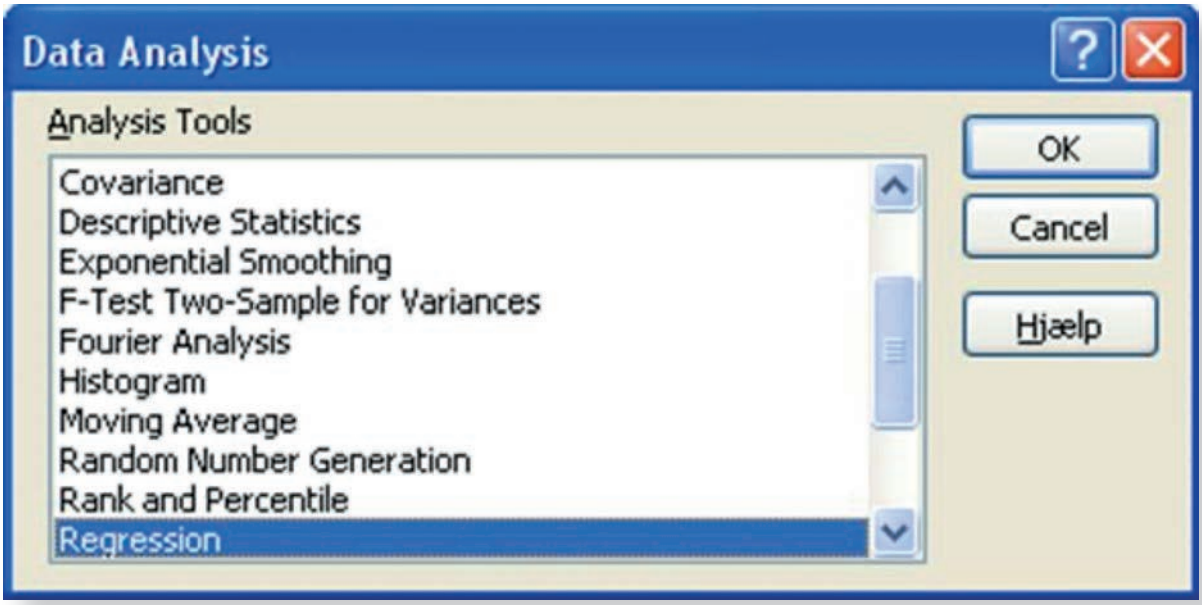
Plot of calibration curve, residual plot, residual output and residual plot can be done by using e.g. Excel spread sheet and the Data Analysis option:

184 M. Thompson, S.L.R. Ellison and R. Wood, "Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report)", *Pure Appl. Chem.*, vol. 74, No. 5, 2002, pp. 835-855.

185 Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002.

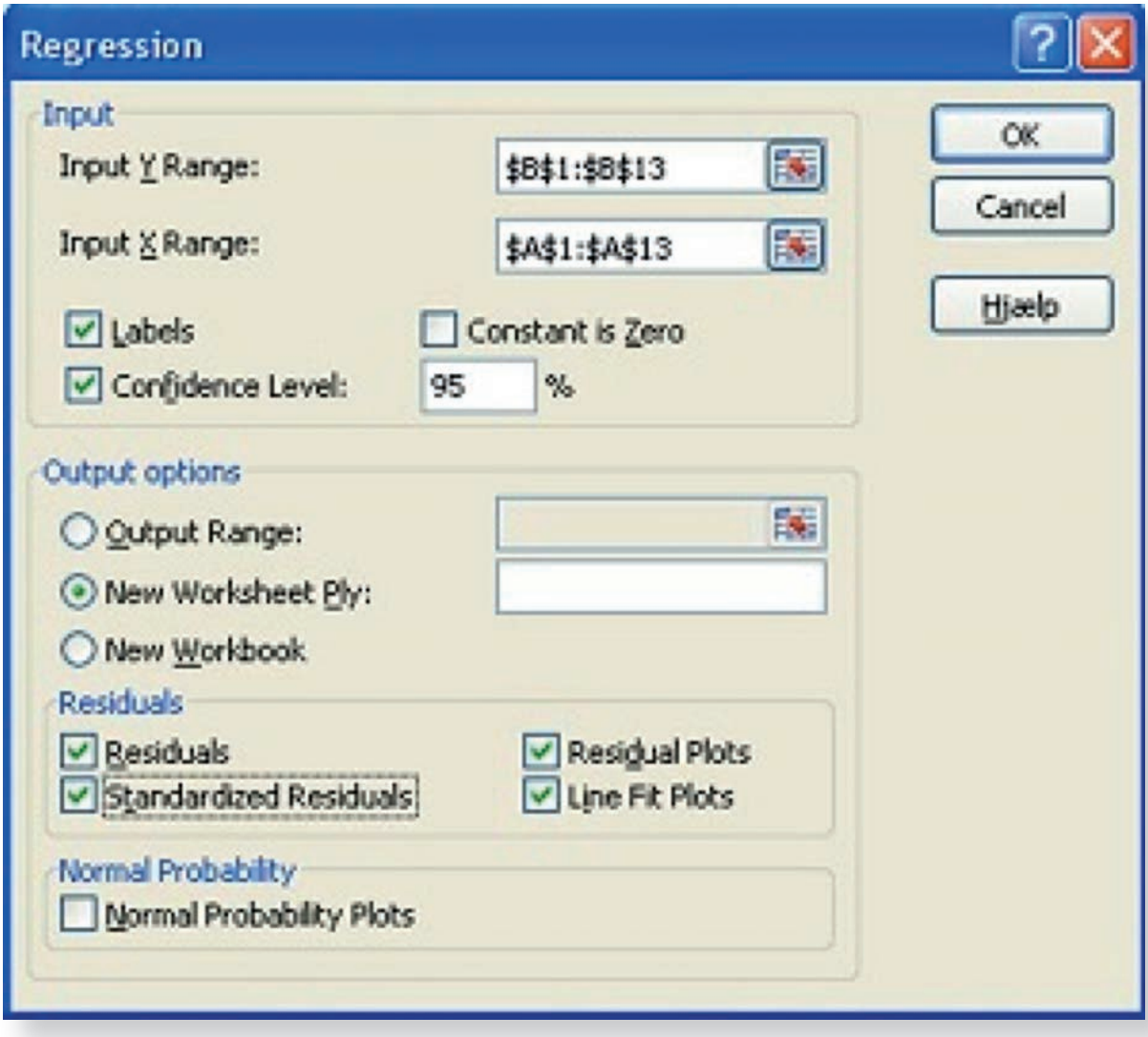
186 ISO 3534-1, *Statistics – Vocabulary and symbols* – Part 1: *Probability and general statistical terms*, 1993.

187 Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002.

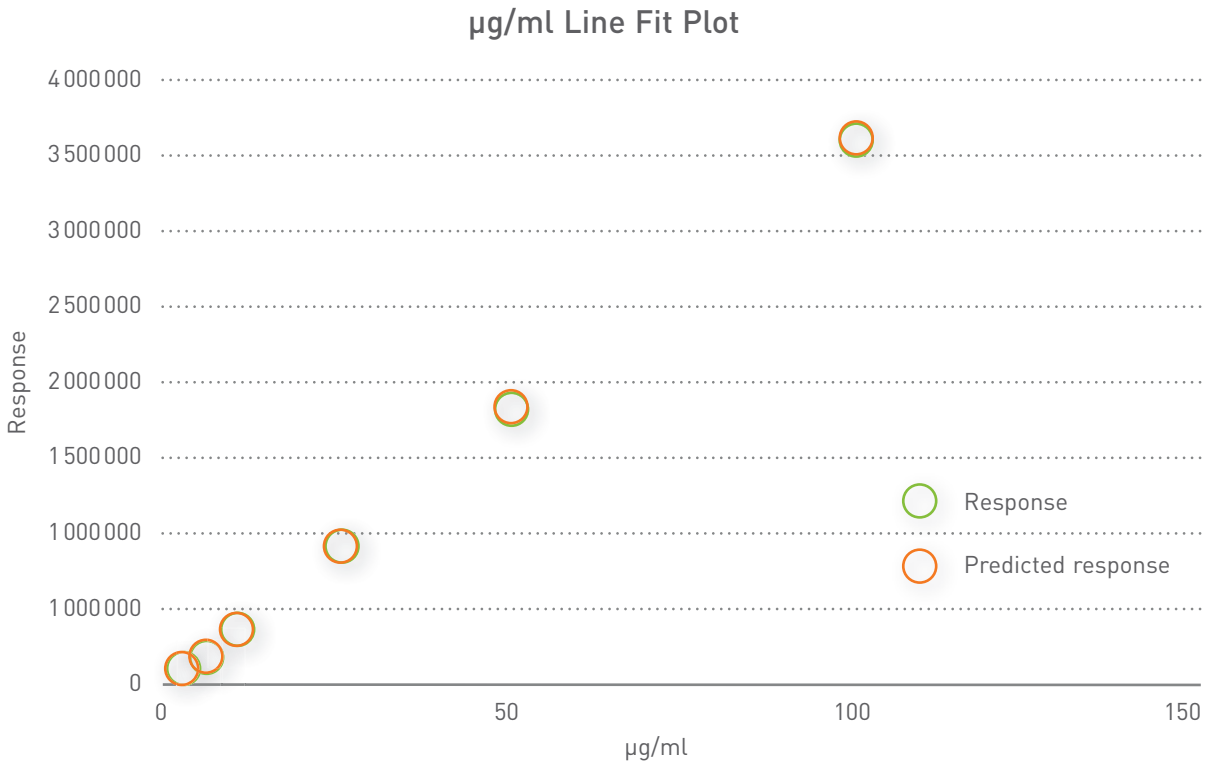


For the Y-range, choose the responses and for the X-range choose the levels. Tick the relevant Output options.

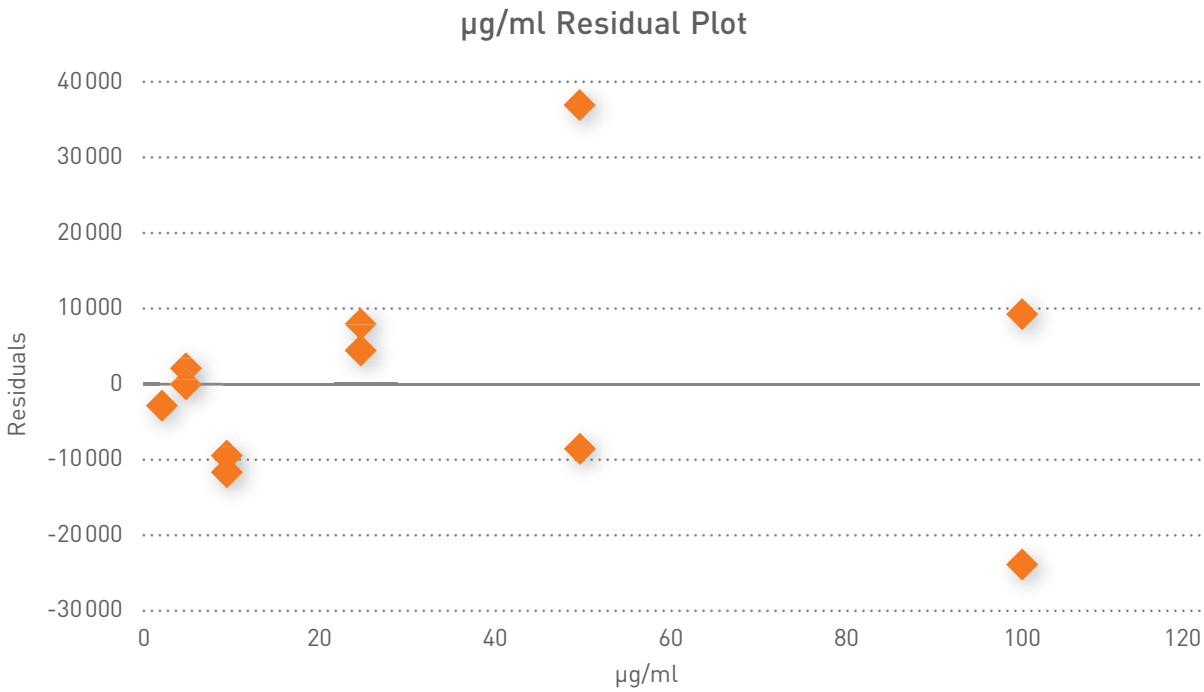
The data is given in tables and figures as shown below.



R^2 = 0.9999 indicating a significant correlation between response and concentration.



The slope coefficient is 36240 and the intercept is 4502 and since the 95% interval for the intercept is including (0, 0) there is more than 95% certainty that the calibration curve includes (0, 0).



The Residual plot shows that the points are randomly distributed around the x-axis, indicating a linear curve.

Linearity can be tested, mathematically, according to the **Tiley test**.

s_2^2 is based on the sum of the difference between the two repetitions (a-b) (see calculations below:

$$s_2^2 = \frac{1}{2n} \sum (a - b)^2 = \frac{1}{2 \times 6} \times 3161113435 = 263426779,6$$

s12 is based on the squared difference between the measured response (y) and the calculated response from the calibration curve (ŷ) (see calculations below):

$$s_1^2 = \frac{1}{n - 2} \sum (y - \hat{y})^2 = \frac{1}{10} \times 2397613089 = 239761309$$

Ratio of the two squared standard deviations is calculated (F-ratio) and compared to the critical F-value (p=0.05), according to the degree of freedom for the two standard deviations (here 10 and 6 degrees of freedom).

µg/mL	Response	(a-b)	(a-b)2	ŷ	(y- ŷ)	(y- ŷ)2
2.5	92023			95101	-3078	9475131
2.5	91892	131	17161	95101	-3209	10298773
5	187248			185701	1547	2394231
5	186126	1122	1258884	185701	425	180906
10	357074			366900	-9826	96543776
10	355749	1325	1755625	366900	-11151	124337425
25	915327			910497	4830	23332113
25	917891	-2564	6574096	910497	7394	54676154
50	1807727			1816492	-8765	76819343
50	1853189	-45462	2066793444	1816492	36697	1346694437
100	3604581			3628482	-23901	571241474
100	3637516	-32935	1084714225	3628482	9034	81619327
Sum			3161113435			2397613089
	s_2^2	(df=12) =	263426119			
				s_1^2	(df=10) =	239761309

$$F = \frac{s_1^2}{s_2^2} = \frac{239761309}{263426119,6} = 0,91$$

with 10 and 6 degrees of freedom.

$F_{critique}$ (p=0,05) = 4,06

→ Linearity is hereby confirmed.

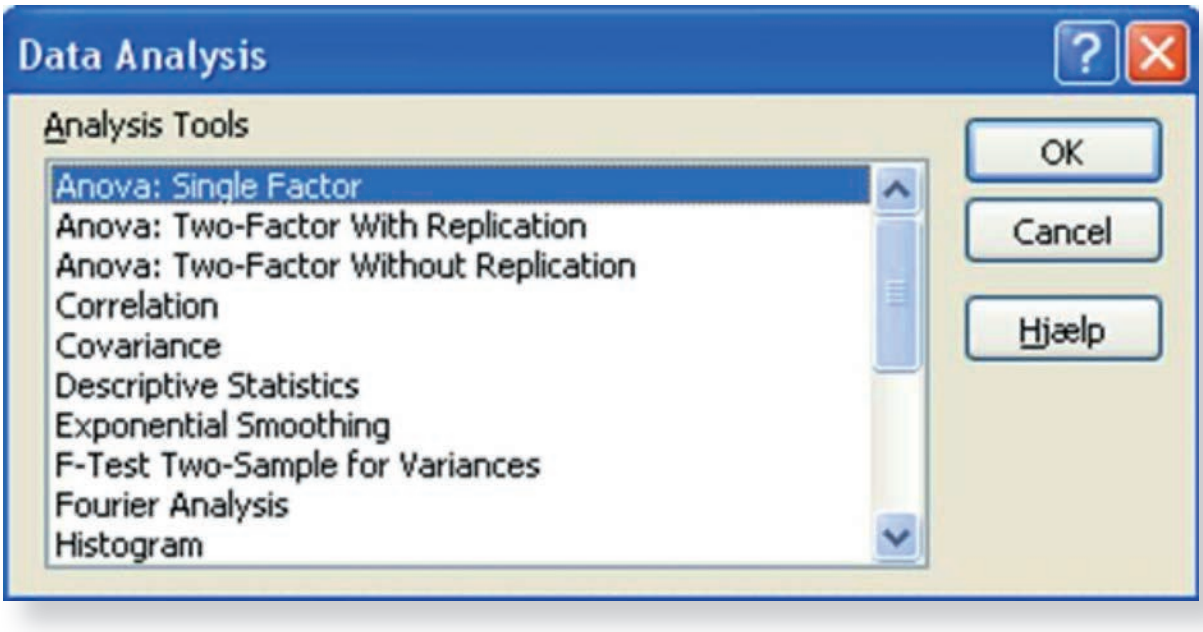
A.3. Precision

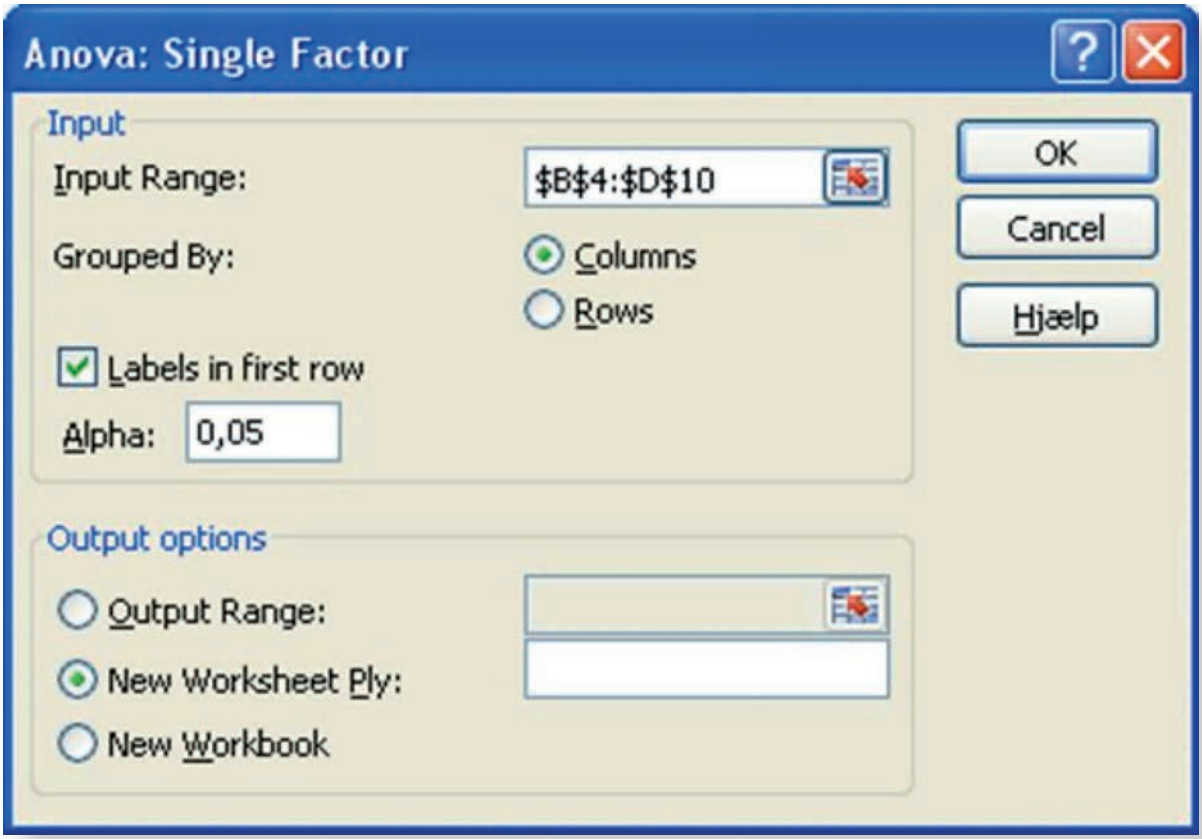
The precision can be calculated in several ways. The two examples below demonstrates a one-sided variance analysis (example 1) and a reduced approach when duplicate analysis has been made (example 2).

Example: Chloramphenicol was spiked 0.3 µg/kg into six samples of fish following analysis. This was repeated on two other days. An ANOVA calculation is used with excel spread sheet and the data analysis option.

	Day 1	Day 2	Day 3
		(µg/kg)	
Conc. 1	0.36	0.28	0.28
Conc. 2	0.31	0.33	0.29
Conc. 3	0.36	0.33	0.35
Conc. 4	0.32	0.33	0.30
Conc. 5	0.34	0.30	0.30
Conc. 6	0.28	0.33	0.29

Repeatability (s_r) and internal reproducibility (S_R) can be calculated by variance analysis using Excel spread sheet and the Data analysis option.





Anova: Single factor						
Summary						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Day 1	6	1.97	0.3283333	0.0009767		
Day 2	6	1.9	0.3166667	0.0004667		
Day 3	6	1.81	0.3016667	0.0006167		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0021444	2	0.0010722	1.5614887	0.2420822	3.6823203
Within Groups	0.0103	15	0.0006867			
Total	0.0124444	17				

sr^2 is shown as Within Groups variance, MS, and the connection between the internal reproducibility and repeatability is:

$$s_R^2 = s_r^2 + s_L^2$$

where:

$$S_L^2 = \frac{S_d^2 - S_r^2}{n}$$

where s_d^2 is the Between Groups variance, MS.

$$s_L^2 = \frac{S_d^2 - S_r^2}{n} = \frac{0.0010722 - 0.0006867}{3} = 0.000129$$

$s_R^2 = \sqrt{0.000815}$ $s_r^2 + s_L^2 = 0.0006867 + 0.000129 = 0.000815$

$s_R = \sqrt{0.0006867} = 0.029$ (~ 9.0 %) with 17 degrees of freedom

$s_r =$ = 0.026 (~ 8.3 %) with 15 degrees of freedom

Example: Deoxynivelanol in wheat flour has been analysed by adding 50 µg/kg to samples of wheat flour and analysed in duplicate. This has been repeated on five other days.

A simplified setup is when analysing samples in duplicate on different days. Results are given in the table and S_r and S_R are calculated as described below.

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Sum
Conc. 1 (y_{i1})		49.0	56.0	62.5	44.0	50.2	52.2	
Conc. 2 (y_{i2})		60.0	64.6	55.5	45.5	44.1	63.0	
$(y_{i1} - y_{i2})^2$	121.00	73.96	49.00	2.25	37.21	116.64	400.06	
$[(y_{i1} + y_{i2})/2]^2$	2970.25	3636.09	3481.00	2002.56	2223.12	3317.76	17630.79	
$(y_{i1} + y_{i2})/2$	54.50	60.30	59.00	44.75	47.15	57.60	323.30	

$$s_r^2 = \frac{\Sigma(y_{i1} - y_{i2})^2}{2n} = \frac{400{,}06}{12} = 33.34 s_r = 5.77\ (\tilde{}10.7\,\%)$$

with 6 degrees of freedom

$$s_L^2 = \left[\frac{n \times \Sigma(\bar{y}_i)^2 - (\Sigma \bar{y}_i)^2}{n \times (n-1)}\right] - \frac{s_r^2}{2} = \frac{6 \times 17630.79 - 323.30^2}{30} - \frac{33.34}{2} = 25.39$$

$s_R^2 = s_r^2 + s_L^2 = 33.34 + 25.39 = 58.73 s_R = 7.66\ (\sim 14.2\,\%)$ with 11 degrees of freedom

A.4. Limit of detection (LOD) and limit of quantification (LOQ)

The detection limit and quantification limit can be calculated in several ways and below two examples are given. In the first example limits are calculated from analysis of blank samples and in the second the limits are calculated from the calibration curve method.

Example 1: Three blank samples of fish tissue has been analysed for metronidazol. This has been repeated on two other days.

An ANOVA calculation is used with Excel spread sheet for calculating S_B (within-laboratory reproducibility).

LOD and LOQ are calculated as 3 times and 6 times the standard deviation of the blank (S_B).

When quantifying blank samples, it is important that the negative results of the blank values are not set to zero, but that the actual values are used in the calculations, as this would otherwise result in an erroneously low limit.

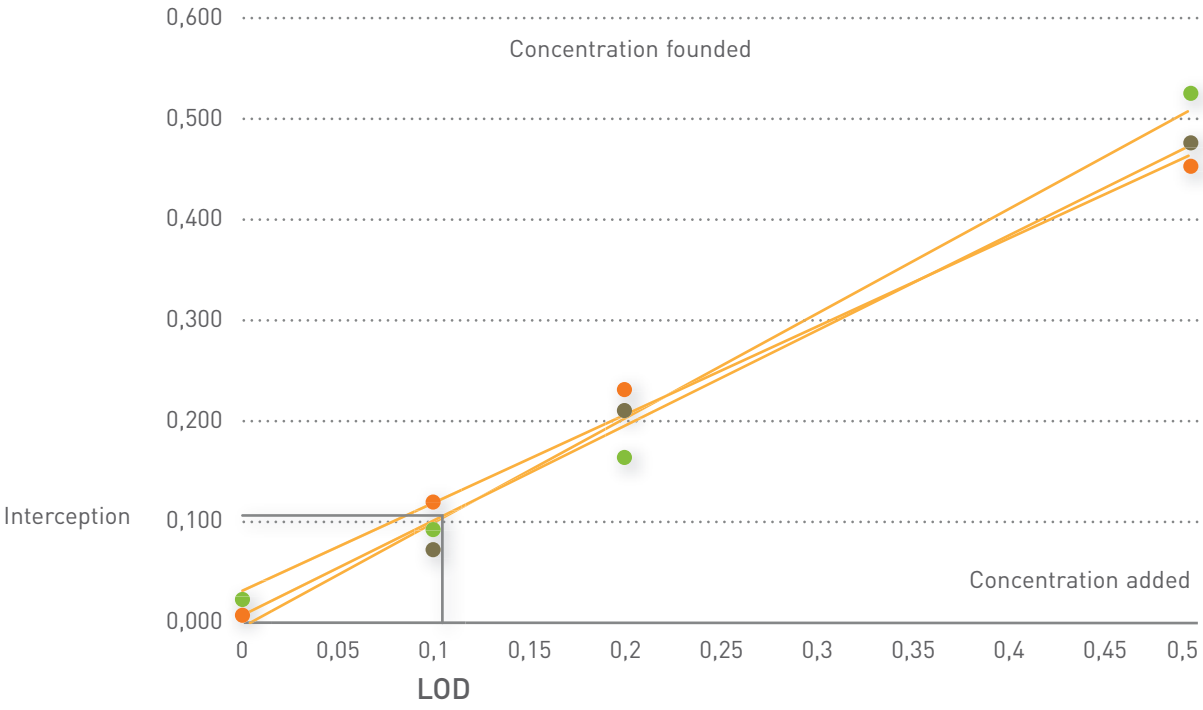
Blanc samples				
	Day 1	Day 2 (µg/kg)	Day 3	
	0.070	0.000	0.000	**Moyenne**
	0.120	0.000	0.030	0.037 µg/kg
	0.070	0.000	0.040	s_B
				0.048 µg/kg
Average	0.087 µg/kg	0.000 µg/kg	0.023 µg/kg	
LOD = 3 * s_B = 3 * 0.048 µg/kg = 0.144 µg/kg				
LOQ = 6 * s_B = 6 * 0.048 µg/kg = 0.288 µg/kg				

Example 2: Four samples of fish tissue has been analysed with different concentrations of chloramphenicol added (0; 0.1; 0.2 and 0.5 µg/kg).

This has been repeated on two other days.

Regression analysis using Excel spread sheet has been done to calculate the calibration curve parameters and LOD is calculated as 3 times the within-laboratory standard deviation, S_{IR}, plus intercept of the calibration curve divided by the slope of the curve.

Calibration curve			
	Day 1 Conc. (µg/kg)	Day 2 Conc. (µg/kg)	Day 3 Conc. (µg/kg)
0 µg/kg	0.020	0.024	0.012
0.1 µg/kg	0.072	0.095	0.120
0.2 µg/kg	0.210	0.165	0.230
0.5 µg/kg	0.475	0.524	0.454
Correlation	0.990	0.984	0.989
Slope	0.938	1.021	0.870
Intercept	0.007	-0.002	0.030
S_{IR}	0.029	0.029	0.029
LOD	0.100	0.083	0.134
Average LOD = 0.108 µg/kg			



A.5. Method documentation protocol

This annex sets out the recommendation approach to documenting analytical methods.

Update and review summary

This section serves two purposes. To enable minor changes to be made to the text of the method without the need for a full revision and reprint of the method. It is recommended that every method should be **reviewed for fitness-for-purpose periodically** and the update and review summary serves as a record that this has been done. The summary typically would be located at the front of the method, just inside the front cover.

Title

Preferred format: determination of A {analyte or measurand} (in the presence of B {interference}) in C {matrix} using D {principle}.

Scope

The following details should be included the analyte(s) which can be determined by the method:

- the form in which analyte(s) are determined – speciation, total/available etc.;
- the sample matrix(es) within which those analyte(s) may be determined;
- the concentration range of analyte(s) over which the method may be used;
- known interferences which prevent or limit the applicability of the method;
- the technique used by the method.

Warning and safety précautions

Detailed precautions may be given in the relevant sections, but an indication of any health and safety issues should be provided.

Provide suitable warnings of any hazards involved with:

- handling the samples;
- handling or preparing solvents, reagents, standards, or other materials;
- operation of equipment;
- requirements for special handling environments e.g. fume cupboards.

Definitions

For example, in a method describing the procedure for determining the 'Nitrogen content of meat and meat products', the nitrogen content may be defined as the quantity of nitrogen corresponding to the ammonia produced and determined under the conditions specified below.

Principle

Outline the principle by which the analytical technique operates. This section should be written so as to allow an at-a-glance summary of how the method works.

Include an explanation on the principle of the calculation. Where appropriate to clarifying the working of the method or calculations, include details of any relevant chemical reactions (for example, this may be relevant where derivatisation is involved, or titrimetry).

The following is an example of a description of the principle of a method for the determination of nitrogen content of a food product, such as meat or milk.

Digestion of a test portion with concentrated sulphuric acid, using copper(II) sulphate as a catalyst, to convert organic nitrogen to ammonium ions; alkalisation, distillation of the liberated ammonia into an excess of boric acid solution, titration with hydrochloric acid to determine the ammonia bound by the boric acid, and calculation of the nitrogen content of the sample from the amount of ammonia produced.

Reagents and materials

List all of the reagents materials, blanks, QC samples and standards and certified reference materials required for the analytical process, numbered for later reference.

List:

- details of any associated hazards including instructions for disposal;
- analytical grade;
- need for calibration and QC materials to come from independent batches;
- details of preparation, including need to prepare in advance;
- containment and storage requirements;
- shelf life of raw material and prepared reagent;
- required concentration, noting whether w/v, w/w or v/v;
- labelling requirements;
- disposal hazards.

Apparatus and equipment

Describe individual equipment and how they are connected in sufficient detail to enable unambiguous set-up. List minimum performance requirements and verification requirements, cross-referenced to the calibration section and any relevant instrument manuals.

Number for later reference. For glassware include grade where applicable (bear in mind that use of a particular grade may require justification and that proof of compliance may be required). Include environmental requirements (fume cupboards etc.).

Sampling

Include sufficient detail to describe how the test portion is arrived at starting with the sample as received by the laboratory. Include storage, conditioning and disposal details.

Calibration

Identify the critical parts of the analytical process. These will have to be controlled by careful operation and calibration. Cross-reference to the relevant sections above. Include calibration of equipment – what needs to be calibrated, how, with what, and how often?

Consider appropriate traceability of calibrants.

Quality control

Explain what form the quality control takes, frequency of quality control checks during batch analysis, pass/fail criteria, action to take in the event of a failure. Cross-reference to the relevant sections above.

Procedure

Describe the analytical procedure, cross-referencing previous sections as appropriate including numbered reagents, apparatus and instrumentation. Where parameters are expressed (time, temperature) which are critical to the procedure, cross-reference to the relevant part of the calibration section. Indicate at which point in the analytical procedure the quality control, and calibration procedures should be performed.

Calculation

Lay out the formulae for calculating the results ensuring all terms are clearly defined and derived. Indicate requirements for checking, cross-reference to QC requirements. The following is an example of the method of calculation of the nitrogen content of a food product, such as meat or milk. Method of calculation and formula:

The nitrogen content of the sample, expressed as a percentage by mass, is equal to:

$$0.0014 \times (V_1 - V_0) \times \frac{100}{m}$$

where:

V_0 is the volume, in millilitres, of 0.1 N hydrochloric acid solution required for the blank test;

V_1 is the volume, in millilitres, of 0.1 N hydrochloric acid solution required for the determination;

m is the mass, in grams, of the test portion.

Note

If the standard volumetric hydrochloric acid solution used is not of exactly the concentration indicated in the relevant section of the standard, a suitable correction factor should be used in calculating the result.

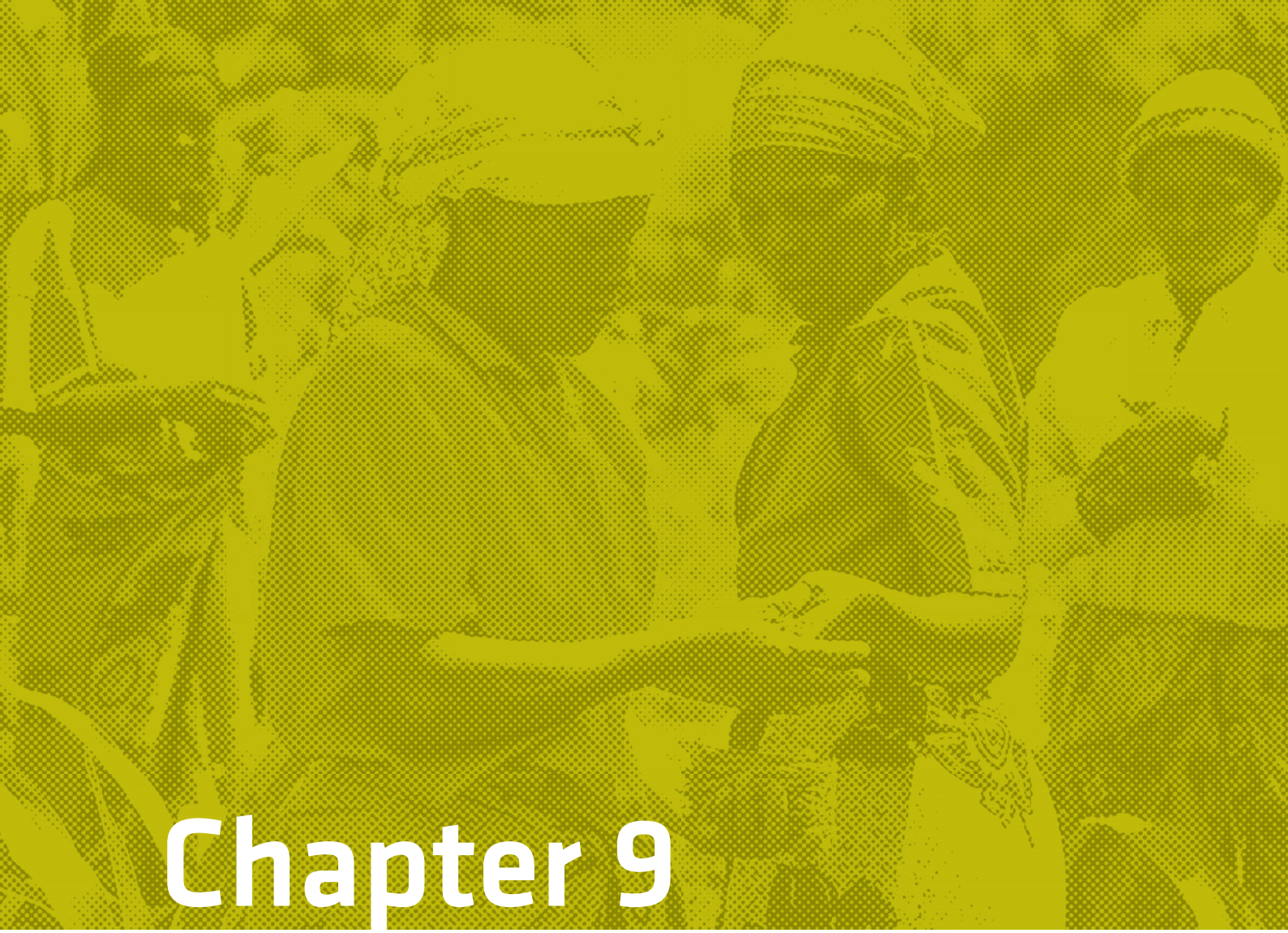
Report the result to the nearest 0.01 g of nitrogen per 100 g of sample.

Reporting procedures including expression of results

Indicate how results should be reported, including: rounding of numbers; final units: ± uncertainty; confidence interval.

Normative references

Any references which provides fundamental background information to the method. Depending on the volume of data in support of the validation, it may be appropriate to list it here or provide reference to a separate file.



Chapter 9

Analytical methods for chemical contaminants

9.1. INTRODUCTION TO CHEMICAL ANALYTICAL METHODS

9.1.1. Context

This Guide sets out guidance on laboratory methods for the analysis of contaminants in foods. It is aimed at helping laboratory analysts and managers in Competent Authorities to select the correct approach to the analysis of different food safety hazards for official controls.

This document sets out the approaches to analysis of heavy metals, mycotoxins, pesticide residues, other process contaminants, and environmental contaminants such as PCBs and dioxins. In each case it provides a summary of the equipment required, outlines the principles of the analytical methods, and describes the key points to be considered by the analyst to ensure valid and reliable test results. The methods described are drawn from various official sources, including EU regulatory requirements where these are specified, but also ISO and US/Canadian analytical methods where appropriate.

The content is particularly based on EU Regulations concerning contaminants in food, as follows:

- Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs;
- Regulation (EC) No. 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin;
- Regulation (EC) No. 470/2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin;
- Regulation (EC) No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

The chapter is divided into sections. Key terms are defined and explained. The main part of the guide then provides a description of each methodology. The chapter is therefore intended to provide practical advice for the implementation of analytical testing for official controls of contaminants in foods.

The methodologies were selected for inclusion on the basis that they were either a) set out in EU legislation specifying a particular method b) ISO or EN standard methods c) other sources (AOAC etc.) where a) and b) not available.

In all cases full references are provided for the method described. Relevant references are provided at appropriate points in the chapter.

9.1.2. Background of analytical method

A chemical analytical method is the description of the analytical techniques and laboratory procedures that is used to identify and determine the concentration of a chemical compound or chemical element in a sample. Chemical analysis may be qualitative or quantitative. Qualitative analysis identifies the compound present in the sample and may express its concentration range as well. Quantitative analysis determines the amount of a compound within a statistical confidence interval

and quantitative analysis is used to verify the result from a qualitative analysis. Modern analytical instruments make it possible to identify hundreds of compounds in a single analysis of a sample and to detect very low concentrations.

Performance of an analytical method **must be verified by proper validation** and the laboratory personal must follow strict **quality assurance and quality control procedures**.

9.1.3. Collections of analytical methods

Development and implementation of analytical methods in a laboratory are often based on published methodology in the scientific literature or analytical method collections from international or national organisations.

In many cases the described method **has to be modified** to accommodate instrumentation and sample preparation equipment available in the actual laboratory. Adjustments may also be required **due to different sample matrices or inclusion of other compounds**.

Examples of **major collections of chemical analytical methods**:

- ISO (International Organization for Standardization). ISO is the world's largest developer of voluntary International Standards. International Standards give state of the art specifications for products, services and good practice (www.iso.org).
- CEN (The European Committee for Standardization). CEN is a major provider of European Standards and technical specifications. CEN's 33 National Members work together to develop voluntary European Standards (ENs) (www.cen.eu).
- AOAC INTERNATONAL (Association of Analytical Communities). AOAC INTERNATIONAL is a worldwide provider and facilitator in the development, use, and harmonization of validated analytical methods and laboratory quality assurance programs. AOAC has two methods validation programs, the AOAC Official Methods Program and the AOAC Performance Tested Methods Program (www.aoac.org).
- *CODEX ALIMENTARIUS* generates international food standards, guidelines and codes of practice (www.codexalimentarius.org).
- US EPA, U.S. Environmental Protection Agency. The US EPA test methods are approved procedures for measuring the presence and concentration of physical and chemical pollutants; evaluating properties, such as toxic properties, of chemical substances (www.epa.gov/fem/methcollectns.htm).

9.1.4. Choosing a chemical analytical method fit for purpose

The data generated using analytical methods is essential in evaluation compliance with respect to e.g. maximum limits for chemical residues in food and feed. To be confident in the reliability of this data it is crucial that the methods are fit for purpose. Analytical method validation is the process of demonstrating that a method is suitable for its intended use. In a particular analytical work the laboratory should evaluate the method in accordance to the defined performance requirements and by how the method performs when used by the laboratory staff and available equipment.

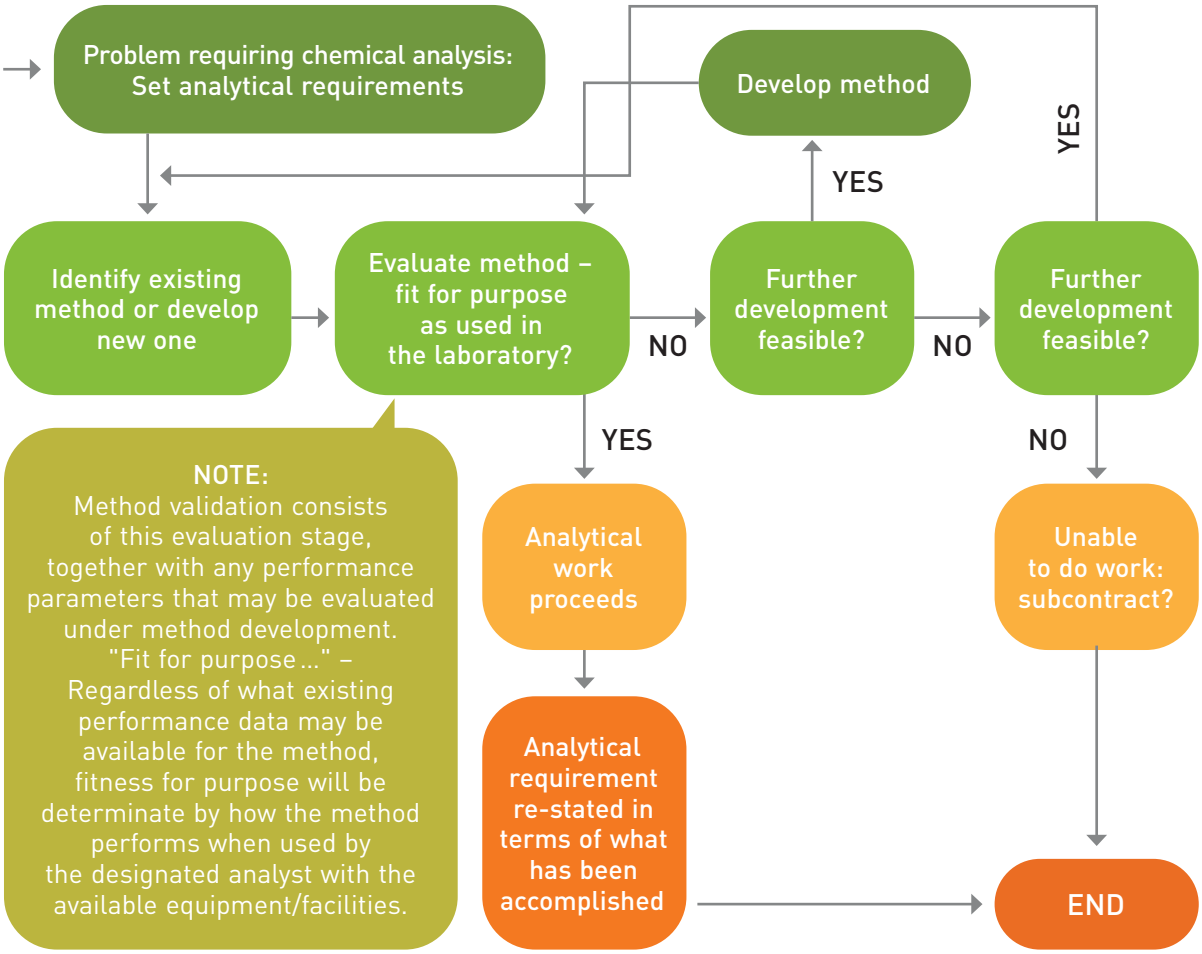


Figure 1 - Choosing, developing and evaluating methods (from EUROCHEM Guide, 1998)

Setting up an analytical method will typical follows these **steps**:

Define method performance criteria or standard method to be used → Analytical method development/establishment → Method validation → (Accreditation) → Routine analysis.

9.1.5. The chemical analytical detection chain

It is important to understand that in analytical chemistry the analytical methodology is not only the instrumental detection and a description of the sample preparation and sample clean-up. The entire analytical detection chain has to be critical considered, evaluated and tested in order to ensure analytical results of high quality:

Sampling → Sample preparation → Sample clean-up → Instrumental detection → Quantification → QA/QC → Reporting

Proper sampling technique is the key to achieve representative and homogeneous samples and without good samples the results will not be reliable no matter how excellent the rest of the analytical method performs.

Proper QA and QC procedures are also vital so the analytical results can be documented as reliable.

9.1.6. Chemical analytical method description

After an analytical method has been developed and validated **it is recommended that the procedure is drawn up in accordance to a standard layout (ISO 78-2:1999)**.

The adoption of a standard form of layout ensures:

- that no important point is overlooked in the preparation of the analytical method description;
- that the various items of information to be included in the method are always given in the same order;
- that any desired paragraph may be found rapidly, whatever the origin or scope of the method;
- simplification, rationalisation and standardisation of methods, reagents and equipment used in the laboratory.

The preferred order for layout as stated in ISO 78-2:1999:

- Foreword;
- Introduction;
- Title;
- Warning;
- Scope;
- Normative references;
- Definitions;
- Principle;
- Reactions;
- Reagents and materials;
- Apparatus;
- Sampling;

- Procedure;
- Calculation;
- Precision;
- Quality assurance and control;
- Special cases;
- Test report;
- Annexes;
- Bibliography;

Additional subdivisions or extensions to consider:

- Interferences
- Samples collection, preservation and storage
- Pollution prevention
- Waste management

It should be remembered that the proposed layout is for guidance only. It should be adapted to suit any special requirements. There may be no need for all subdivisions provided.

9.2. ANALYTICAL METHODS FOR TOXIC ELEMENTS

9.2.1. Introduction

Elements are natural components of the Earth's crust which are not degraded withtime. Their amount in food depends on the natural content and on the condition under which the food is produced and processed. Some elements have nutritional functions to life (e.g. selenium, iodine and zinc) and are essential to humans and animals to maintain a good health. Other elements, e.g. **lead, cadmium and mercury** have no nutritional properties and **exposure to these elements can lead to severe adverse health effects**.

In 2010 the World Health Organization (WHO) listed the **four elements arsenic, lead, mercury and cadmium** on their prioritized list of the top ten chemicals of major public health concern (WHO, 2010). These elements and other elements with toxic properties are often named '**heavy metals**' and this term is well perceived in the general population although due to contradictory definitions in the literature and lack of coherent scientific basis, it is not an accepted term in the scientific community (Duffus, 2002). An alternative term is '**toxic elements**' for which no general scientific consensus or exact definition exists either, but it seems to have a more general broad acceptance and therefore used here.

9.2.2. Toxic metals, sources and levels in foodstuffs

Cadmium, lead and mercury are usually the toxic metals, which have drawn most attention, with regards to dietary exposure and many countries have established maximum levels for these toxic elements in their food legislation. However, also there are also other examples of toxic metals with relevance to food, including e.g. arsenic, (organo-) tin and aluminium. The most important sources for metals in food include both anthropogenic (e.g. industrial release, agricultural practices) as well as from natural activities (e.g. weathering of minerals, volcanic activity). Table below provides selected examples of various sources of toxic elements and their routes to contamination of food produce.

Examples on sources of toxic elements and the routes to food contamination

Toxic element	Sources (selected examples)
Cadmium	• Atmospheric precipitation → soil → crops • Use of phosphate fertilizers → soil → crops • Contaminated feed → animals → meat
Lead	• Atmospheric precipitation of Pb containing dust from industrial processes → vegetables (especially with large surface area) • Use of Pb containing ammunition → game animals → meat
Mercury	• Natural sources (e.g. water surfaces, forest fires, volcanic activity) • Anthropogenic sources (e.g. agriculture, incineration, fossil fuels) • Bioaccumulation in the aquatic environment (seafood)
Arsenic	• Weathering of minerals → groundwater → rice • Poultry growth promoter v manure → soil → crops • Bioaccumulation in the aquatic environment (seafood)
Aluminium	• Food additive to e.g. biscuits and noodles • Food packaging materials → migration to food
Organotin	• Antifouling agents → marine environment → seafood • PVC stabilizer in food contact materials → migration to food

The concentration of the toxic elements will vary widely depending on element-foodstuffs combination. In general the levels will typically range from the low ppb (µg/kg) to ppm (mg/kg) range.

This figure shows some examples of food items, where elevated contents of toxic elements typically have been reported. The reason for the elevated levels may be due to natural/biological causes, *i.e.* the particular food item has a capability of accumulating the element (e.g. mercury in predator fish) or contamination by a man-made source, *i.e.* use of cadmium-contaminated fertilizers.

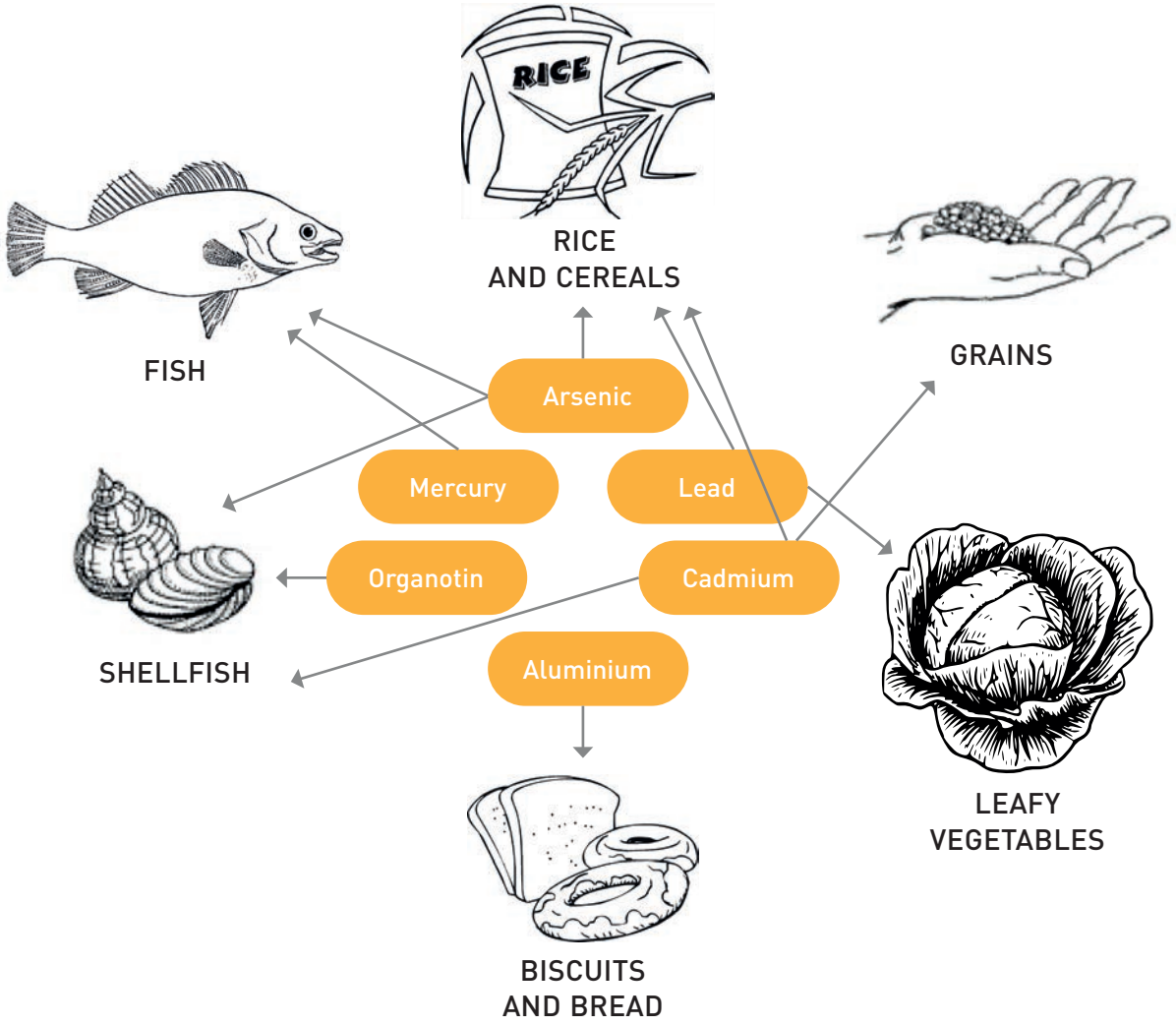


Figure 2 - Selected examples of foodstuffs with links to toxic elements, which have been reported at significant levels in them.

9.2.3. Analytical procedure for determination of toxic elements in food

This figure gives on overview of the various main steps in the analytical procedure for determination of toxic elements in foodstuffs. It is important that all steps in the procedure are controlled to ascertain a reliable result for the sample in question. **Some examples of analytical methods for toxic elements are also given in the Annex.**

Figure 3 - Overview of the steps in an analytical procedure for element detection

It is of course highly important to avoid contamination of the sample in all the steps of the analytical procedure as well as during storage. Hence it is important to ensure that all equipment, e.g. containers, cutting knifes and homogenization tools that comes into contact with the sample does not release any of the elements to be determination to the samples. Soft glassware equipment may release substance or elements may absorb to the walls. Hence, it is usually recommended to replace glassware (e.g. flasks, beakers, vessels, dishes etc.) with similar equipment made of quartz, fluoro-polymers (e.g. PTFE) or polyolefines (e.g. PE or PP). It is also important to ensure a clean laboratory environment and facilities, so that samples and solutions are not contaminated from dust or by contact to equipment used in the procedure. Furthermore all reagents used throughout the procedure should be as clean as possible with regards to the elements in question to minimize contamination from this source. The concentration of trace elements in water should be low enough not to affect the results of the determination and typically purified water is used (e.g. doubly-distilled, de-ionised or similar). It is important to analyse a reagent blank solution, which has been subjected to the same procedure and reagents as the samples in order to be able to correct the results for any contribution to the signal from other sources than the sample.

9.2.4. Sampling

Sampling is often an overlooked step in the analytical procedure, which has not been paid so much attention. It is important that the collected samples are representative of the parent original population. All procedures used for acquisition, reduction and preservation of the sample may affect the reliability of the analytical result. For the sampling of foodstuffs intended for elemental analysis it is important to pay special attention to avoid contamination and analyte loss during handling and transport to the laboratory. In the EU the requirements for sampling foodstuff samples for official control has been laid down in an EU directive (EU, 2007). Furthermore a proper labelling of samples with all the needed information (e.g. sample type, sampling place, date, amount etc.) is required to keep track on the sample throughout the whole procedure.

9.2.5. Sample preparation

The basic requirement in the sample preparation step is to ensure a sufficient homogenization and representative subsample for further analysis. Usually the samples are prepared in a way as usual in the normal household and only the part intended for eating is subjected to analysis. Parts which are not intended for eating should be discarded from the sample, e.g. outer leaves, shell, skin and bones. Furthermore gross surface contamination like soil or rotten parts of plants should be removed. If a washing step is needed, leaching effects from cut surfaces should be avoided and to avoid contamination from tap water a final rinsing with de-ionised water is recommended. Rinsing water is removed by draining or tapping with soft tissue paper. The European standard EN13804 provides suggestions on sample preparation procedures for selected foodstuffs (CEN, 2002). After preparation the samples should be homogenized, e.g. by grinding

or milling. Freeze-drying of samples following milling to a fine powder is sometimes used to enable proper homogenization. Frozen food may be homogenized prior to defrosting and loss of liquid in the defrosting stage should be avoided. For some samples (e.g. dry samples) it may be beneficial to add a known amount of water to aid the homogenization process. Any equipment that comes into direct contact with the sample should be properly cleaned with e.g. detergent and hot water followed by rinsing with purified water. From the final homogenate representative subsamples can be taken for further analysis. If storage of the (sub-) samples is needed, it must be carried out in such a way that the composition does not change, e.g. by drying, evaporative loss or spoilage. For most samples it is advisable to preserve samples under cooled conditions.

9.2.6. Digestion

Prior to the determination of the elements, the analytes in the sample have to be brought into solution. The composition of foodstuffs may vary greatly with varying proportions of fat, proteins and carbohydrates as the main components and in addition the foodstuffs contain a varying amount of salt and different other minerals. Due to this large variation, proper precautions should be taken to ensure a complete digestion of the sample digestion prior to analysis.

The two most commonly used digestion techniques include dry ashing and wet digestion.

9.2.6.1. Dry ashing

The samples are dry ashed in crucibles at elevated temperatures (e.g. 450 °C) under a gradual increase in temperature in a furnace oven (usually starting from <100 °C and increasing at 50°C/h). Usually 10-20 gram of test sample is weighed in and suitable ashing aids (e.g. $Mg(NO_3)2$ or HNO_3) may be added to speed up the process. However, ashing aids should be used with caution as they may contaminate the samples. It is important to avoid loss of elements by volatilisation due to too high temperature or too rapid temperature increase. Cross contamination between samples may happen since open vessels are used, but is usually not considered to be a major problem. Usually the ashing is complete overnight, but several days may be required for complete ashing. Following ashing the ash is dissolved in hydrochloric acid, and the solution obtained evaporated to dryness. The final residue is re-dissolved in dilute nitric acid, and the contents of toxic elements can then be determined.

Table 1: Advantages and disadvantages of dry ashing

Advantages	Disadvantages
Large test sample sizes => reduced limits of detection => less problem with inhomogeneity	Contamination problems
	Volatilisation of analytes
High sample throughput with little sample manipulation	Long durations (up to several days)

9.2.6.2. Wet digestion

Digestion using strong acids is another main technique for bringing food samples into solution. Use of clean acids is highly recommended to avoid contamination. Usually analysis grade acids are used or following a sub-boiling purification step. The predominant acid used is HNO_3, either alone or in combination with H_2O_2. The digestion may be done in closed vessels made of plastic or glass/quartz contained in steel cylinders and heated under pressure in an autoclave or another heating device. However, the dominating technique nowadays is digestion in closed vessels (e.g. quartz or PTFE) and heating under pressure by means of microwave irradiation. Both volatile and more refractory elements can be analysed in the digests. The technique provides highly reproducible results, however only small test portions (typically < 0.5 gram dry matter) are used, which may result in homogeneity problems for certain food samples.

Table 2: Advantages and disadvantages of microwave wet digestion

Advantages	Disadvantages
Short time required (typically < 1 h incl. cooling)	Large dilution factors => Higher limit of detection
Low risk of contamination	Small sample test portions => Inhomogeneity problems
Good reproducibility between replicates	

9.2.7. Detection techniques

There are several different detection techniques available for the determination of the toxic elements. The most commonly used are techniques based on atomic absorption spectrometry (AAS techniques) or inductively coupled plasma (ICP techniques). Table 3 shows a comparison of different detection techniques for toxic element determination. Information is provided about commonly detected elements, limit of detection (LOD) range as well as the advantages and disadvantages of the different approaches.

Table 3: Features of atomic spectrometry techniques

Technique	Elements	LOD range	Advantages	Disadvantages
ICPMS	Most elements (both metals and non-metals)	ppt	Rapid, sensitive, multi-element, wide dynamic range, good control of interferences	Limited total dissolved solids tolerance
ICPOES	Most metals and some non-metals	Mid ppb to mid ppm	Rapid, multi-element, high tolerance for total dissolved solids	Complex interferences, relative poor sensitivity

ETAAS	Many elements (commonly Pb, Cd, As, Se, Ni, Cu, Co)	ppt	Sensitive, few interferences	Single element technique, limited dynamic range
HGAAS	Hydride forming elements (As, Se, Tl, Pb, Bi, Sb, Te)	Ppt to ppb	Sensitive, few interferences	Single element technique, slow, complex
CVAAS	Hg	ppt	Sensitive, simple, few interferences	Single element technique, slow

9.2.7.1. Atomic absorption based techniques

In Atomic Absorption Spectroscopy (AAS) the determination of elements is based on their absorption of light at element-specific wavelengths by free atoms in a gaseous phase. More than 70 different elements can be determined by this technique. The quantification relies on Lambert-Beers law, which describes the relationship between analyte concentration and absorbance. A disadvantage of the AAS technique is the non-linearity of the calibration curves when absorbance becomes higher than 0.5 to 1. Figure below shows a block diagram of an AAS instrument.

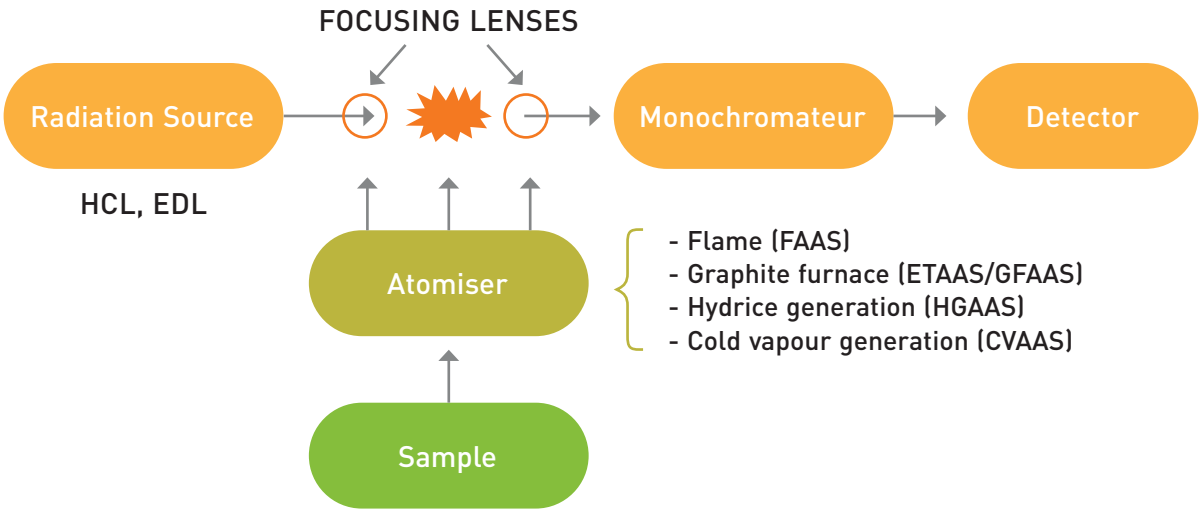


Figure 4 - Schematics of an Atomic Absorption Spectrometer

Several different atomizers exist. The oldest and most simple set-up is the flame AAS (FAAS), where an acetylene-air flame at 2300°C is used. The liquid sample is aspirated by a nebuliser and the aerosol and sent to the flame via a spray chamber, where the analytes are atomised and ionised. Electrothermal atomisers (ETAAS) use graphite furnaces, where the sample is placed (typically 10-50 µL or 1 mg), and then subjected to a temperature program. There are normally four steps in the procedure:

1. drying step – to remove solvent;
2. pyrolysis – to remove matrix;
3. atomisation – to release analyte to gaseous phase;
4. cleaning – to remove residues at high temperature.

The sensitivity for ETAAS is typically 2-3 orders of magnitude better than FAAS. For the hydride-forming elements (e.g. As, Se, Sb) the use of hydride-generation AAS (HGAAS) will enhance the sensitivity by 1-2 orders of magnitude compared to alternative methods. The gaseous hydrides are formed by reaction with sodium borohydride and swept into the atomisation chamber by an inert gas. The gaseous analytes are measured by absorption or emission spectrometry. For mercury the Cold-Vapour technique (CVAAS) may be used for enhanced sensitivity, due to the high vapour pressure of mercury at ambient temperature. In this approach mercury compounds are converted to Hg2+ ions by oxidation followed by a reduction step to Hg0 with tin(II)chloride. The concentration of mercury is determined by measuring the absorbance at 253.7 nm.

The radiation sources include hollow cathode lamps (HCL) and electrodeless discharge lamps (EDL), which emit light at the element-specific wavelengths. A spectrometer includes a wavelength selector (typically a monochromator) and the detector. The monochromator resolves the analytical line from other radiation emitted by the lamp, typically in a bandpass of 0.2 to 2 nm.

In atomic spectroscopy background correction is necessary to distinguish analyte signal from absorption, emission and optical scattering of sample matrix. Several approaches are used (e.g. D2 lamp, Zeeman) and in modern instruments this feature is built-in the instrument as a standard.

9.2.7.2. ICP based techniques

There are two forms of instrumental techniques based on inductively coupled plasma – namely ICP-OES (coupling to an optical emission spectrometer) and ICPMS (coupling to a mass spectrometer).

In the ICP argon gas is ionized in an intense electromagnetic field and a stable, high temperature plasma of about 7000-10000 K is generated as the result of the inelastic collisions created between the neutral argon atoms and charged particles. In ICP-OES the argon-plasma is used to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample. As detector in ICPOES instruments, photomultipliers have been used to measurement the light intensity at element-specific wavelengths. In more modern units, an array of semiconductor photodetectors such as charge coupled devices (CCDs) has been used. In instruments using these detector arrays, the intensities of all wavelengths (within the system's range) can be measured simultaneously, allowing the instrument to analyse for the elements to which the unit is sensitive all at once. Consequently, samples can be analysed very quickly.

In ICPMS the argon-plasma is used to atomise and ionise the isotopes of the elements, which then can be determined according to their mass in the mass spectrometer.

Figure below shows a schematic diagram of an ICPMS instrument. The sample solution is introduced via a peristaltic pump to a nebuliser, which forms an aerosol in the spray chamber. Here the larger droplets are discarded and only the smaller droplets are sent to the ICP via the torch. In the interface region a rotary pump makes a vacuum and the atomised and ionised elements are drawn through the sample

and skimmer cones. In the following section several lenses are responsible for focusing of the ion-beam. The quadrupole acts as a mass filter, which allows only the isotopes with a pre-selected mass-to-charge ratio (m/z) to reach the detector. The detector is most often an electron multiplier, which means that it can generate a measurable signal pulse from the impact of a single ion (unit: counts per second [cps]).

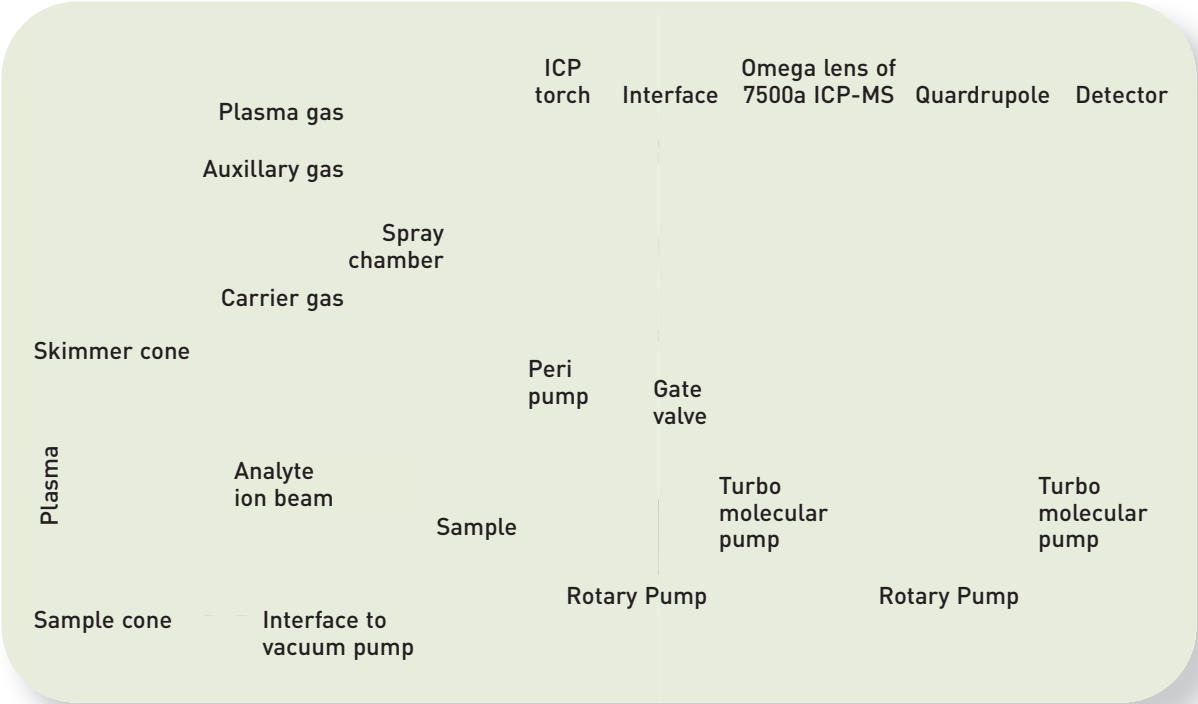


Figure 5 - Schematic diagram of a quadrupole-based ICPMS instrument

To summarise the functions of an ICPMS instrument the various operations can be divided into the following distinct parts:

- sample introduction (nebuliser and spray chamber);
- ion generation in the ICP;
- plasma/vacuum interface;
- ion focusing (lenses);
- ion separation (quadrupole);
- ion measurement (detector).

The next figure shows **an ICPMS mass spectrum** of the mass range with the mercury (m/z 198, 199, 200, 201, 202, 204) and lead (m/z 204, 206, 207, 208) isotopes as determined in a coffee sample. It can be seen that both elements have an isotope at m/z 204.

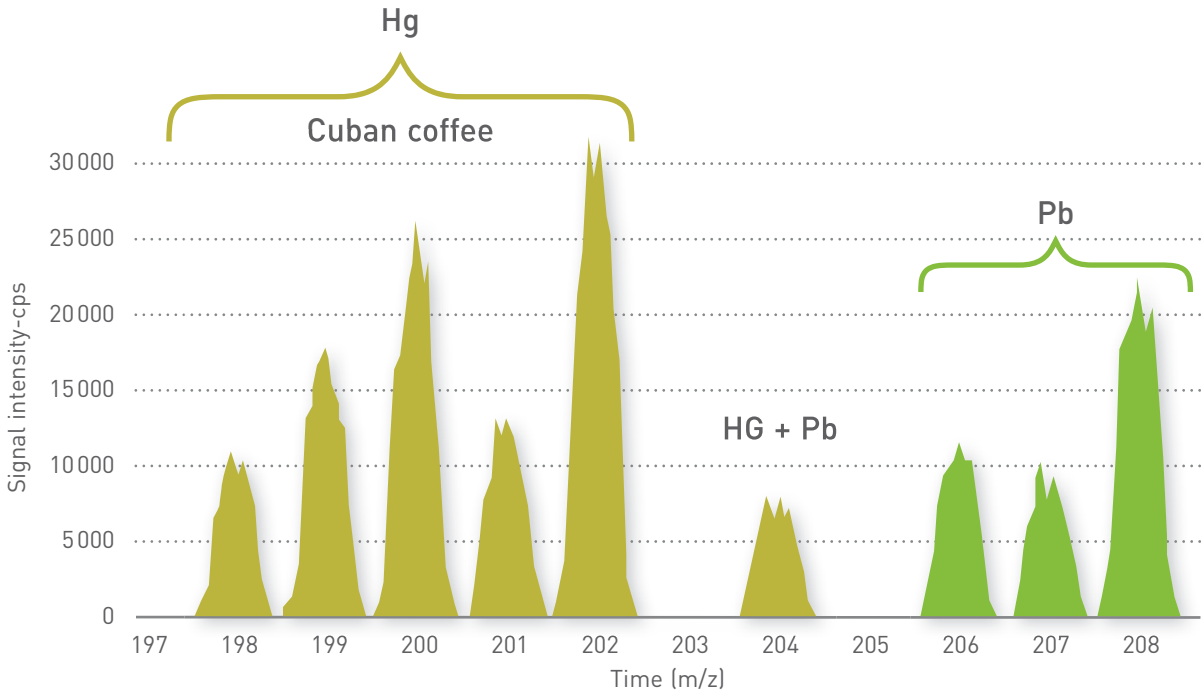


Figure 6 - Mass spectrum of a sample of coffee showing the mercury and lead isotopes

9.2.7.3. Comparison of the performance of AAS and ICPMS

This table shows a comparison of various typical performance characteristics for atomic analysis method as well as indication of sample throughput, sample volume and purchase cost of instruments. There are of course large differences between various instrument models and the figures also depend on the application.

Table 4: Comparison of analytical performance of atomic analysis methods

	FAAS	ETAAS	ICPOES	ICPMS
LOD (ng/g)	10-1000	0.01-1	0.1-10	0.001-0.1
Linear range	10^2	10^2	10^5	10^8
Precision				
• **short term (5-10 min)**	0.1-1 %	0.5-5 %	0.1-2 %	0.5-2 %
• **long term (hours)**	1-10 %	1-10 %	1-5 %	<5 %
Interferences				
• **spectral**	very few	very few	many	few
• **chemical**	many	many	very few	some
• **mass**	-	-	-	many
Sample throughput	10-15 s /element	3-4 min /element	6-60 elements /min	all elements /2-5 min
Sample volume	large	very small	medium	medium
Relative purchase cost	1-2	2-3	5-6	8-10

9.2.8. Trace element speciation analysis

In the standard set-up the ICPMS can only determine the total amount of the elements. In order to be able to determine different element-containing compounds (element species), the ICPMS can be coupling to various chromatographic techniques (e.g. HPLC or GC) (speciation analysis). For some element the toxicity varies a lot depending on the chemical form and for food safety evaluations it is important to be able to distinguish analytically between the different chemical forms. As an example HPLC-ICPMS can be used to determine different arsenic compounds in foodstuffs.

This figure shows a chromatogram of an extract of a rice sample, where inorganic arsenic (toxic form) and organic arsenic (less toxic form) has been separated by anion-exchange chromatography HPLC and determined by ICPMS.

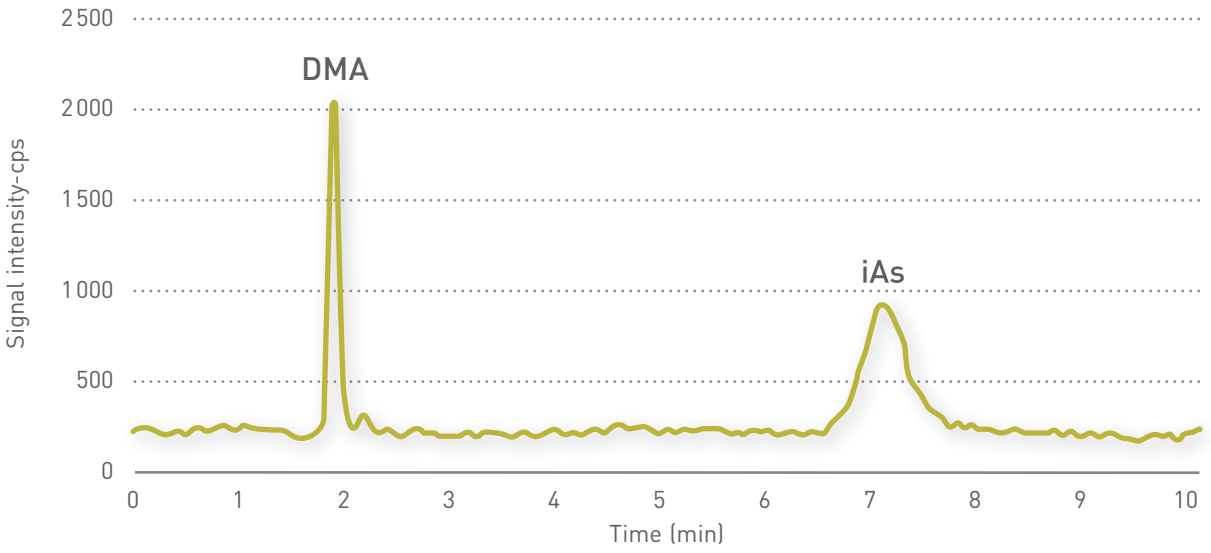


Figure 7 - HPLC-ICPMS chromatogram of arsenic compounds in a rice sample. DMA = dimethylarsinic acid (an organic arsenic compound); iAs = inorganic arsenic

9.2.9. Quality assurance and quality control procedures

In order to guarantee the credibility of the analytical result some sort of quality assurance and quality control procedures must be followed. The ISO17025 standard provides information of the required content in a quality manual in analytical laboratories, often the first step towards an accreditation. The main items of a quality control system are the use of certified reference materials (CRMs) and regular participation in proficiency tests (PTs). A suitable CRM (ideally matching matrix composition, analytes and concentration level) should be analysed regularly together with the samples. The results should be compiled and used to evaluate whether the method is in control, e.g. by a control chart. The CRMs can also be used to evaluate the trueness of the results, although caution should be taken her, since it should be kept in mind that the analyst knows the certified values prior to the analysis and this may make him/her biased. There are a large number of commercially available food-based CRMs on the market, covering many different types of foodstuff sample type and many different elements.

Another important quality control tool is the participation in PTs. In this case the concentration of the analyte is not known to the analyst on beforehand and is an independent way to estimate the analyst competence. PTs only provide a "snapshot" at a specific time, but after several PTs the performance of the analytical method can be evaluated.

9.3. ANALYTICAL METHODS FOR VETERINARY DRUGS

9.3.1. Introduction

In modern agricultural practice, veterinary drugs are being used on a large scale and administered as feed additives or via the drinking water in order to prevent the outbreak of diseases and they are given in cases of disease, for dehydration or to prevent losses during transportation. Growth-promoting agents (e.g., hormones and certain veterinary drugs, mainly antibiotics) are applied to stimulate the growth of animals. The European Union (EU) has strictly regulated controls on the use of veterinary drugs, including growth-promoting agents, particularly in food-animal species, by issuing several Regulations and Directives, and, since 1998, has prohibited antibiotics used in human medicine from being added to feed.

Council Directive 96/23/EC contains guidelines for controlling veterinary drug residues in animals and their products with detailed procedures for EU member states to set up national monitoring plans, including details on sampling procedures.

For any type of animal or food, there are two main groups of substances that must be monitored: Group A comprises prohibited substances (in conformity with Council Directive 96/22/EC and table 2 of Regulation 37/2010) and it is divided into 6 subgroups (A1-A6); and Group B which contains residues of many pharmacologically active substances which may be authorized for use in food-producing animals in the EU *i.e.* Table 1 of Annex to Regulation 37/2010.

Table 5: List of veterinary drugs and substances with anabolic effect (with examples)

Group A. Substances havinganabolic effect and unauthorized substances
A1 Stilbenes (diethylstilbestrol)
A2 Antithyroid agents (thiouracils)
A3 Steroids (androgens, gestagens, estrogens)
A4 Resorcylic acid lactones (zeranol)
A5 Beta-agonists (clenbuterol)
A6 Other compounds (nitrofurans, chloramphenicol)
Group B. Veterinary drugs and contaminants
B1 Antibacterial substances (sulphonamides and quinolones)
B2 Other veterinary drugs
B2a Anthelmintics

	B2b Anticoccidials, including nitroimidazoles
	B2c Carbamates and pyrethroids
	B2d Sedatives
	B2e Non-steroidal anti-inflammatory drugs (NSAIDs)
	B2f Other pharmacologically active substances (dexamethasone)

Regulation No. 470/2009 lays down the procedure for setting Maximum Residue Limits (MRLs) for residues of pharmacologically active substances in food of animal origin and a complete list of substances and their MRLs are given in table 1 to the Regulation and substances for which no MRL values could be established (*i.e.* prohibited substances) are listed in table 2.

Minimum required performance limit (MRPL) applies to several substances prohibited or not authorised in food-producing animals in the EU e.g. chloramphenicol, nitrofurans or e.g. malachite green (EU, 2004/25; 2003/181). MRPLs are "the minimum content of an analyte in a sample, which at least has to be detected and confirmed". They are the reference point for action ('Action levels') when evaluating food consignments (EU, 2005/34).

Group A and B subgroups which must be tested for in the commodities/animal species are listed in Annex II to Directive 96/23 and EU countries must follow these rules strictly. Non-EU countries can be given some flexibility, though, where testing for Group B substances should be chosen likely to be used or misused in their livestock production systems. Refer to an EU guidance for more detail (EU, Manual).

The prohibition of the use of growth promoting agents (e.g., hormones and beta-agonists) is laid down in Council Directive 96/22/EC. Control for Group A is more critical (*i.e.* has a higher priority) because of public-health concern: relatively large numbers of samples have to be analysed and more stringent analytical criteria have to be used in view of the serious implications of positive results for public health. Technical guidelines and performance criteria (e.g. detection level, selectivity and specificity) for residue control in the framework of Directive 96/23/EC are described in Commission Decision 2002/657/EC.

9.3.2. Samples

For monitoring drugs having an MRL, animal tissues (e.g., muscle, liver, kidney, fat and milk) are selected most frequently. Since the drug concentration in the consumable parts of an animal has to be below the MRL, these matrices are of interest. For control of the use of unauthorized substances, urine, liver and meat are normally the matrices to analyse. Hair and retina are the new sample materials to be chosen as matrices to analyse. In these matrices, residues of certain growth-promoting agents can be detected even months after treatment. Residues in urine, liver and to some extent also meat will no longer be detectable after that time.

Also matrices like eggs, honey and fish are important products to test and according to EEC legislation they should be included in the residue control plan (EU, 97/747). Three examples are given here on these matrices.

Some fluoroquinolones and polyether antibiotics are approved for treatment of disease in poultry but are banned for egg-laying hens. Analysis of these substances in eggs is therefore done.

In fish malachite green and its metabolite leucomalachite green has been found. Malachite green is a fungicide and parasiticide and is readily being absorbed by fish and these substances are often included in residue control of fish.

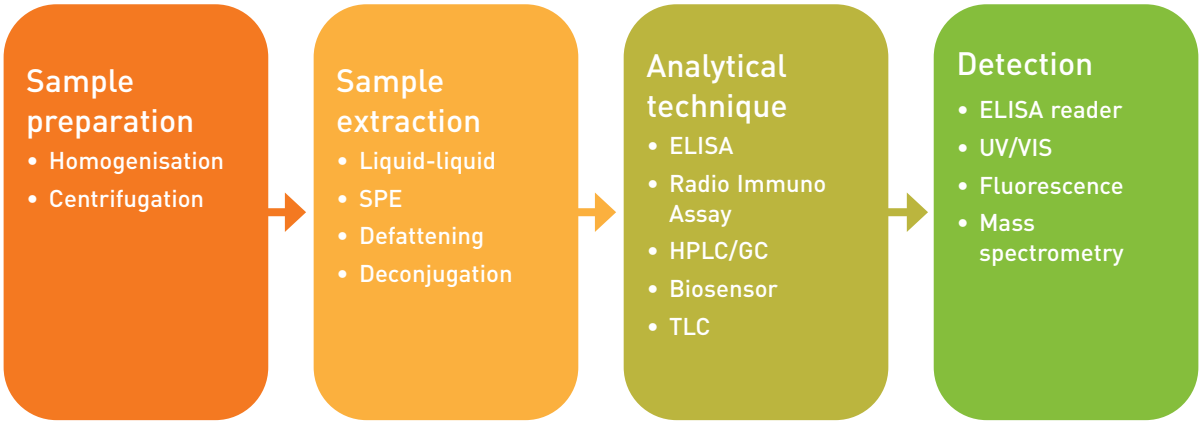
Use of antibiotics in beekeeping has been known for a long time and e.g. nitrofurans and macrolide antibiotics are of interest for preventing American foulbrood disease. In honey no MRL values has been established but minimum required performance levels, MRPL, has been proposed in the range 20-50 µg/kg for many classes of antibiotics. Official MRPL's for nitrofurans and chloramphenicol in honey is 1 µg/kg and 0.3 µg/kg respectively.

9.3.3. Sample extraction

Selecting a suitable method of residue analysis will, in many instances, depend on the problem at hand as well as on the final goal. When large sample series have to be monitored for a group of antibiotics such as sulphonamides and tetracyclins, sample throughput will be an important criterion since speed is of the essence. In this situation a screening method is selected because high sample throughput and speed are the characteristics of such a method. When, on the other hand, samples are suspected to contain an illegal growth-promotor such as, e.g., stanozolol, method selectivity will no doubt be the main criterion because avoiding false non-compliant results now is of overriding importance. In this situation a confirmatory method is of interest because it provides full or complementary information enabling to confirm the identity of the substance.

An analytical procedure depends on the analytical technique choice, but in most cases it consists of a sample preparation, sample extraction, analytical technique with a detection.

Table 6: Analytical procedure



During sample preparation the sample is prepared for analysis; urine is centrifuged, fat and tendons is removed from meat, egg is homogenized etc. During sample extraction an enzymatic or chemical deconjugation step can be relevant when

analytes are in a conjugated for (steroids in urine conjugated with one or more glucuronide- or sulfategroup), and defattening with heptane if the sample contains fat substances that interfere in the analysis.

During sample extraction, solid-phase extraction (SPE) is the most popular technique followed by liquid extraction (liquid-liquid extraction and liquid extraction of homogenized tissues).

Solid-phase extraction is a method for rapid sample preparation in which a stationary phase is typically packed in a barrel. The most popular sorbent has been silica-based where different functional groups have been attached. Hydrophobic sorbent with C18 or C8 are often used but for some applications ion exchange sorbent are useful for removal of interferences.

The introduction of various types of co-polymeric sorbents has helped to make SPE a more robust extraction technique with a wider application range than the conventional silica-based sorbents (Kinsella, 2009). The most widely used polymeric sorbents are the (poly)styrenedivinylbenzene co-polymers. A polymeric sorbent frequently used for the extraction of drug residues from biological samples is Oasis-HLB. This is a hydrophilic-lipophilic balanced copolymer (HLB) and the hydrophilic properties increase the water wettability of the polymer, and the lipohilic divinylbenzene provides the reversed-phase retention necessary to retain analytes. Like the silica based sorbent the polymeric sorbents also have a range of sorbents with different polarity.

Also use of immunoaffinity extraction are used. It can be seen that IAC is particularly advantageous when low detection levels in the ngkg–1 to μgkg–1 are required for banned substances, particularly when using less selective HPLC based detection systems. IAC columns are available for multi steroid analysis, but it must be mentioned that with regards licensed drugs, IAC is perhaps too specific since there is now a trend to move towards multi-class residue methods with detection by LC–MS/MS.

A wide range of IAC columns are commercially available from vendors such as Rhone Diagnostics Technologies, Biocode, r-Biopharm, Tecna, Randox and Euro-Diagnostica.

Molecular imprinted polymers (MIP) are used more and more. These MIP's are engineered crosslinked polymers that exhibit high affinity and selectivity towards a target compound or class of structurally related compounds. MIPs can be tailored to selectively extract analytes present in complex matrices such as blood, urine, tissue or feed. These materials have demonstrated binding to trace levels of target analytes, and display high selectivity in the presence of other compounds that have similar physico-chemical properties, as well as being extremely stable. Like the immunoaffinity columns they are very specific which can be a problem in multi residue analysis. But for analysis of chloramphenicol and beta agonists they have proven very useful but many other applications are available.

9.3.4. Analytical techniques

Laboratories involved in residue control must face a large number of samples, with a variety of analytes, to be analysed in relatively short periods of time. Thus,

there is a need for screening methods that allow the analysis of such a large number of samples in short periods of time. This means that high through-put methods with low cost must be available. These methods must be able to detect an analyte or class of analytes at the level of interest (Van Peteghem, Daeselaire, & Heeremans, 2001). Some false positives (false non-compliant) are acceptable, as they will be further submitted for confirmatory analysis but the method must avoid or reduce to a minimum the number of false negative results (false compliant) because they will not be further analysed.

There are different techniques available for the screening of residues in animal foods. In general, the limits of detection will depend on the previous extraction and clean-up of the sample.

The immunological methods mainly consist of ELISA test kits. There are many kits commercially available. Other immunological methods are based on radioimmunoassay and, more recently, several methods using biosensors are commercially available. The chromatographic methods mainly consist in two types, GC and HPLC, coupled to different detection systems. Biosensor methods, antimicrobial inhibitor tests and TLC methods are also methods used for screening of veterinary drug residues but they will not be discussed further. In table 3 examples of analytical methods used is shown.

Table 7: Methods used for residue control (examples)

Compound/Group	Method – Screening	Method – Confirmation
Steroids	ELISA (Estradiol, Testosteron or Trenbolon etc.) RIA (Testosteron) GC-MS (Multimethod) LC-MS/MS (Multimethod)	GC-MS LC-MS/MS
Beta-Agonists	ELISA GC-MS LC-MS/MS	LC-MS/MS GC-MS
Antibiotics	Microbial inhibitor tests (commercial or in-house) ELISA (Sulfonamides, Tetracyclins, Macrolides or Aminoglycosides etc.) HPLC (see above) LC-MS/MS (Multimethod)	HPLC (with DAD or Fluorescence) LC-MS/MS
Chloramphenicol	ELISA GC-MS LC-MS/MS	GC-MS LC-MS/MS
Nitrofurans	HPLC LC-MS/MS	LC-MS/MS

9.3.4.1. Immunological detection methods (ELISA and RIA)

The immunological technique is based on the antigen and antibody reaction. This interaction antigen–antibody is very specific and useful for the detection of residues of chemical and veterinary drugs in animal foods and the most usual technique consists in the enzyme-linked-immunosorbent assay (ELISA) and the detection system is usually based on enzyme-labelled reagents where colour is developed during incubation and measured with a microplate reader, which is proportional to the amount of analyte in the sample. Radioimmunoassay (RIA) implies the measure of radioactivity of immunological complex using a counter. These kits offer important advantages like the large number of samples to be analysed per kit, fast to operate and its high specificity and sensitivity in comparison to conventional detection methods. Another advantage is the possibility to use the kit within the food-processing facility without the need to transport the sample to the laboratory. Many diagnostic companies have marketed ELISA test kits for the detection of such residues. Thus, ELISA kits are available for a large number of substances within each group like b-agonists, corticosteroids, steroids, stilbenes, resorcylic acid lactones and several antibiotics (sulfonamides, tetracyclines, chloramphenicol etc.).

In general, though, these methods require some time of manual operation for the addition of sample, incubation, washing and discarding of liquids, reagents for colour development etc. This has prompted the development of automated ELISA tests by some companies.

9.3.4.2. HPLC

The use of high performance liquid chromatography (HPLC) expanded during the 1990s and the availability of automation somehow facilitated its use as a screening technique. HPLC is a separative technique and its ability to detect compounds depends on the type of detector used. The choice of the detection system is very important for selectivity and sensitivity. Some analytes, not detected by absorbance or fluorescence, may require chemical modifications to render chromophore, fluorescent or UV-absorbing compounds. Usually, the detection of multi-residues is based on a SPE extraction clean-up (discussed previously) followed by filtration and injection into a reversed phase HPLC with UV-diode array detection.

When using HPLC results are often obtained in short time (few min/sample) and the method is very sensitive and specific. Of course you also have drawback when using HPLC. You need experts to run the samples and sample preparation like extraction and filtration is often necessary. Furthermore you have a high initial investment for the equipment.

For all classes of veterinary drugs HPLC methods have been published and it is by far the most used method for analysis of veterinary drugs in animal food.

9.3.4.3. LC-MS

HPLC coupled to a mass spectrometer is nowadays used routinely for screening and confirmation. When confirming Group A substances (banned substances and hormones) mass spectrometry is a must and for confirmation of Group B substances (e.g. substances with MRL like antibiotics) it is widely used. In this

sense, the coupling of high speed HPLC with MS/(MS) can substantially reduce the analysis time. The mass spectrometer is an instrument that will sort out charged gas molecules (ions) according to their masses and in short when using LC-MS you separate the molecules chromatographically on the LC-system and according to their mass on the mass spectrometer. In the mass spectrometer the molecules are firstly ionized and accelerated by an electric field. The ions are dispersed according to their mass-to-charge ratio and finally they are detected giving an electrical signal. For ionization of the molecules either electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI) is used. Both ionisation techniques facilitate the analysis of small to relatively large and hydrophobic to hydrophilic molecules and are thus very adequate for the analysis of veterinary drug residues.

In Commission Decision 2002/657 the performance criteria are shown for the different types of detectors for confirmatory methods. Suitable confirmatory methods are based on GC or LC in combination with detectors like MS, DAD and fluorescence. Criteria include use of internal standard, if possible, and when using full-scan UV/VIS (DAD) the absorption maxima should be the same as those of the calibration standard and the spectra should be comparable. When evaluating confirmatory results the concept of identification points (IP's) are introduced. During confirmatory analysis, a specific number of IPs has to be collected. For confirmation of the identity of Group A substances a minimum of four IPs is required. For confirmation of the identity of Group B substances, a minimum of three IPs is required. The number of IPs earned by a specific analysis depends on the technique used. A low-resolution mass spectrometer (e.g., a triple quadrupole (QqQ) or an ion trap [IT]) is able to acquire 1.0 IP for the precursor ion and 1.5 IPs for each product ion (*i.e.* with the selection of two multi-reaction-monitoring (MRM) transitions, 4.0 IPs are acquired). When using single quadrupole MS one identification point is earned for each ion measured and therefore four mass fragments has to be acquired.

9.3.5. References

1. Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC.
2. Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stock farming of certain substances having a hormonal or thyrostatic action and of ß-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC.
3. Commission Regulation (EU) No. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.
4. Regulation No. 470/2009/EC of the European parliament and of the council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No. 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No. 726/2004 of the European Parliament and of the Council.

5. Commission Decision 2004/25/EC of 22 December 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin.
6. Commission Decision 2003/181/EC of 13 March 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin.
7. Commission Decision 2005/34/EC of 11 January 2005 laying down harmonised standards for the testing for certain residues in products of animal origin imported from third countries.
8. European Commission. Manual on residue requirements for non-EU countries exporting to the EU (ec.europa.eu/food/food/chemicalsafety/residues/third_countries_en.htm).
9. Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.
10. Commission Decision 97/747 of 27 October 1997 fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products.

9.4. ANALYTICAL METHODS FOR PESTICIDES

9.4.1. Introduction

POPs, which it defined as "chemical substances that persist in the environment, bio-accumulate through the food web, and pose a risk of causing adverse effects to human health and the environment". Stockholm Convention on Persistent Organic Pollutants is an international environmental treaty, signed in 2001 and effective from May 2004, which aims to eliminate or restrict the production and use of persistent organic pollutants (POPs), including a number of organochlorine pesticides such as DDT, aldrin, chlordane and heptachlor. Most of the chlorinated organic pesticides can be found in food items, as the compounds are fat soluble accumulated up through the food chain and biomagnified in higher species. Therefore, the most important human intake sources for the organochlorine pesticides in foodstuffs are fatty foods including fish, meat, eggs and dairy products whereas pesticides used today are often found in fruits and vegetables, cereals and at some frequency in product of animal origin.

Pesticides are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides are also classified on the basis of their actions, namely, algaecides (control algae in lakes, canals, swimming pools etc.), antifouling agents (kill or repel organisms that attach to underwater surfaces such as boat bottoms), antimicrobials (kill organisms such as bacteria and viruses, attractants (attract pests, for example, to lure an insect or rodent into a trap), biopesticides (pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals), biocides (kill microorganisms), disinfectants and sanitizers (kill or inactivate disease-producing microorganisms on inanimate objects), fungicides (kill fungi including blights, mildews, molds and rusts), fumigants (produce gas or vapour

intended to destroy pests in buildings or soil), herbicides (kill weeds and other plants that grow where they are not wanted), insecticides (kill insects and other arthropods), miticides/acaricides (kill mites that feed on plants and animals), microbial pesticides (microorganisms that kill, inhibit, or outcompete pests, including insects or other microorganisms), nematicides (kill nematodes: microscopic, worm-like organisms that feed on plant roots), ovicides (kill eggs of insect and mites), pheromones (biochemicals used to disrupt the mating behaviour of insects), repellents (repel pests, including insects such as mosquitoes and birds), rodenticides (control mice and other rodents), defoliants (cause leaves or other foliage to drop from a plant, usually to facilitate harvest), desiccants (promote drying of living tissues, such as unwanted plant tops), insect growth regulators (disrupt the molting, maturity from pupal stage to adult or other life processes of insects), and plant growth regulators (substances excluding fertilizers or other plant nutrients that alter the expected growth, flowering, or reproduction rate of plants). Insecticides are usually classified into the following three classes according to their mode of action (Handbook of Pesticides, 2010).

The European Union (EU) has strictly regulated controls on the use of pesticides and guidelines for controlling of pesticides residues are found Council Directive 96/23/EC for animal products and their products with detailed procedures for EU member states to set up national monitoring plans, including details on sampling procedures. Furthermore the EU Commission implemented Regulation No. 788/2012 on 31 August 2012 concerning a coordinated multiannual control programme of the Union for 2013, 2014 and 2015 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin, which all member states are obelised to follow.

EU pesticide residue maximum residue limits (MRLs) are assembled in a database with public access directly via ec.europa.eu/sanco_pesticides/public/index.cfm. However attention should be paid to the EU Commission regulation no. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin as some compounds can be used both as pesticide or as a veterinary drug.

Le lien

9.4.2. Monitoring of pesticides

For any type of animal or food, there are two main groups of substances that must be monitored in all EU member states according to the EU legislation Council Directive 96/23/EC Annex 1.

Group B – Veterinary drugs and contaminants

- *B2 Other veterinary drugs*
- *B2c Carbamates and pyrethroids*
- *B3 Other substances and environmental contaminants*
- *B3a Organochlorine compounds including PCBs*
- *B3b Organophosphorus compounds*
- *B3f Others*

The matrixes to be included can be found in Annex II.

Table 8: Annex II

Residue or substance group to be detected by type of animal, their feeding stuffs, including drinking water, and primary animal products							
Type of animal feeding-stuffs or animal products Substance groups	Bovine, ovine, caprine, porcine, equine animals	Poultry	Aqua-culture animals	Milk	Eggs	Rabbit meat and the meat of wild(*) game and farmed game	Honey
A1	X	X	X			X	
2	X	X				X	
3	X	X	X			X	
4	X	X				X	
5	X	X				X	
6	X	X	X	X	X	X	
B1	X	X	X	X	X	X	X
2a	X	X	X	X		X	
b	X	X			X	X	
c	X	X				X	X
d	X						
e	X	X		X		X	
f							
3a	X	X	X	X	X	X	X
b	X			X			X
c	X	X	X	X		X	X
d	X	X	X	X			
e			X				
f							

(*) Only chemical elements are relevant where wild game is concerned

Commission Regulation (EC) No. 788/2012 concerning a coordinated multiannual control programme ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin, which all member states are obliged to follow.

9.4.3. Analytical techniques

Analytical method used for the determination of **pesticides in food samples** and their compliance with EU regulation **shall be validated according to the guidance provided in Document SANCO/10684/2009**.

The analysis of pesticide residues normally involves assessment of the pesticides and food matrixes to analyse, extraction of the pesticide from the sample matrix, removal of interferences (clean-up), concentration of pesticides residues as low limit of quantification is often needed, determination and quantification of pesticides, sometimes including metabolites or breakdown products and reporting of results.

Several extraction techniques have been developed which might be used for extraction of pesticides from food samples. Liquid-liquid partitioning methods, Single-drop and liquid micro-extraction (SDME), Pressurized liquid extraction or accelerated solvent extraction (PLE or ASE), Microwave-assisted extraction (MAE), Sorptive and Membrane-Based Extraction Methods, Solid-phase micro-extraction (SPME), Sorptive-phase developments, SPE Disk extraction, Stir-bar sorptive extraction (SBSE) and Membrane micro-extraction are examples of extraction and/or clean-up methods used in analytical method for determination of pesticides from food samples.

The selection of the extraction procedure to be employed is governed by the type of pesticide and the nature of the matrix/sample under examination. The extraction procedure should provide high recoveries and preferable give higher sample throughput combined with sufficiently selective and consume small amounts of organic solvent, and require minimum clean-up before the determination. PLE/ASE can be used for the determination of residues of organophosphorus, carbamates and organochlorine pesticides in fruits and vegetables, but also for extraction of pesticides from products of animal origin.

A relatively simple procedure termed 'QUECHERS' for the quantitative determination of pesticides in fruits and vegetables has been developed. The QUECHERS method compared favourably with traditional methods and is under consideration by regulatory bodies. The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, Safe) is today the most common used analytical method for fruits and vegetables and is the acronym for a highly beneficial analytical approach that vastly simplifies the analysis of multiple pesticide residues in fruit, vegetables, cereals and processed products thereof (www.quechers.com). Within a relatively short time after the publication of the QuEChERS-method the method has experienced a widespread adoption around the globe and is today probably the most used sample preparation approach in pesticide residue analysis worldwide. The QuEChERS procedure entails a number of simple analytical steps and is thus fast and easy to perform and little susceptible to errors. QuEChERS provides high recoveries for a very broad scope of pesticides belonging to various chemical classes and the final extract, being solved in acetonitrile, gives full flexibility in the choice of the determinative analysis technique. Direct connection with liquid- and gas-chromatography is

possible. The procedure entails the following steps; Weigh 10 g of sample and add 10 mL acetonitrile and internal standard, agitate intensively and add NaCl, $MgSO_4$ and buffering salts for phase-separation and pH-adjustment. Agitate intensively and centrifuge to give the raw extract, from which an aliquot is taken of the upper organic phase and subject it to dispersive SPE clean-up (d-SPE) by mixing it with $MgSO_4$ and a sorbent e.g. PSA, to remove water and undesired co-extractives. Agitate shortly and centrifuge (optionally add Analyte Protecting Agents) to achieve the final extract that can be analysed directly by GC-MS and LC-MS.

Soxhlet extraction has been widely used for determination of pesticides especially organochlorine pesticides from fish. The best results have often been proven when using a combination of polar and non-polar solvent on the Soxhlet apparatus.

SPME determination can be used for different screening applications. The principle of the SPME device is placing a stationary phase coated extraction fibre that enables a direct injection into the gas chromatography injection port. The extraction of the pesticides can be performed either directly from a liquid phase or as headspace solid-phase micro-extraction (HS-SPME) to the determination of the pesticides in the headspace above a sample.

Gel permeation chromatography (GPC) is a clean-up and purification step often used for determination of pesticides from matrixes containing fat, for example products of animal origin. The technique uses a chromatographic column to separate components in a complex mixture based upon their molecular size or shape. The size exclusion column used for this procedure separated lipids and high molecular weight components from target pesticides in tissue extracts. An automated gel permeation chromatographic technique using a high performance liquid chromatography system consisting of an auto-¬sampler and an isocratic pump to purify sample extracts. However in most cases, the GPC clean-up is insufficient and a further SPE clean-up step is required.

Solid-phase extraction (SPE) phases are more similar to liquid chromatography stationary phases and can be used as for example normal or reverse phase separation of the pesticides from the matrix. As regards sample treatment, solid-phase extraction (SPE) was found to be the most popular techniques followed by liquid extraction (liquid–liquid extraction and liquid extraction of homogenized tissues). Solid-phase extraction is a method for rapid sample preparation in which a stationary phase is typically packed in a barrel. The most popular sorbent has been silica-based where different functional groups have been attached. Hydrophobic sorbent with C18 or C8 are often used but for some applications ion exchange sorbent are useful for removal of interferences.

Matrix solid-phase dispersion (MSPD) has been used for clean-up of organochlorine, organophosphorus, and carbamate pesticides in dairy and fatty foods. MSPD eliminates the need for the tedious homogenization and centrifugation steps found in traditional solvent extraction and also reduces both the analytical time and the amount of solvent used.

9.4.3.1. Gas chromatography

Gas chromatography has been proved to be a largely used, versatile and sensitive method for pesticide residue analysis. Determination of pesticides using GC-ECD (electro capture detector) or GC-NPD (nitrogen phosphorus detector) has been widely used for several years, however today most methods uses GC coupled to a mass spectrometry for the determination of pesticide residues all kind of food samples.

Table 9: Comparison of different detection techniques

Detection technique	Advantages	Disadvantages
Electron capture detector (ECD)	High sensitivity for halogenated compounds	Low dynamic range (linearity)
Nitrogen Phosphorous detector (NPD)	High sensitivity for nitrogen and phosphorus High specificity	Only sensitive for nitrogen and phosphorus
Mass spectrometer (MS)	High sensitivity Chemical or structural information	Expensive

Different injection techniques are used on the gas chromatograph. The Split/Splitless injector is commonly used, introducing a sample into the heated injection port via a syringe through a septum, The sample is vaporised and the carrier gas either force the sample onto the column (splitless mode) or only a portion of the sample onto the column (split mode). Split injection is preferred when working with dirty samples or samples having high concentrations of the analytes whereas splitless injection is best suited for trace analysis with low amounts of analytes and therefore the method often used for pesticide determination.

On-column injection introduces the sample directly into the chromatographic column in without heat.

The PTV injector is a temperature-programmed-vaporising technique, which allow introduction of large sample volumes in capillary GC. The temperature of the liner is chosen slightly below the boiling point of the solvent allowing the solvent to continuously evaporate and vented through the split line whereas the pesticides in retained in the liner. When the solvent is vented at high temperature rate is applied to the injector, vaporising the pesticides and introducing them onto the column.

The Time-of-flight mass spectrometry (TOF-MS) is a method of mass spectrometry in which an ion's mass-to-charge ratio is determined via a time measurement and has the advantages that is measure full scan spectra for all compounds.

A triple quadrupole mass spectrometer or a tandem mass spectrometer consisting of two quadrupole mass spectrometers in series, with a (non-mass resolving) collision cell quadrupole between them for collision-induced dissociation. The advantages is a very specific determination of the compounds.

The nitrogen phosphorus detector (NPD) is a type of detector commonly used with gas chromatography, in which thermal energy is used to ionize an analyte. With this method, nitrogen and phosphorus can be selectively detected. A concentration of hydrogen gas is used such that it is just below the minimum required for ignition. A rubidium or caesium bead, which is mounted over the nozzle, ignites the hydrogen (by acting catalytically), and forms a cold plasma. Excitation of the alkali metal results in ejection of electrons, which in turn are detected as a current flow between an anode and cathode in the chamber. As nitrogen or phosphorus analytes exit the column, they cause a reduction in the work function of the metal bead, resulting in an increase in current. Since the alkali metal bead is consumed over time, it must be replaced regularly.

The electron capture detector is used for detecting electron-absorbing components (high electronegativity) such as organochlorine pesticides in the output stream of a gas chromatograph. The ECD uses a radioactive beta particle (electron) emitter in conjunction with a so-called makeup gas flowing through the detector chamber. As the detector is specific the determination of organochlorine pesticide used two capillary columns with different polarity to verify the identity of the pesticide.

9.4.3.2. Liquid chromatography

The application of liquid chromatography for pesticide detection depends on the pesticides having sufficiently different partition coefficients in the selected solvent system. As non-volatile pesticides are analysed by this technique, an extremely wide range of pesticides can be separated.

Liquid chromatography–mass spectrometry (LC-MS) for the purpose of simultaneous determination of carbamate and OPPs in fruits and vegetables has been used for several years. A coupled-column reversed-phase, liquid chromatography was used for the determination of pesticide residues. Ultra-performance liquid chromatography and HPLC with tandem quadrupole mass spectrometry were used to determine priority pesticides in baby food.

Liquid chromatography–mass spectrometry (LC-MS) is a chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and selectivity. Generally its application is oriented towards the general detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture).

For ionization of the molecules either electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI) is used. Both ionisation techniques facilitate the analysis of small to relatively large and hydrophobic to hydrophilic molecules and are thus very adequate for the analysis of veterinary drug residues.

In Commission Decision 2002/657 the performance criteria are shown for the different types of detectors for confirmatory methods. Suitable confirmatory methods are based on GC or LC in combination with detectors like MS, DAD and fluorescence. Criteria include use of internal standard, if possible, and when using full-scan UV/VIS (DAD) the absorption maxima should be the same as those of the calibration standard and the spectra should be comparable.

9.4.4. References

1. Council Directive 96/23/EC.
2. Commission regulation No. 37/2010.
3. Commission regulation No. 788/2012.
4. Commission Decision 2002/657.
5. Validation guidance Document No. SANCO/10684/2009.

9.5. ANALYTICAL METHODS FOR MYCOTOXINS

9.5.1. Introduction

Mycotoxins are secondary fungal metabolites with diverse structures and toxicological properties that induce a variety of toxic effects in humans and animals when foods and feed contaminated with these compounds are ingested. The toxic effects include acute toxicity, carcinogenicity, immunotoxicity, mutagenicity and teratogenicity.

Several hundreds of mycotoxins, produced by a wide range of different fungi are known today, however, only about 30 to 40 have been shown to be contaminants of human or animal food. In particular, the fungi of the genera *Aspergillus*, *Penicillium* and *Fusarium* are significant in foods and feed all over the world.

The Food and Agricultural Organization of the United Nations (FAO) has estimated that **up to 25% of the world's food crops are significantly contaminated with mycotoxins**. In many developing countries mycotoxins present not only a health problem, but also a considerable economic problem. Requirements for compliance to the food safety standards can be a barrier for developing countries to participate in the international trade and, more seriously, may lead to the exclusion of small scale producers in developing countries from global supply chains. The present food safety standard requirements should not be sought as barrier to market entry, but as motivator to enable developing countries to improve their market competitiveness and agricultural practices.

The occurrence of mycotoxins in agricultural commodities depends on such factors as region, season, and conditions under which a particular crop is grown, harvested, and stored. Crops grown in warm and moist weather in the tropical and subtropical countries are much more prone to mycotoxin contamination than those in temperate zones. In addition to specific growth conditions, the fungal spoilage of crops and their grains is enhanced by drought, insect damage and cracking or breaking of kernels during harvesting. During the entire postharvest period, food crops are essentially in a state of storage, and fungal growth on them is preventable only by careful regulation of moisture content, temperature, and other environmental conditions.

Mycotoxins with regulated EU maximum limits in different food matrices include the aflatoxins, deoxynivalenol, fumonisins, patulin, ochratoxin A and zearalenone (Table 10), where aflatoxins and fumonisins raise most concern in the warmer regions.

Aflatoxins are widely distributed, often at high levels, in staple foods such as maize, peanuts and other commodities. The widespread occurrence of aflatoxins in Africa and other tropical countries make contamination a major potential hazard to human health, which is highlighted with an attributed outbreak of aflatoxicosis as recent as 2004 in Kenya. Fumonisins have carcinogenic properties and are primarily produced by *F. proliferatum* and *F. verticillioide*s. These species are mainly present in tropical and subtropical areas and fumonisin contaminations of pre-harvest crops, and high concentrations have been observed in maize and different maize products. There are several groups of fumonisins with several members, but fumonisin B1 is the predominant and best studied analog OTA is regarded as an important and most commonly occurring of a group of structurally related compounds essentially produced by species of only two genera of fungi namely *Penicillium* and *Aspergillus*. These fungi grow in a wide range of environmental conditions in terms of pH, substrate, temperature and moisture. OTA is the major compound found as a natural contaminant of plant material, including cereals, fresh grapes, dried vine fruit, wine, beer, coffee, and cocoa. *Penicillium* is considered to be responsible for production of OTA in colder climates while *Aspergillus* in tropical and subtropical regions. Other examples of fungi and their production of mycotoxins are shown in Table 10.

Table 10: Toxigenic fungi, their toxic metabolites, toxic effects and known contaminated foods

Fungi	Mycotoxin	Toxic effects	Foods
• *Aspergillus flavus* • *Aspergillus parasiticus*	• Aflatoxin B1 and B2 • AflatoxinG1 and G2 • Aflatoxin M1	• Acute toxicity • (especially to the • liver) • Liver cancer • Immune suppression • Growth abnormalities, • Lowered productivity	• Maize • Nuts (pistachio nuts, peanuts, Brazil nuts) • Spices • Dried figs • Milk and milk products
• *Aspergillus ochraceus* • *Penicillium* spp. • *Aspergillus fumigatus*	• Ochratoxin A • Patulin • OTA • Gliotoxin	• Acute toxicity (especially to the kidney) • Cancer	• Cereals • Beer • Coffee • Cocoa • Dried vine fruit • Spices • Raisin and apples
• *F. verticillioides* • *F. proliferatum* • *F. avenaceum*	• Fumonisins • Enniatins • Beauvericin	• Leuko encephalomalcia • Neural tube defects • Pulmonary oedema • Esophagealcancer	• Maize • Maize products

Fungi	Mycotoxin	Toxic effects	Foods
• *Fusarium graminearum* • *F. culmorum,* • *F. poae,* • *F. sporotrichioides*	• Deoxynivalenol, • Nivalenol • Zearalenone	• Acute toxicity • Immune suppression • Reproductive dysfunction	• Cereal • Cereal products

9.5.2. Sampling

The sampling procedure is of great importance due to the heterogeneous distribution of mycotoxins in lots of raw agricultural products. If the sampling is not carefully performed the analytical result would be of no value. In EU legislation, the maximum level for a contaminant is always tied to sampling protocols and requirements for analytical methods. EU policy is that the sampling procedure must be practical and must minimize the consumer's risk without rendering trade impossible.

No methods are specified in EU legislation but different performance criteria are set: reliable analytical results require the systematic application of quality assurance measures, including documentation, trained personnel, appropriate and calibrated instrumentation, validated methods and adequate laboratory infrastructure.

Method performance is demonstrated through proficiency or inter laboratory studies, use of reference materials, and statistical evaluation (repeatability, reproducibility values and accuracy and precision control charts). Adequate quality assurance procedures enable both the identification of problems and their correction.

9.5.3. Sample clean-up

An important aspect in the extraction of mycotoxins is the clean-up procedure, since they affects the purity of sample and hence the sensitivity of the results. In clean-up of mycotoxin samples, several methods have been used. They include liquid-liquid extraction (LLE) that utilizes the solubility advantage of the toxin in aqueous phase and in immiscible organic phase to extract the compound into one solvent.

Others are supercritical fluid extraction (SFE) that uses a supercritical fluid like CO_2, and solid phase extraction (SPE) that uses the chromatographic principles using cartilages packed with bonded phases which form the stationary phase. Several SPE columns, immunoaffinity chromatography or combination of both are usually used for clean-up procedures.

Antibody based immunoaffinity column (IAC) are favoured by many analysts because of its specificity. However, the disadvantages for using IAC have been associated with its relatively high costs, limited shelf life and the required maximum amount of toxins that can be applied on it. When compared to LLE, the SPE offers the advantages of using fewer solvents and are faster in operations. They can also be used to pre-

concentrate the samples hence providing better detection results in addition to cleaning sample. Their main disadvantage is its less robustness hence makes difficult to get a single universal type of cartilage to be used for extraction of all toxins. Notwithstanding, they are cheap when compared to IAC and nowadays they are very popular techniques used in analysis of mycotoxins.

9.5.4. Analytical techniques

9.5.4.1. HPLC

The most frequently and widely used method of mycotoxin analysis is high-performance liquid chromatography (HPLC). HPLC reference methods that are quite sensitive and have reasonably low levels of detection has been developed for most of the major mycotoxins and hence regarded as the good quantitative methods.

HPLC separates a mixture of compounds, usually present in an extract of a sample by relative affinity of the compounds for a stationary column and a mobile phase. Compounds eluted from the column pass through a detector that helps the quantitation for the specific compounds in the original sample injected onto the column.

Detectors operate based on selective response for solute, such as UV-absorbance or fluorescence, or on bulk property of mobile phase which is modified by the solute, such as refractive index. The most widely used types of detectors and their characteristics are shown in table 11.

Table 11: Comparison of commercial HPLC detectors

Detector	Approximate limit of detection (ng)
Ultraviolet	0.1–1
Refractive index	100–1 000
Evaporative light-scattering	0.1–1
Charged aerosol	1
Electrochemical	0.01–1
Fluorescence	0.001–0.01
Nitrogen (N $\xrightarrow{\text{combustion}}$ NO $\xrightarrow{O_3}$ NO_2 $\longrightarrow$ *hv*)	0.3
Conductivity	0.5–1
Mass spectrometry	0.1–1
Fourier transform infrared	1 000

HPLC methods are relative expensive and require well-equipped laboratories and considerable analytical expertise. In some circumstances, it would be advantageous with simpler, faster methods based on competitive Enzyme Linked Immuno Sorbent Assay (competitive ELISA) as would be advantageous under circumstances where there is a need for screening large number of samples as for example in order to obtain information on sample variability.

They would also enable small and middle-sized grain processing industries to perform a better self-control of their products, because these methods do not require expensive and specialised techniques. It is, **however absolutely necessary that the method is reliable** and that the limit of detection enables the user to pinpoint grain with mycotoxin contents above the relevant EU limit. In essence, the major challenges for any simple method are to fulfil the demands on accuracy and sensitivity that are accomplished by the standard methods.

Today **ELISA kits are commercially available for the EU regulated mycotoxins**: the aflatoxins, fumonisin B1 and B2, DON, OTA, patulin, zearalenone in various food and feed matrices.

9.5.4.2. Multi-mycotoxin analysis

Today multi-mycotoxin methods are going to be more and more popular and highly relevant because real samples will most often contain a mixture of different mycotoxins.

Due to the chemical differences of the mycotoxins it will not be possible to use the same clean-up procedure nor the same detection method. For these reasons different more genetic methods have recently been developed **based on the QuEChERS method** (Quick, Easy, Cheap, Effective, Rugged and Safe).

Briefly, the method uses acetonitrile (ACN) for extraction of the analytes followed by the addition of high concentrations of $MgSO_4$ and NaCl. The salts induce a phase separation between ACN and water, keeping extremely polar contaminants in the water. Buffering can be applied to overcome pH effects of the matrix on the extraction efficiency of chargeable compounds. Most of the multi-methods use LC-MS/MS on triple quadrupole systems, although LC-TOF-MS and LC-Orbitrap based methods are also looking promising. A major drawback with the multi-methods is that matrix interferences often occur which might lead to low sensitivity and other problems.

9.5.5. References

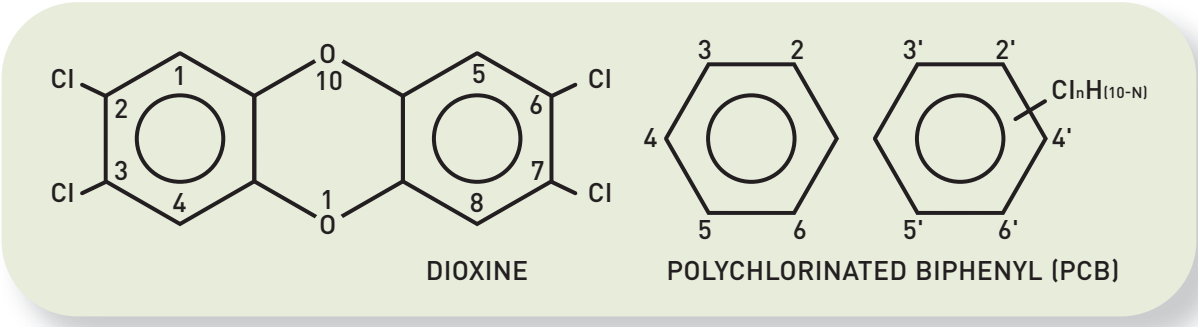
1. The EU regulation is strict and described in several Commission regulations:
   - Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
   - Commission Regulation (EU) No. 165/2010 of 26 February 2010 amending Regulation (EC) 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins.
   - Commission Regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.
   - Commission Regulation (EU) No. 178/2010 of 2 March 2010 amending Regulation (EC) 401/2006 as regards groundnuts (peanuts), other oilseeds, tree nuts, apricot kernels, liquorice and vegetable oil.

2. Special conditions governing certain foodstuffs imported from certain third countries due to contamination risks of these products by aflatoxins are laid down in:
   - Commission Regulation (EC) No. 1152/2009 of 27 November 2009 imposing special conditions governing the import of certain foodstuffs from certain third countries due to contamination risk by aflatoxins and repealing Decision 2006/504/EC.
3. In order to assist the competent authorities on the official control of aflatoxin contamination in food products which are subject to Commission Regulation (EC) No. 1152/2009, a guidance document has been prepared:
   - "Guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins", ec.europa.eu/food/food/chemicalsafety/contaminants/guidance-2010.pdf.

9.6. ANALYTICAL METHODS FOR DIOXINS AND PCB

9.6.1. Introduction

Dioxins are a short expression for **a group of 210 compounds** including polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofuranes (PCDF). Dioxins are a group of polychlorinated aromatic compounds with similar structures and chemical and physical properties. This group of compounds consists of 75 dibenzo-p-dioxins (PCDDs) and 135 dibenzofurans (PCDFs). Of these compounds, **2,3,7,8-TCDD is the most toxic**. Dioxins are **formed during industrial and household combustion processes and as by-products** by certain industrial productions e.g. metal manufacturing and metal reclamation.



DIOXINE POLYCHLORINATED BIPHENYL (PCB)

A **subgroup of PCB congeners** has the same toxicological effects as dioxins and they are referred to as '**dioxin-like PCB**' (they have been found to resemble TCDD in its biochemical and toxicological properties). PCB, polychlorinated biphenyls, is a group of 209 compounds that, due to their physical and chemical properties, were utilized for industrial purposes since the 1930s. PCB has been widely used as an isolating material in capacitors and transformers. Furthermore, because of its chemical stability and fire-retarding properties, PCB has been used in hydraulic systems and as an additive to paints, printing inks, coolants, and cutting oils, and PCB has been used as a plasticizer in plastics.

Dioxin-like PCB

12 PCB congeners exhibit **toxicological properties similar to dioxins** and are therefore often termed "dioxin-like PCBs". The other PCBs do not exhibit dioxin-like toxicity but have a different toxicological profile. Most work on the development of analytical methods has therefore focused on dioxins and dioxin-like PCBs.

Once released into the environment these organic pollutants will persist for decades in soils, waters and the atmosphere and hence continue to be of concern for a very long time after the release has ceased. These pollutants cause impairment of the immune system, the nervous system, the endocrine system and the reproductive functions and are also suspected of causing cancer. Foetuses and new-born children are most sensitive to exposure. There is considerable public, political and scientific concern over the negative effects on human health and on the environment of long-term exposure even to very small amounts of these chemicals.

Dioxins and PCB have regain attraction as persistent organic contaminants in feed and food during the last decade. In the late 1990's several incidents with contaminations of feed and food occurred and they initiated a more systematic monitoring of dioxins and PCB in many countries. In 2001 the European Union (EU) established maximum levels for dioxins in certain feed and food, and a revision in 2006 included maximum levels for the sum of dioxins and dioxin-like PCB and in 2012 non-dioxin-like PCB. Food is the major source for the human intake of dioxins and PCB and with feed being an important contributor of the food contamination. In many industrialised countries human exposures of dioxins and PCB are high compared to toxicological tolerable levels and for that reason it is of interest to follow any trend in contamination of animal feed and food over the years.

9.6.2. Compounds, matrices and levels

Maximum levels are established for the most toxic dioxins and PCB congeners, which have been assigned dioxin toxicity equivalency factors, TEF (EU 2012). This include 17 2,3,7,8 chlorine substituted PCDDs and PCDFs and 12 dioxin-like PCB. The TEF-values are used to weight the concentrations of the individual congeners before summing to produce the total toxic equivalency for dioxins, TEQ (Table 12).

Maximums levels are also established for 6 non-dioxin-like PCBs (EU 2012). The maximum levels are defined for the sum of PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180.

The most important matrices are fatty food items such as meat, fish, eggs and dairy products. For animal feed the matrices include both fat containing ingredients and minerals. The concentrations in the samples are typical in the low pg TEQ/g range for the dioxin-like compounds and ng/g for the non-dioxin-like PCBs.

Table 12: Dioxin toxicity equivalency factors (TEF) as defined by WHO in 2005 for dioxins (PCDD and PCDF) and dioxin-like PCB

WHO-TEF for dioxins		WHO-TEF for dioxin like PCB	
PCDD		**Non-ortho PCB**	
2,3,7,8-TCDD	1	PCB 77	0.0001
1,2,3,7,8-PeCDD	1	PCB 81	0.0003
1,2,3,4,7,8-HxCDD	0.1	PCB 126	0.1
1,2,3,6,7,8-HxCDD	0.1	PCB 169	0.03
1,2,3,7,8,9-HxCDD	0.1	**Mono-ortho PCB**	
1,2,3,4,6,7,8-HpCDD	0.01	PCB 105	0.00003
OCDD	0.0003	PCB 114	0.00003
PCDF		PCB 118	0.00003
2,3,7,8-TCDF	0.1	PCB 123	0.00003
1,2,3,7,8-PeCDF	0.03	PCB 156	0.00003
2,3,4,7,8-PeCDF	0.3	PCB 157	0.00003
1,2,3,4,7,8-HxCDF	0.1	PCB 167	0.00003
1,2,3,6,7,8-HxCDF	0.1	PCB 189	0.00003
1,2,3,7,8,9-HxCDF	0.1		
2,3,4,6,7,8-HxCDF	0.1		
1,2,3,4,6,7,8-HpCDF	0.01		
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0003		

9.6.3. Sample extraction, clean-up and detection techniques

Analytical methods for dioxins and PCB are performance based methods (EPA 1613, EN 16215). In the European legislation screenings methods (chemical and bioassays) are allowed but positive samples has to be analysed by a verification method, which is based on GC/HRMS (gas chromatography interfaced to a high resolution mass spectrometer).

Samples extraction involves extraction with organic solvents by e.g. soxhlet or PLE (pressurised liquid extraction) and a comprehensive clean-up of the extract by multilayer/multi column chromatography. The extract is finally fractionated into two fractions containing dioxins plus non-ortho PCB and mono- plus di-ortho PCBs, which is analysed separately by GC/HRMS. On most matrices it is possible to analyse the mono- and di-ortho PCB on GC/LRMS (low resolution mass spectrometer) or dual column GC/ECD (electron capture detector). It is possible to automate a major part of the sample clean-up procedure (Focant, 2001).

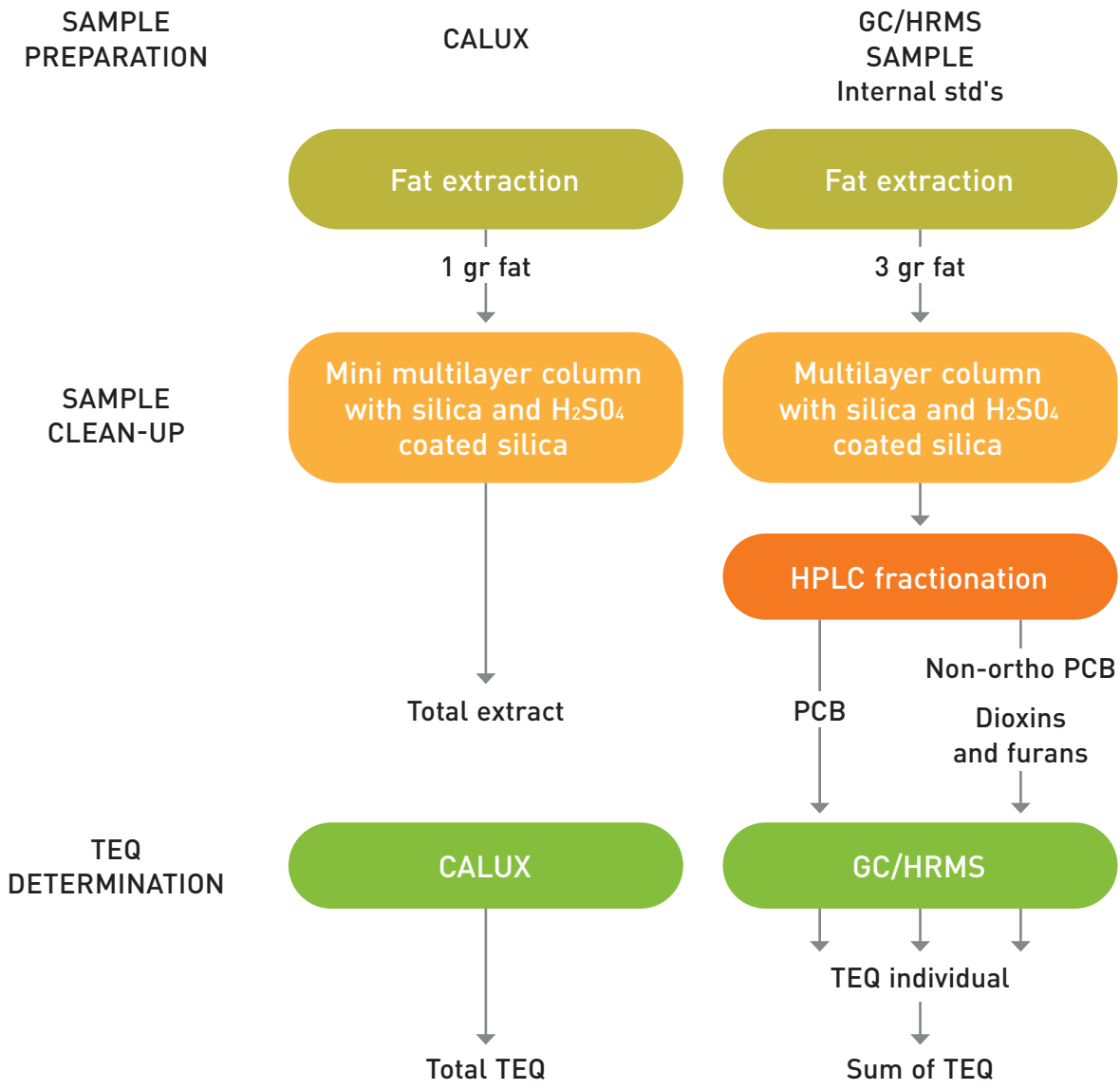


Figure 8 - Flow diagram describing the analytical principle for determination of dioxins and PCB. The CALUX bioassay as a screening method and GC/HRMS as a verification method

9.6.4. References

1. Official methods:
   - EN 16215:2012 Animal feeding stuffs – Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS.
   - EPA 1613: 1994 Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope dilution HRGC/HRMS.
2. EU legislation:
   - Commission regulation (EU) 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006.

- Commission regulation (EU) 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs
- Commission recommendation of 23 August 2011 on the reduction of the presence of dioxins, furans and PCBs in feed and food.

3. Other:
   - EU strategy 2010 progress report (dioxins). ec.europa.eu/environment/dioxin/index.htm

9.7. METHODS FOR THE DETERMINATION OF NITRATES

Regulation (EC) No. 1881/2006 sets maximum levels for certain contaminants in foodstuffs and includes limits for nitrates in vegetables.

A number of EN standards have been published that provide methods for the determination of nitrates and/or nitrites, in foodstuffs, including vegetables. These include:

- EN 12014-2:1997 Foodstuffs – Determination of nitrate and/or nitrite content – Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products – Ion Chromatography with conductivity detector/ HPLC with UV detector.
- EN 12014-5:1997 Foodstuffs – Determination of nitrate and/or nitrite content – Part 5: Enzymatic determination of nitrate content of vegetable containing food for babies and infants.
- EN 12014-7:1997 Foodstuffs – Determination of nitrate and/or nitrite content – Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after cadmium reduction.

Methods based on the use of cadmium reduction of nitrate to nitrite and colorimetric determination of the nitrite are generally considered to be less reliable than those based on the use of HPLC or Ion chromatography. These methods give significantly lower recoveries than those obtained by the use of HPLC or ion chromatography, probably as a result of variations in the efficiency of the cadmium reduction process and incomplete colour development attributable to poor control of the pH after addition of the acidic colour development reagent.

For this reason **it is recommended that laboratories use methods based on the use of HPLC or ion chromatography**. To carry out the analysis of vegetables for the determination of their nitrate content, laboratories will require HPLC equipment with a conductivity detector or UV detector, capable of measuring absorbance at 205 nm or a dedicated Ion Chromatograph.

9.8. METHODS FOR THE DETERMINATION OF PROCESS CONTAMINANTS

9.8.1. Introduction

Process contaminants include 3-monochloropropane-1,2-diol (3-MCPD) and acrylamide arising from the treatment of foods during processing. Food may also be intentionally adulterated with melamine to boost nitrogen content and apparent protein content.

Regulation (EC) No. 1881/2006, as amended, setting maximum levels for certain contaminants in foodstuffs and includes limits for 3-MCPD in hydrolysed vegetable protein and in Soy sauce. Currently no limits are set for acrylamide. Acrylamide is found in a wide range of foodstuffs including products such as potato chips, French fries, roast potatoes, breakfast cereals, crisp bread and roasted coffee. Acrylamide (CH_2=$CHCONH_2$) may be formed in foods during cooking or other thermal processing such as frying, baking or roasting at temperatures of 120°C or higher.

9.8.2. Methods for the determination of 3-MCPD

The method is applicable to the determination of 3-chloropropane-1,2-diol in hydrolysed vegetable protein (HVP), soups and stocks, stock cubes, soy sauce, malt extract, salami, fish, cheese, flour, starch, cereals, and bread. The method is available in AOAC Official Method 2000.01.

9.8.2.1. Principle

An internal standard 3-chloropropane-1,2-diol-d5 (3-MCPD-d5) and sodium chloride solution are added to the test portion and the resultant mixture is homogenised. After sonication, the contents of an Extrelut™ refill pack are added and mixed thoroughly. The mixture is transferred to a glass chromatographic column and the nonpolar components are eluted with a mixture of n-hexane and diethyl ether. The 3-MCPD is then eluted with diethyl ether, and the extract is concentrated to a small volume. A portion of the concentrated extract is derivatised and analysed by gas chromatography with mass spectrometric detection (GC/MS). The concentration of 3-MCPD is expressed in mg/kg.

The following information regarding a method for the determination of 3-MCPD has been taken from an application report entitled 'Improving the Sensitivity of Detection of 3-chloropropane-1,2-diol (3-MCPD) using Gas Chromatography with Negative Chemical Ionisation Mass Spectrometry detection'.[188]

9.8.2.2. Sample preparation

A specified amount of a soy sauce sample was weighed into a beaker and 5M sodium chloride solution was added. This was followed by sonication to achieve homogeneity.

After sonication, the contents of an Extrelut™20 refill were mixed with the sample and the mixture was transferred into a glass chromatography column.

Le lien ne fonctionne pas

188 www.shimadzu.com.sg/sap/appli/sap_appdat/C-GCGCMS/AD0005GM%203-MCPD%20NCI%20(GCMS-001).pdf.

The non-polar components were eluted using 75 mL of hexane – diethyl ether (90:1). The 3-MCPD was subsequently eluted using 250 mL of diethyl ether. The 3-MCPD extract was concentrated to 10 mL and 2 mL of this extract was evaporated to dryness using a gentle stream of nitrogen. 1 mL of 2,2,4- trimethylpentane was added to the dried extract and derivatisation was carried out using the following procedure.

9.8.2.3. Derivatisation

50 µL of N-heptafluoro-butyrylimidazole (HFBI) was added to the 1 mL solution of the sample extract. The mixture was then heated at 70 °C for 20 min. After cooling the mixture to room temperature, 1 mL of water was added. The mixture was then stirred on a Vortex stirrer for 30 s and the phases were allowed to separate and the process of mixing on the Vortex stirrer and separation of the phases was repeated. The upper organic layer was transferred to a new vial, dried over anhydrous sodium sulphate and injected into the GCMS.

Table 13: GC operating parameters were as follows

Column:	DB-5, 30m length × 0.25 mm ID × 0.25 µm film thickness
Injector temperature:	270 °C
Oven temperature:	50 °C (1 min) → 90 °C at 2 °C/min → 270 °C at 40 °C/min
Carrier gas:	Helium
Inlet pressure:	100 kPa
Injection:	Splitless, sampling time 0.6 min
Injection volume:	1 µL

Table 14: Mass spectrometer operating parameters were as follows

Ionisation mode	**Electron Impact**
Interface temperature:	270 °C
Acquisition mode:	Selected Ion Monitoring (SIM)
m/z:	253, 275, 289, 291, 453
Ionisation mode:	Negative Chemical Ionisation
Reagent gas:	Isobutane
Reagent gas pressure:	0.5 bar
Interface temperature:	200 °C
Acquisition mode:	Full scan and SIM
m/z range (Scan):	154 – 514 amu
m/z (SIM):	446, 482, 502 (3-MCPD-d_0) 449, 486, 507 (3-MCPD-d_5)

9.8.3. Methods for determination of acrylamide

Methods for the determination of acrylamide in food are based on the use of GC/MS/MS or LC/MS/MS. For example, in a survey for the presence of a number of process contaminants carried out in the UK in 2008 and reported in a Food Standards Agency, Food Surveillance Information Sheet (Number 03/09 July 2009) a GC/MS/MS method was used. The principle of the method was as follows.

Acrylamide was determined as 2-bromopropenamide using Gas Chromatography tandem Mass Spectrometry (GC/MS/MS) following extraction of samples with water and bromination. Carbon-13 labelled acrylamide was used for quantification.

In a report from the EU's Joint Research Centre, details are provided of a collaborative study of a method for the determination of acrylamide in coffee by isotope dilution LC/MS/MS.

The collaborative study was carried with the intention of validating an existing method that was standardised for the determination of acrylamide in bakery and potato products and extending its scope to include the determination of acrylamide in roasted coffee.

The method is based on aqueous extraction of the roasted coffee matrix and solid phase extraction (SPE) clean-up followed by isotope dilution high performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

The test portion of the sample was spiked with isotope labelled acrylamide and extracted on a mechanical shaker with n-hexane and water for one hour. The sample extract was centrifuged, the organic phase was discarded, and a portion of the aqueous extract was further cleaned-up by solid phase extraction on Isolute Multimode followed by Isolute ENV+ columns. The acrylamide-containing fraction eluted from the second SPE column was evaporated to about 500 µL and analysed by reverse phase HPLC with tandem mass spectrometric detection.[189]

9.8.4. Methods for determination of melamine

In some countries, limits for melamine are specified in foods. The introduction of limits for the presence of melamine was in response to a number of incidents where melamine has been deliberately added to food and to animal feed, to enhance the apparent protein content.

In the EU these requirements are set out in Regulation (EC) No. 594/2012 amending Regulation (EC) No. 1881/2006 as regards the maximum levels of the contaminants Ochratoxin A, non-dioxin-like PCBs and melamine in foodstuffs, published in July 2012. These specify limits for a) food with the exception of infant formulae and follow-on formulae and b) powdered infant formulae and follow-on formulae.

The majority of methods that have been published for the determination of melamine in food, are based on the use of LC/MS/MS. For example, DD ISO/TS 15495:2010 – provides guidelines for the quantitative determination of melamine and cyanuric acid by LC-MS/MS in milk, milk products and infant formulae.

Le lien ne fonctionne pas

189 irmm.jrc.ec.europa.eu/activities/acrylamide/Documents/eur_23403_en.pdf.

The principle of the method is described as follows. The sample is made homogenous (or optionally reconstituted in the case of powdered samples). A suitable solvent is added to the test sample to precipitate proteins from the matrix and to extract melamine and cyanuric acid. After centrifugation, an aliquot of the supernatant is analysed by LC-MS/MS.

LC-MS/MS includes any method combining either high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC), with either triple quadrupole or ion-trap mass spectrometric detection. Chromatographic separation is based on hydrophilic interaction liquid chromatography (HILIC) to ensure good separation of melamine and cyanuric acid. Ionisation of the substance is accomplished by electrospray ionization (ESI) and the mass spectrometric detection utilises the selected reaction monitoring (SRM) mode.

Quantification of both melamine and cyanuric acid is based on isotope dilution using stable isotope internal standards for both analytes.

9.9. ANNEXES

A.1. References and regulations

1. Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs.
2. Regulation (EC) No. 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.
3. EN 13804:2002 Foodstuffs – Determination of Trace Elements – Performance Criteria, general considerations and Sample Preparation.
4. EN 14083:2003 Foodstuffs – Determination of trace elements – Determination of lead, cadmium, chromium and molybdenum by graphite furnace atomic absorption spectrometry (GFAAS) after pressure digestion.
5. BS EN 14084:2003 Foodstuffs – Determination of trace elements – Determination of lead, cadmium, zinc, copper and iron by atomic absorption spectrometry (AAS) after microwave digestion.
6. BS EN 13806:2002 Foodstuffs – Determination of trace elements – Determination of mercury by cold-vapour atomic absorption spectrometry (CVAAS) after pressure digestion.
7. BS EN ISO 11212-1:1997 Starch and derived products – Heavy metals content – Part 1: Determination of arsenic content by atomic absorption spectrometry.
8. BS ISO 17239:2004 Fruits, vegetables and derived products – Determination of arsenic content – Method using hydride generation atomic absorption spectrometry.
9. BS EN 14332:2004 Foodstuffs – Determination of trace elements – Determination of arsenic in seafood by graphite furnace atomic absorption spectrometry (GFAAS) after microwave digestion.
10. BS ISO 17240:2004 Fruit and vegetable products – Determination of tin content – Method using flame atomic absorption spectrometry.
11. BS ISO 14377:2002 Canned evaporated milk – Determination of tin content – Method using graphite furnace atomic absorption spectrometry.
12. EC Regulation No. 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.
13. Guidance Document for Competent Authorities for the Control of Compliance with EU Legislation on Aflatoxins.
14. EN 12955:1999 Foodstuffs – Determination of aflatoxin B1, and the sum of aflatoxins B1, B2, G1 and G2 in cereals, shell-fruits and derived products – High performance liquid chromatographic method with post column derivatisation and immunoaffinity column clean-up.
15. EN 14123:2007 Foodstuffs – Determination of aflatoxin B1 and the sum of aflatoxin B1, B2, G1 and G2 in hazelnuts, peanuts, pistachios, figs, and paprika powder – High performance liquid chromatographic method with post-column derivatisation and immunoaffinity column clean-up.

16. ISO 16050:2003 Foodstuffs – Determination of aflatoxin B1, and the total content of aflatoxins B1, B2, G1 and G2 in cereals, nuts and derived products – High performance liquid chromatographic method.
17. EN 15851:2010 Foodstuffs – Determination of aflatoxin B1 in cereal based foods for infants and young children. HPLC method with immunoaffinity column clean-up and fluorescence detection.
18. EN ISO 14501:2007 – Milk and milk powder – Determination of aflatoxin M1 content – Clean-up by immunoaffinity chromatography and determination by high performance liquid chromatography.
19. EN ISO 15141-1:1998 Foodstuffs – Determination of ochratoxin A in cereals and cereal products – Part 1: High performance liquid chromatographic method with silica gel clean up.
20. EN ISO 15141-2:1998 Foodstuffs – Determination of ochratoxin A in cereals and cereal products – High performance liquid chromatographic method with bicarbonate clean up.
21. EN 14133:2009 Foodstuffs – Determination of ochratoxin A in wine and beer – HPLC method with immunoaffinity column clean-up
22. EN 14132:2009 Foodstuffs – Determination of ochratoxin A in barley and roasted coffee – HPLC method with immunoaffinity column clean-up
23. EN 15829:2010 Foodstuffs – Determination of ochratoxin A in currants, raisins, sultanas, mixed dried fruit and dried figs – HPLC method with immunoaffinity column clean-up and fluorescence detection.
24. EN 15835:2010 Foodstuffs – Determination of ochratoxin A in cereal based foods for infants and young children – HPLC method with immunoaffinity column clean-up and fluorescence detection.
25. BS EN 14177:2003 Foodstuffs – Determination of patulin in clear and cloudy apple juice and puree. HPLC method with liquid/liquid partition clean-up.
26. AOAC Method 2000.02 Patulin in Clear and Cloudy Apple Juices and Apple Puree – Liquid Chromatographic Method.
27. BS EN 15891:2010 Foodstuffs – Determination of deoxynivalenol in cereals, cereal products and cereal based foods for infants and young children – HPLC method with immunoaffinity column clean-up and UV detection.
28. JRC 44148 EN – 2008 Validation of an Analytical Method to Determine the Content of Fumonisins in Baby Food, Breakfast Cereals and Animal Feed – Report on the Collaborative Trial.
29. EUR 23559 EN – 2008 Validation of an Analytical Method to Determine the Content of T-2 and HT-2 Toxins in Cereals and Baby Food by Immunoaffinity Column Clean-up and GC-MS.
30. AOAC Official Method 2000.01 Determination of 3-Chloropropane-1,2-diol in Foods and Food Ingredients Gas Chromatography/Mass Spectrometric Detection.

31. AD-0005-GM Improving the Sensitivity of Detection of 3-chloropropane-1,2-diol (3-MCPD) using Gas Chromatography with Negative Chemical Ionisation Mass Spectrometry detection.
32. UK Food Standards Agency, Food Surveillance Information Sheet (Number 03/09 July 2009) – Survey of Process Contaminants in Retail Foods 2008.
33. EUR 23403 EN– 2008 Validation of an Analytical Method to Determine the Content of Acrylamide in Roasted Coffee – Report on the Collaborative Trial.
34. EC Regulation No. 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin.
35. ISO 3890-1:2009 Milk and milk products – Determination of residues of organochlorine compounds (pesticides) – Part 1: General considerations and extraction methods.
36. ISO 3890-2:2009 Milk and milk products – Determination of residues of organochlorine compounds (pesticides) – Part 2: Test methods for crude extract purification and confirmation.
37. EC Decision 657-2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.
38. ISO 8260:2008 / IDF 130 – Milk and milk products – Determination of organochlorine pesticides and polychlorobiphenyls – Method using capillary gas liquid chromatography with electron capture detection.
39. BS EN 1528-1: 1997 Fatty Food – Determination of Pesticides and Polychlorinated Biphenyls (PCBs) – Part 1. General
40. BS EN 1528-2: 1997 Fatty Food – Determination of Pesticides and Polychlorinated Biphenyls (PCBs) – Part 2. Extraction of fat, pesticides and PCBs and determination of fat content.
41. BS EN 1528-3: 1997 Fatty Food – Determination of Pesticides and Polychlorinated Biphenyls (PCBs) – Part 3. Clean-up methods.
42. BS EN 1528-4: 1997 Fatty Food – Determination of Pesticides and Polychlorinated Biphenyls (PCBs) – Part 4. Determination, confirmatory tests, miscellaneous.
43. EN 12393-1: 1999 Non fatty food – Multi-residue methods for the gas chromatographic determination of pesticide residues – Part 1. General considerations.
44. EN 12393-2: 1999 Non fatty food – Multi-residue methods for the gas chromatographic determination of pesticide residues – Part 2. Methods for extraction and clean-up.
45. EN 12393-3: 1999 Non fatty food – Multi-residue methods for the gas chromatographic determination of pesticide residues – Part 3. Determination and confirmatory tests.
46. EN 12396-1: 1998 Non Fatty Foods – Determination of dithiocarbamate and thiuram disulphide residues – Part 1: Spectrometric method.
47. EN 12396-2: 1998 Non-fatty foods – Determination of dithiocarbamate and thiuram disulfide residues – Part 2: Gas chromatographic method.

48. EN 12396-3: 2000 Non-fatty foods – Determination of dithiocarbamate and thiuram disulfide residues – Part 3: UV spectrometric xanthogenate method.
49. EN 13191-1: 2000 Non-fatty food – Determination of bromide residues – Part 1: Determination of total bromide as inorganic bromide.
50. EN 13191-2: 2000 Non-fatty food – Determination of bromide residues – Part 2: Determination of inorganic bromide.
51. EN 15662:2009 Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS-method
52. PMR-001 Determination of Pesticides in Fruits and Vegetables (with Solid Phase Extraction Clean-Up and GC/MSD and HPLC Fluorescence Detection) – Canadian Food Inspection Agency.
53. PMR-006 Determination of Pesticides in Infant Foods using Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC/ESI-MS/MS) – Canadian Food Inspection Agency.
54. SPR-001 Determination of Formetanate in Fruits (HPLC Method) – Canadian Food Inspection Agency.
55. SPR-002 Determination of EBDC in Fruits and Vegetables (HPLC With Fluorescence Detection) – Canadian Food Inspection Agency.
56. SPR-003 Determination of Benomyl in Fruits and Vegetables (HPLC Method) – Canadian Food Inspection Agency.
57. SPR-004 Determination of Daminozide in Apples (GC-MSD Method) – Canadian Food Inspection Agency.
58. SPR-005 Determination of Thiabendazole in Fruits and Vegetables (HPLC Method) – Canadian Food Inspection Agency.
59. SPR-006 Determination of EBDC in Fruit and Vegetables By GC/MSD – Canadian Food Inspection Agency.
60. SPR-007 Determination of Abamectin in Fruits and Vegetables using HPLC with Fluorescence Detection – Canadian Food Inspection Agency.
61. SPR-008 Determination of 2-Imidazolidinethione in Fruits and Vegetables by GC/MSD – Canadian Food Inspection Agency.
62. EN 12014-2:1997 Foodstuffs – Determination of nitrate and/or nitrite content – Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products – Ion Chromatography with conductivity detector/HPLC with UV detector.
63. EN 12014-5:1997 Foodstuffs – Determination of nitrate and/or nitrite content – Part 5: Enzymatic determination of nitrate content of vegetable containing food for babies and infants –
64. EN 12014-7:1997 Foodstuffs – Determination of nitrate and/or nitrite content – Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after cadmium reduction

65. Regulation (EC) No. 470/2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin.

66. Regulation (EC) No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

67. Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products.

68. CLG-CAM.04 – Determination and Confirmation of Chloramphenicol – USDA FSIS September 2009.

69. CLG-NFUR2.01 – Screening and Confirmation of Nitrofuran Metabolites by Liquid Chromatography – Tandem Mass Spectrometry – USDA FSIS March 2010.

70. CLG-SUL4.01 – Quantitation and Confirmation of Sulfonamides by Liquid Chromatography – Tandem Mass Spectrometry (LC-MS-MS) – USDA FSIS May 2011.

71. CLG-BLAC.03 – Screening and Confirmation of ß-Lactam Antibiotics by HPLC-MS/MS – USDA FSIS July 2011.

A.2. Analytical methods for toxic elements (metallic contaminants)

Sampling and sample preparation

As with any analysis for contaminants at trace levels, care must be taken to avoid cross contamination of samples either via sampling, sample preparation or through the analytical procedure.

Regulation (EC) No. 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs, includes advice on the avoidance of cross contamination during the sampling process.

Part B of the regulation sets out the procedures to be followed when samples are taken for analysis.

Paragraph B.1.3 concerning precautions to be taken requires that, in the course of sampling, precautions must be taken to avoid any changes which would affect either the levels of contaminants originally present in the foodstuff being sampled, adversely affect the analytical determination or make the aggregate samples unrepresentative.

B.1.7 concerning packaging and transmission of samples requires that each sample be placed in a clean, inert container that provides adequate protection from contamination, from loss of analytes by adsorption to the internal wall of the container and from damage in transit. It also requires all necessary precautions be taken to avoid any change in the composition of the sample which might occur during the transportation or storage of the sample.

Part C of Regulation (EC) No. 333/2007 sets out the procedures to be followed during sample preparation and analysis.

Paragraph C.2.1 concerning the precautions and general considerations that apply to sample preparation, clearly states that the basic requirement of sample preparation is to obtain a representative and homogeneous laboratory sample without introducing secondary contamination.

The Regulation also requires that all of the sample material received by the laboratory must be used for the preparation of the laboratory sample.

Paragraph C.2.2.1 provides the procedures that must be followed in preparing samples for the determination of lead, cadmium, mercury and inorganic tin. In particular, the analyst is required to ensure that samples do not become contaminated during sample preparation. Analysts are advised that wherever possible, apparatus and equipment coming into contact with the sample should not contain those metals to be determined and be made of inert materials e.g. plastics such as polypropylene, polytetrafluoroethylene (PTFE) etc.

All equipment used for sample preparation should be acid cleaned to minimise the risk of contamination. High quality stainless steel may be used for cutting edges.

In Regulation (EC) No. 333/2007 reference is made to EN 13804:2002 – Foodstuffs – Determination of Trace Elements – Performance Criteria, general considerations and Sample Preparation.

EN 13804:2002 provides more detailed guidance on sampling, sample preparation and the specific requirements that apply to the analysis of foods for trace elements. With regard to sample preparation, EN 13804:2002 includes the following guidance.

In trace element analysis only the edible part of the sample should be analysed. Reported results should relate to the edible part of the sample.

For preparation of the test sample a sample mass of at least 200 g should be available from the edible part of the laboratory sample. Parts which are normally not intended for eating should be discarded from the food, e.g. outer leaves, shell, skin, bones.

Additionally gross surface contamination like soil, rotten parts of plants or leaves must be removed.

Most of the food samples need to be cleaned more or less intensively, depending on the degree of their dirtiness. During washing of the samples a leaching effect from cut surfaces must be avoided. In order to avoid contamination by tap water, final rinsing with de-ionised water is recommended. The rinsing water should be removed from the samples, by draining, tapping on a soft tissue paper (e.g. fish, mushrooms) or using a separator (e.g. crinkly vegetables).

Equipment and reagents for the determination of trace elements

Since the levels of metallic contaminants to be determined are rather low, precautions must be taken to ensure that results of analysis are not unduly influenced by cross contamination from various potential sources.

Laboratory environment

Since there is considerable risk of contamination from the environment, all analyses for trace metals should be conducted in a dedicated laboratory.

Air intakes to the laboratory should incorporate filters to remove dust and other particles, greater than 5 µm in size.

Items such as muffle furnaces must not be located in the same laboratory.

Equipment such as atomic absorption spectrometers and ICP spectrometers must be located in a completely separate area to that which is used for digestion of samples, preparation of calibration solutions etc. This is particularly important since these types of equipment can also release trace elements. It is also important to consider whether operations such as equipment maintenance and repair can affect the analytical result.

Similarly the initial preparation of samples, including the removal of outer packaging, must be carried out in area that is again separated from the laboratory where trace element analysis is performed.

Muffle furnaces

Where muffle furnaces are used for dry ashing of samples prior to the determination of trace metals, it must be remembered that they can be a source of contamination by dust from the furnace linings. Furnace chambers should be regularly cleaned using a vacuum cleaning device. The furnace should also be regularly heated to a higher than normal temperature (*i.e.* a few hundred degrees higher than the maximum temperature that is normally used for dry ashing of samples).

Microwave digestion vessel cleaning

Digestion vessels must be acid cleaned after each digestion.

Where digestion vessels are being used for the first time or if a previous digestion was incomplete, digestion vessels must be cleaned with liquid laboratory grade detergent and then subjected to the acid cleaning procedure described below.

Incomplete digestions are usually dark coloured (yellow to brown), have a bad odour and may contain undissolved material.

The manufacturer of the microwave digestion equipment may provide additional information on cleaning vessels and other components of the equipment. In particular, care must be taken not to use anything that can scratch the vessel walls. The Teflon material is relatively soft and can scratch easily.

- Detergent cleaning – The digestion vessels should be taken apart and its components left to soak for at least 2 hours in a solution of liquid laboratory grade detergent and hot water. Thermo-wells should be wiped down with a paper towel and detergent solution. After being left to soak for an appropriate period, thermo-wells and vessel components should first be rinsed with warm tap water and then rinsed thoroughly with reagent grade water. The rinsed vessels and components should be left to dry in a clean area.
- Acid cleaning – Add 10 mL of nitric acid to each vessel and microwave them, taking account of any guidance provided by the manufacturer. After the vessels have cooled to less than 50 °C remove them from the microwave oven and vent excess pressure slowly in a fume hood. The digestion vessels should then be taken apart and the covers and liners rinsed with copious quantities of reagent

quality water. The rinsed vessels and components should be left to dry in a clean area. Outside surfaces of vessels may be dried with laboratory tissues. If the digestion vessels are not to be used after drying, they should be stored assembled in an appropriate contamination free environment.

Glassware and equipment

To minimise the risk of cross contamination from other laboratory activities, all glassware and equipment used for trace metal analysis must be kept separate from other glassware and equipment used for other analyses.

All glassware used for trace metal determinations must be made of borosilicate glass and wherever possible, glassware such as flasks, volumetric flasks, beakers and other vessels, should be replaced by ones made of quartz, fluor polymers [e.g. polytetrafluoroethylene (PTFE), perfluoroalkoxyfluorocarbons (PFA)] or polyolefines (e.g. polyethylene, polypropylene).

Cleaning of glassware and other equipment

All reusable laboratory ware (glass, polyethylene, PTFE etc.) must be sufficiently clean for trace element analysis. The recommended cleaning procedure for all laboratory ware includes:

- washing in a laboratory detergent that is easily removed by rinsing;
- rinsing in reagent quality water to remove all traces of laboratory detergent;
- soaking for at least 4 hours in 10% nitric acid, followed by a final rinse with reagent quality water.

In addition all glassware or other laboratory ware should be rinsed with 1% nitric acid immediately before use.

N.B. All pipettes used for the preparation of calibration standards or to produce further dilutions of sample solutions, must be soaked for at least 4 hours in 10% nitric acid, followed by rinsing in reagent quality water.

Disposable laboratory ware

All disposable laboratory ware such as auto-sampler cups and bottles/tubes for analytical solution storage should be rinsed with 1% nitric acid immediately before use. Disposable laboratory ware should be tested for contamination or pre-cleaned before using a particular lot.

Pipettors

Colourless disposable plastic tips should be used with pipettors since coloured tips may be a source of contamination.

Gloves

Where gloves are used, they should be powder free vinyl, polyethylene, or nitrile gloves.

Latex gloves should not be used as these are known to be a possible source of contamination.

Gloves manufactured for clean room use are available, that are free from trace metals contamination.

Reagents

Water used in trace element determinations must be of high quality, for example twice distilled water, distilled in quartz apparatus, de-ionised water etc. The concentration of trace elements in water must be low enough as not to affect the results of the determination.

Acids and other chemicals of analytical grade are generally not sufficiently pure.

All acids, hydrogen peroxide and chemicals used as matrix modifiers, must be ultrapure with respect to their trace metal content.

The major suppliers of laboratory chemicals offer suitable grades of reagents, acids etc. These are normally referred to as ultrapure and specifically provided for use in trace analysis.

Preparation of calibration solutions

Commercially prepared lead, cadmium, mercury, arsenic and tin standard solutions, containing 1,000 mg/L of each element are available. These solutions may be used for the preparation of calibration solutions, provided that they are supplied with adequate certification and are traceable to a recognised standard.

When preparing calibration solutions from the concentrated stock, standard solutions, a complete record of their preparation must be maintained. The record must include details of all dilutions, together with the name of the analyst responsible for preparing the calibration standards.

All calibration standards must be clearly marked with preparation and expiry dates.

Storage of calibration solutions

It is preferable to store calibration solutions in a refrigerator but care should be taken to ensure that they are at room temperature, before use.

The shelf life of the most dilute calibration solutions should be set at no more than 30 days.

Since elements such as lead and cadmium can be adsorbed on to the surface of glass, low-density polyethylene bottles are recommended for storage of standard and analytical solutions. In addition use of plastic bottles is recommended because of low trace metals contamination. Other types of plastic bottles can be used such as high-density polyethylene, polypropylene, polystyrene, Teflon etc. Teflon FEP bottles are preferred from a contamination standpoint, particularly for storage of intermediate and standard solutions.

Reagent blanks

Reagent blanks must be included with every batch of samples analysed.

Reagent blanks serve two purposes:

- long term evaluation, which requires a large number (> 20) of blanks, from which the mean and standard deviation is calculated. The mean may be used to correct the result for contamination and the standard deviation for establishing the limit of detection. Both parameters must be regularly recalculated using new blank results, in order to reflect the current situation in the laboratory;
- batch evaluation. Each batch of samples must contain a sufficient number of blanks and the results obtained for each blank should be assessed for excessive (random) contamination. If severe contamination is detected for a particular batch of analyses, the analyst must decide whether or not the whole batch may have been contaminated to such a degree that the results must be discarded.

Methods for the determination of lead and cadmium

Statutory and guideline limits set for lead in food are generally in the range 0.02 to 0.1 mg/kg with limits for cadmium being in the same range. The detection of such low levels is unlikely to be possible by the use of Inductively Coupled Plasma Spectrometry (ICP) or Flame Atomic Absorption. Normally these elements would be determined by the use of a graphite furnace in conjunction with Atomic Absorption spectrometry. It is recommended that Zeeman background correction is used.

Whichever instrument is eventually used, samples require pre-treatment to destroy organic matter. The use of dry ashing is not appropriate in this case and so wet oxidation with nitric and sulphuric acids is required. It is recommended that this is carried out using a Microwave Digestion system in which the sample is digested in sealed bombs. Such a system is both more convenient and rapid than conventional wet oxidation.

An alternative to microwave digestion is pressure digestion. Both microwave digestion and pressure digestion are carried out in sealed digestion vessels. In the case of pressure digestion, the sealed vessel and its contents are heated by convection in a temperature controlled oven or heating block, to temperatures up to 250 °C.

By contrast, in microwave digestion, the sealed digestion vessel is heated in a microwave oven. The digestion vessels used are constructed of chemically inert materials but as these materials are transparent to microwaves, the sample solution is heated directly. As a result, the digestion time for microwave digestion is considerably shorter.

Since samples are analysed against relatively low limits, the use of high purity acids is required.

Many of the EN/ISO methods for the determination of lead and cadmium specify the use of graphite furnace atomic absorption spectrometry (GFAAS). Such methods frequently require the use of a chemical matrix modifier to stabilise the analyte or volatilise the bulk of the sample matrix, prior to volatilisation of element to be

determined. Matrix modifiers also prevent loss of the analyte during the ashing step by converting the analyte to a less volatile form. The use of matrix modifiers in this way reduces the background signal or interference from other chemicals that may be present in the sample matrix.

EN 14083 describes a method for the determination of lead, cadmium, chromium and molybdenum by graphite furnace atomic absorption spectrometry (GFAAS) after pressure digestion.

A number of options for matrix modifiers are available including:

- palladium/magnesium nitrate solution;
- ammonium phosphate/magnesium nitrate solution;
- palladium/ascorbic acid solution.

The analyst is advised that during optimisation of the graphite furnace programme the selected matrix modifier must be included.

The following data regarding the repeatability and reproducibility of the method is provided in Annexe A of the standard.

Element	Sample	Mean Concentration mg/kg	Repeatability (r) mg/kg	Reproducibility (R) mg/kg
Pb	Bovine liver, lyophilised	4.40	0.53	1.85
	Wholemeal wheat flour	0.37	0.12	0.26
	Bovine muscle, lyophilised	0.23	0.04	0.09
	Green paprika, lyophilised	0.10	0.04	0.13
	Tomato powder	0.64	0.21	0.44
	Spinach powder	1.24	0.38	0.62
Cd	Bovine liver, lyophilised	2.04	0.33	0.68
	Wholemeal wheat flour	0.16	0.03	0.05
	Bovine muscle, lyophilised	0.014	0.004	0.008
	Green paprika, lyophilised	0.38	0.06	0.22
	Tomato powder	0.19	0.02	0.08
	Spinach powder	0.40	0.05	0.13

EN 14084:2003 describes a method for the determination of lead, cadmium, zinc, copper and iron, in foodstuffs, by atomic absorption spectrometry (AAS) after microwave digestion.

Methods for determination of mercury

Mercury is normally determined by a method based on cold vapour generation of any mercury from the sample. Mercury present in the sample as Hg2+, is reduced to elemental mercury using a reducing agent such as tin (II) chloride. The mercury vapour is then passed into a silica cell which is placed in the light path of an atomic absorption spectrometer and the absorption of the mercury vapour measured using a mercury hollow cathode lamp.

BS EN 13806:2002 describes a method for determination of mercury in foodstuffs, by cold-vapour atomic absorption spectrometry (CVAAS) after pressure digestion.

Instruments are available that can be used for the direct determination of mercury in solid samples. Typically the instrument consists of a nickel boat into which the sample is placed. The nickel boat then passes into a quartz combustion tube containing a catalyst mixture. The sample is initially dried prior to combustion in an oxygen atmosphere. The mercury vapour produced by combustion is trapped on the surface of gold by amalgamation. The mercury is then released from the gold by heating to a temperature of 900 °C and the concentration of mercury in the vapour is determined by atomic absorption spectrometry using a silicon dioxide detector, at 253.6 nm.

Methods for determination of arsenic

The determination of arsenic in food is normally carried out using a method based on hydride generation in conjunction with atomic absorption spectrometry. Any arsenic present in the sample is converted to arsenic hydride by reaction with sodium borohydride. The arsenic hydride is then passed into a heated silica cell placed in which is placed in the light path of an atomic absorption spectrometer. The arsenic hydride decomposes on heating to produce elemental arsenic and the absorption of the arsenic vapour is measured using an arsenic hollow cathode lamp.

The following standards are also relevant:

- BS EN ISO 11212-1:1997 describes a method for the determination of arsenic content by hydride generation atomic absorption spectrometry, for starch and derived products.
- BS ISO 17239:2004 describes a method using hydride generation atomic absorption spectrometry for determination of the arsenic content of fruits, vegetables and derived products.
- BS EN 14332:2004 describes a method for the determination of arsenic in seafood by graphite furnace atomic absorption spectrometry (GFAAS) after microwave digestion.

Methods for determination of tin

Many of the methods for the determination of tin are based on the use flame atomic absorption spectrometry and require the use of a nitrous oxide, acetylene flame.

BS ISO 17240:2004 describes a method using flame atomic absorption spectrometry for the determination of the tin content of fruit and vegetable products.

Alternatively, BS ISO 14377:2002 specifies a method for the determination of the tin content of canned evaporated milk, based on the use of graphite furnace atomic absorption spectrometry. The method is applicable to samples with a tin content of more than 5 mg/kg.

The principle of the method is as follows. A test portion is diluted 100 fold with water and then further diluted (1:1) with 15% ascorbic acid solution as a matrix modifier. The atomic absorption is measured at a wavelength of 286.3 nm following electrothermal atomisation from the wall of the tube in a graphite furnace.

A.3. Analytical methods for antibiotics and other residues of veterinary medicines

Methods for the determination of chloramphenicol

ELISA methods

A number of commercially available test kits are available for the determination of chloramphenicol in different sample matrices.

Typically these tests are described as competitive enzyme immunoassays for the quantitative analysis of chloramphenicol. The methods are normally applicable to products such as milk, milk powder, honey, shrimps, meat, fish meal and eggs. The principle of the test is as follows.

The wells of a microtitre plate are coated by the manufacturer of the kit with antibodies directed against chloramphenicol. Chloramphenicol standards or sample solution and chloramphenicol enzyme conjugate are added to individual wells.

As the assay is a competitive enzyme immunoassay, free chloramphenicol and chloramphenicol enzyme conjugate compete for the chloramphenicol antibody binding sites. Any unbound enzyme conjugate is then removed in a washing step. After the addition of a substrate/chromogen solution to the wells, bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution causes a change in colour from blue to yellow. The intensity of the yellow colour is measured spectrophotometrically at 450 nm. Since this is a competitive assay, the absorption is inversely proportional to the chloramphenicol concentration in the sample.

Milk samples can be analysed directly but other samples such as milk powder, meat products etc., require extraction with ethyl acetate. The ethyl acetate extract is then evaporated to dryness and the residue re-dissolved in a buffer solution, prior to the ELISA assay.

Confirmatory methods

The method for the determination and confirmation of chloramphenicol is based on the use of gas chromatography with an electron capture detector for quantification of any chloramphenicol present in the sample. Confirmatory analysis is by GC/MS. The principle of the method is as follows.

Meta-chloramphenicol is added to the sample as a recovery index. The sample is then incubated with ß-glucuronidase to convert any chloramphenicol monoglucuronide to free chloramphenicol. Chloramphenicol is extracted from muscle with ethyl

acetate and the ethyl acetate is concentrated. A solution of sodium chloride is added and the remaining ethyl acetate is purged with nitrogen. The salt solution is applied to the top of a C18 SPE column, the cartridge is washed with methanol: water (20:80) and the chloramphenicol is eluted with acetonitrile. The eluate is evaporated to dryness and silanised. The amount of chloramphenicol is quantitatively determined by GC/ECD. Confirmation of chloramphenicol is achieved by the use of GC/MS, using negative ion chemical ionization.

The method is described in detail at: www.fsis.usda.gov/PDF/CLG_CAM_04.pdf.

Methods for the determination of nitrofurans

Methods for the detection of nitrofurans are based on detection of their corresponding metabolites in the sample.

The metabolites of nitrofurans are as follows:

- **Furazolidone** metabolite: 3- amino- 2-oxazolidinone (AOZ)
- **Furaltadone** metabolite: 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ)
- **Nitrofurantoin** metabolite: 1-aminohydantoin (AHD)
- **Nitrofurazone** metabolite: semicarbazide =(SEM)

ELISA assays

ELISA assays are available for the following nitrofuran metabolites.

Determination of AMOZ

ELISA assays for AMOZ are normally based on a competitive enzyme immunoassay and most of the commercially available kits can be used for the quantitative analysis of AMOZ in samples such as shrimp, meat (chicken, pork, and beef), liver, fish and whole egg.

The samples are homegenised and then any AMOZ present in the sample is derivatised by incubation at 37°C with a solution of 2-nitrobenzaldehyde in dimethyl sulphoxide. After incubation, the nitrobenzaldehyde derivative is then extracted into ethyl acetate and the ethyl acetate extract is then evaporated to dryness.

The residue is then dissolved in n-hexane and thoroughly mixed with a buffer solution. The mixture is the centrifuged and an aliquot of the lower aqueous phase is pipetted into a well of the microtitre plate. The wells of the microtitre plate are coated with capture antibodies directed against anti-AMOZ antibodies. AMOZ standards or sample solution, AMOZ enzyme conjugate and anti-AMOZ antibodies are added to individual wells.

As the assay is a competitive enzyme immunoassay, free AMOZ and AMOZ enzyme conjugate compete for the AMOZ antibody binding sites. At the same time, the anti-AMOZ antibodies are also bound by the immobilized capture anti-bodies. Any unbound enzyme conjugate is then removed in a washing step.

After the addition of a substrate/chromogen solution to the wells, bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop

solution causes a change in colour from blue to yellow. The intensity of the yellow colour is measured spectrophotometrically at 450 nm. The measured absorption is inversely proportional to the AMOZ concentration in the sample.

Determination of AOZ and SEM

The principle of ELISA assays and the test procedures for AOZ and SEM are the same as that which applies to AMOZ. The only difference is that the wells of the microtitre plate are coated with capture antibodies directed against anti-AOZ antibodies anti-SEM antibodies respectively.

Confirmatory methods of analysis for nitrofuran metabolites

The method for the confirmation of nitrofuran metabolites uses a Liquid Chromatography – Tandem Mass Spectrometry (LC-MS-MS). The method is applicable to the determination of AOZ and AMOZ in bovine, porcine, and avian (poultry) liver at levels ≥ 5 ppb, and fish muscle at levels ≥ 1 ppb.

The principle of the method is as follows. Nitrofuran antibiotics, furazolidone and furaltadone, are analysed as their respective metabolites, 3-amino-2-oxazolidinone (AOZ) and 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ). These metabolites are obtained from blended tissue samples using incubation under acid hydrolysis conditions and simultaneously derivatised using 2-nitrobenzaldehyde. The extract is neutralized, and the derivatised metabolites (2-NP-AOZ and 2-NP-AMOZ) are isolated using liquid-liquid extraction with ethyl acetate followed by screening and confirmation using liquid chromatography-tandem mass spectrometry (LC/MS/MS).

The method is described at: www.fsis.usda.gov/PDF/CLG_NFUR_2_01.pdf.

Methods for determination of sulphonamides

ELISA assays

ELISA assay kits are commercially available for the quantitative analysis of sulphonamides in egg, meat (chicken and pork), fish, shrimps, honey and milk.

After dilution milk can be analysed directly but for meat, fish, shrimps and eggs, any sulphonamides that may be present are extracted using methanol or acetonitrile. The resultant extract is evaporated to dryness and the residue is dissolved in a buffer solution. Any remaining fat is removed by extraction with n-hexane. Honey requires clean up using a C18 SPE cartridge.

The principle of the test is as follows. The wells of the microtitre plate are coated with antibodies directed against anti-sulphonamide antibodies. Sulphonamide standards or sample solution, sulphonamide enzyme conjugate and anti-sulphonamide antibodies are added to individual wells. As the assay is a competitive enzyme immunoassay, free sulphonamides and sulphonamide enzyme conjugate compete for the sulphonamide antibody binding sites. At the same time, the anti-sulphonamide antibodies are also bound by the immobilised capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. After the addition of a substrate/chromogen solution to the wells, bound enzyme conjugate converts

the chromogen into a blue product. The addition of the stop solution causes a change in colour from blue to yellow. The intensity of the yellow colour is measured spectrophotometrically at 450 nm. Since this is a competitive assay, the absorption is inversely proportional to the sulphonamide concentration in the sample.

Whilst ELISA assays can be used to detect a wide range of sulphonamides, the percentage recovery of individual sulphonamides varies widely. It is therefore necessary to take account of the differences in recovery when reporting quantitative results.

Confirmatory methods of analysis

The method for the confirmatory analysis of sulphonamides uses Liquid Chromatography – Tandem Mass Spectrometry (LC-MS-MS). Sample extracts containing sulphonamides are reconstituted in a 80:20 mixture of 0.1% formic acid and iso-propanol. The extracts are injected into a reverse phase liquid chromatography system followed by simultaneous quantification and confirmation by tandem mass spectrometry.

The method can be used for the quantitative determination and confirmation of s the following sulphonamides in tissue (muscle and liver tissues of porcine, bovine, and avian species), processed products and catfish at levels ⩾ 0.05 ppm with the exception of Sulfaquinoxaline which quantitates at levels ⩾ 0.10 ppm.

Sulphaquinoxaline	Sulphamethazine
Sulphathiazole	Sulphamerazine
Sulphaethoxypyridazine	Sulphamethoxazole
Sulphadiazine	Sulphisoxazole
Sulphadimethoxine	Sulphamethoxypyridazine
Sulphachloropyridazine	Sulphadoxine
Sulphamethizole	

Sulfapyridine is used as an internal standard.

Le lien ne fonctionne pas

The method is described at http://www.fsis.usda.gov/PDF/CLG_SUL_4_01.pdf.

Methods for determination of beta-lactam antibiotics

Beta-lactam antibiotics include penicillins and cephalosporins.

Screening methods

A number of ELISA test kits based on the use of competitive immunoassays are available for the detection of penicillins and cephalosporins in milk and in other animal tissues.

In one such test that is particularly relevant to milk and dairy products, beta-lactam antibiotic molecules are conjugated to alkaline phosphatase. The substrate added when the reaction between the antibiotic and antibodies is complete, comprises a monoester of orthophosphoric acid and an aromatic compound. When acted upon by alkaline phosphatase, the phosphate radical is split off to produce a highly fluorescent molecule, the concentration of which is determined by means of fluorimetry.

Other immunoassays are based on the use of an enzyme labelled betalactam which reacts with a substrate to produce a coloured reaction product which is then measured sprectophotometrically. For example, the principle of the method of at least one commercially available test kit is as follows.

The sample extract is added to individual wells of a microtitre plate pre-coated with a beta-lactam antibody. Any beta lactams that may be present in the sample and in the standard, compete with horseradish peroxidase labelled conjugate for capture antibody binding sites on the microtitre plate. As with all competitive assays, the absorbance measured is inversely proportional to the concentration of the analyte.

Confirmatory methods of analysis

Confirmation of ß-Lactam Antibiotics uses an HPLC-MS/MS method. Beta-Lactams are extracted from tissues using acetonitrile/water. Interfering substances are removed using solid phase extraction (SPE). The eluate is reduced in volume and analysed for the presence of ß-Lactams by LC/MS/MS using a triple quadrupole mass spectrometer under electrospray ionization (ESI) conditions. Analytes are identified and/or confirmed by comparison against external or matrix-matched standards.

The method may be used for confirmation of the following ß-Lactams in bovine and porcine kidney and muscle:

- ampicillin ⩾ 10 ppb;
- nafcillin ⩾ 20 ppb;
- cefazolin, desfuroylceftiofur cysteine disulfide metabolite of ceftiofur (DCCD), penicillin G each ⩾ 50 ppb;
- desacetyl cephapirin ⩾ 100 ppb.

The method may also be used to screen for amoxicillin and cloxacillin at ⩾10 ppb and dicloxacillin and oxacillin at ⩾ 50 ppb.

The method is available via the following link:
www.fsis.usda.gov/PDF/CLG_BLAC_03.pdf. Le lien ne fonctionne pas

A.4. Determination of residues of organochlorine compounds in milk and milk products

Introduction

ISO 3890 / IDF 75 – 2009, Parts 1 and 2 (Milk and Milk Products) specify methods for the determination of residues of organochlorine compounds in milk and milk products. Part 1 of the standard covers the general requirements that apply to pesticide residue analysis together with a number of options for the extraction of residues from different product types. The methods are applicable to a range of dairy products, including milk, evaporated milk, sweetened condensed milk, powdered milk products, butter and butterfat, cheese and other milk products. The methods provided in the standard are suitable for the determination of: α-HCH; ß-HCH; γ-HCH; aldrin / dieldrin; heptachlor and heptachlorepoxide; isomers of DDT, DDE, TDE; chlordane and oxychlordane; and endrin.

Principle of the method

Residues of any organochlorine compounds that may be present are extracted from the sample by the use of appropriate solvents. Since any organochlorine compounds present in the sample are associated with the fat, the initial sample extract normally includes fat as well as organochlorine compounds. Substances which interfere in the gas chromatographic analysis are removed from the extract. By using suitable clean-up methods, a solution of the extracted residues is obtained, in a solvent which is suitable for quantitative analysis to determine the content of organochlorine compounds, by gas-liquid chromatography (GLC) with electron capture detection. Since residues of organochlorine compounds are normally associated with the fat component of dairy products, MRLs for such residues are normally expressed as mg/kg of fat. The first stage in any analysis of milk and dairy products is to extract the fat. The ISO standard provides three options.

Methods for extraction of fat

There are different methods of fat extraction that may be used.

- **Soxhlet extraction.** For solid products such as cheese or whole milk powder, it is appropriate to use the Soxhlet extraction method and a suitable procedure is provided in Part 1 of ISO 3890.
- **Column extraction.** This method involves mixing the liquid or semi liquid product with anhydrous sodium sulphate and sand so as to obtain a dry product. This mixture is then transferred to an extraction column the bottom of which has been plugged with glass wool and a layer of anhydrous sodium sulphate. The column is then eluted with a mixture of n-hexane and acetone. The eluate is collected and concentrated by use of a rotary evaporator.
- **AOAC extraction method.** This method is normally used for extraction of fat from milk. In this method, a quantity of milk is mixed with methanol and sodium oxalate in a separating funnel and then mixed by thorough shaking. After mixing, a quantity of diethyl ether is added and the separating funnel is then shaken to extract the fat from the milk. This extraction is repeated by adding a quantity of light petroleum. After separating the phases by centrifuging, the organic phase is transferred to another separating funnel. The remaining aqueous phase is extracted twice by using a 50:50 mixture of diethyl ether and light petroleum. The combined solvent phases are then washed with water and solvent extract is dried over anhydrous sodium sulphate and evaporated to constant weight, using a rotary evaporator.
- **Extraction for butter.** A portion of the butter sample is heated to about 50 °C and decanted through a dry, warm filter. The separated fat from the butter is then dissolved in a suitable solvent. Having extracted the fat from the sample it is then necessary to extract any organochlorine compounds from the fat and to clean-up the pesticide residue extracts, prior to determination of any extracted organochlorine compounds, by gas chromatography.

Methods for clean-up of sample extracts

Part 2 of ISO 3890 / IDF 75 provides details of a number of methods for clean-up of fat extracts containing any organochlorine compounds extracted from the sample.

- **Method A:** Liquid-liquid partitioning with acetonitrile and clean-up on a Florisil column The sample extract obtained by using one of the procedures specified in Part 1 of ISO 3890 is concentrated almost to dryness and then re-dissolved in light petroleum. Any organochlorine compounds in the extract are partitioned into acetonitrile. After mixing the acetonitrile extract with an excess of water, the organochlorine compounds are partitioned back into light petroleum. The petroleum extract is purified by passing the extract through a column containing a Florisil and any organochlorine compounds are eluted from the Florisil by using a mixture of light petroleum and diethyl ether as the eluting solvent. The eluates are concentrated and analysed by gas liquid chromatography (GLC).
- **Method B:** Liquid – Liquid Partitioning with Dimethylformamide (DMF) and Clean-Up on an Alumina Column. Any organochlorine compounds included in the fat extract are partitioned into dimethylformamide. Sodium sulphate solution is then added and the organochlorine compounds are further partitioned into n-hexane. The organic phase is purified by chromatography on neutral aluminium oxide using n-hexane as the eluting solvent. The eluate is concentrated and analysed by GLC.
- **Method C:** Liquid – Liquid Partitioning with Dimethylformamide (DMF) and Clean-Up on a Florisil column. The sample extract obtained by using one of the procedures specified in Part 1 of ISO 3890 is concentrated almost to dryness and then redissolved in light petroleum. Any organochlorine compounds present in the extract are partitioned into dimethylformamide. Sodium sulphate solution is then added and the organochlorine compounds are further partitioned into light petroleum. The organic phase is purified by chromatography on Florisil, using a mixture of light petroleum and diethyl ether as the eluting solvent. The eluate is concentrated and analysed by GLC.
- **Method D:** Column chromatography on Aluminium Oxide of Precisely Defined Activity. The organochlorine compounds are extracted from the sample using acetone and then further extracted by the addition of n-hexane. The acetone in the resultant extract is partitioned into a solution of sodium sulphate. The n-hexane fraction is separated and dried and concentrated. A specified amount of the n-hexane fraction is purified by chromatography on neutral aluminium oxide of precisely defined activity using n-hexane as the eluting solvent. The eluate is concentrated and then analysed by GLC.
- **Method E:** Column Chromatography on an Alumina Column. Organochlorine compounds, together with fat, are extracted from the sample using light petroleum. A specified amount of the fat extract is purified by chromatography using a column containing basic aluminium oxide of precisely defined activity, using light petroleum as the eluting solvent. The eluate is concentrated and then analysed GLC.

- **Method F:** Column Chromatography on Partially Deactivated Florisil. Organochlorine compounds, together with fat, are extracted from the sample. The extract is concentrated almost to dryness and re-dissolved in light petroleum. A specified amount of fat extract is purified by chromatography on a column of partially deactivated Florisil using a mixture of light petroleum and dichloromethane as the eluting solvent. The eluate is concentrated almost to dryness, redissolved in light petroleum and then analysed by GLC.
- **Method G:** Column Chromatography on Partially Deactivated Silica Gel. Organochlorine compounds, together with the fat, are extracted from the test sample. A weighed amount of the extracted fat is dissolved in light petroleum and quantitatively transferred to the top of a silica gel column. The extract is purified by chromatography on the silica gel column, using a mixture of light petroleum and dichloromethane as an eluting solvent and analysed by the use of GLC.
- **Method H:** Gel-Permeation Chromatography. The organochlorine compounds, together with fat, are extracted from the test sample and the resultant extract is evaporated to a low volume. The concentrated extract is diluted by the addition of a mixture of ethyl acetate and cyclohexane and purified by chromatography using a gel-permeation column, with the ethyl acetate and cyclohexane mixture as the eluting solvent. The eluate is concentrated and then analysed by the use of GLC.

Analysis using gas chromatography

ISO 3890 specifies that a gas chromatograph, with an electron-capture detector and provided with a capillary injection system, be used. The use of capillary columns is recommended, both for the initial analysis and any confirmatory analysis that may be required. Capillary columns with an effective column length of at least 25 m are recommended. Suitable stationary phases include CP-Sil 7, SE 30, OV1 or equivalent.

Confirmatory methods of analysis

In those cases where the results of the initial analysis indicate that organochlorine residues are present in the sample at levels that are close to or exceed MRLs, further confirmatory analysis of such samples should be undertaken.

ISO 3890 – Part 2, includes a number of suitable confirmatory methods such as:

- thin layer chromatography;
- chemical modification;
- photochemical modifications;

The most widely used confirmatory methods are those based on the use of GC-MS and GC-MS-MS or gas chromatography with electron capture detection and using at least two capillary columns of different polarity.

For example, EC Decision 657-2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results includes gas chromatography with mass-spectrometric detection as a suitable

confirmatory method for residues of organochlorine compounds in products of animal origin.

EC Decision 657-2002 also regards the use of gas chromatography with an electron capture detector and separation by the use of capillary columns of different polarity, as an acceptable confirmatory method for residues of organochlorine compounds in products of animal origin.

A.5. Determination of some relevant mycotoxins in foodstuffs

Important note: sampling for mycotoxin analysis

Regulation (EC) No. 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs provides methods for sampling of different commodities for analysis for aflatoxins, aflatoxin M1, patulin, Fusarium toxins and ochratoxin A.

Since mycotoxins are very heterogeneously distributed in a lot, it is important to use appropriate sampling methods since sampling has a significant influence on the precision of methods used for their determination.

As with other mycotoxins, aflatoxins are also very heterogeneously distributed in a lot. This is particularly true for a lot of food products with a large particle size such as dried figs or groundnuts. Consequently for food products with a large particle size, the weight of the aggregate sample needs to be greater than the weight of the aggregate sample for food products with a smaller particle size, in order to obtain the same representativeness. Since the distribution of mycotoxins in processed products is generally less heterogeneous than in the unprocessed products, the sampling procedures specified in Regulation 401/2006 for processed products are normally simpler than those required for the sampling of unprocessed products.

Annex I of Regulation (EC) No. 401/2006 provides the "Methods of Sampling for Official Control of the Levels of Mycotoxins in Foodstuffs".

Section A3 of the annex concerns the general provisions that apply to sampling of foodstuffs for analysis of mycotoxins.

Paragraph A.3.3, regarding 'precautions to be taken' requires that in the course of sampling and preparation of the samples, precautions must be taken to avoid any changes, which would affect the mycotoxin content, or adversely affect the analytical determination or make the aggregate samples unrepresentative.

Paragraph A.3.4, requires that as far as possible incremental samples must be taken at various places distributed throughout the lot or sublot.

Paragraph A.3.5 concerning the 'preparation of the aggregate sample' requires that the aggregate sample shall be made up by combining the incremental samples.

Paragraph A.3.6 concerning 'replicate samples' requires that the replicate samples for enforcement, trade (defence) and reference (referee) purposes shall be taken from the homogenised aggregate sample, unless such procedure conflicts with Member States' rules as regards the rights of the food business operator.

Paragraph A.3.7 regarding the 'packaging and transmission of samples' requires that, each sample be placed in a clean, inert container that provides adequate protection from contamination and damage during transit. All necessary precautions must be taken to avoid any change in composition of the sample, which might arise during transportation or storage.

Paragraph A.3.8 concerning the 'sealing and labelling of samples' requires that, each sample taken for official use shall be sealed at the place of sampling and be clearly identified.

It is also required that a record be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

Methods of analysis for aflatoxins

Sample preparation for aflatoxins analysis

Whilst Regulation (EC) No. 401/2006 includes detailed procedures for sampling of various commodities, for the purposes of Official Control more specific advice on sample preparation is given in a document entitled 'Guidance Document for Competent Authorities for the Control of Compliance with EU Legislation on Aflatoxins'.[190] It is widely accepted that obtaining a truly representative sample of a commodity for aflatoxins analysis is particularly difficult and so this document provides more in depth guidance regarding sampling for aflatoxins and the preparation of samples prior to analysis.

Of particular relevance to the analyst, is the guidance concerning:

- sample preparation / for direct human consumption / to be subjected to sorting and/or other physical treatment;
- mixing of the sample;
- treatment of the sample as received in the laboratory;
- homogenisation procedure;

Aflatoxin B1 and total aflatoxins

Methods for the determination of Aflatoxins B1 and total aflatoxins (B1 + B2 + G1 + G2) are provided in a number of ISO or EN standards and are applicable to different types of food. Essentially each of these methods is based on the use of HPLC with post column derivatisation and fluorescence detection. In each of these methods aflatoxins are extracted from the sample using a suitable extraction solvent.

The sample extract is filtered, diluted with phosphate buffered saline (PBS) and applied to an immunoaffinity column (IAC) containing antibodies specific to aflatoxins B1, B2, G1 and G2. The aflatoxins are isolated, purified and concentrated on the column and then eluted from the immunoaffinity column with methanol.

190 ec.europa.eu/food/food/chemicalsafety/contaminants/guidance-2010.pdf.

The aflatoxins are then determined by reverse phase HPLC with post column derivatisation and fluorimetric detection. Whilst the native fluorescence of aflatoxins B2 and G2 is relatively high that of aflatoxins B1 and G1 is rather low. To achieve the required limits of detection, it is necessary to derivatise aflatoxins B1 and G1 so as to enhance their fluorescence.

Post column derivatisation is carried out either by bromination, using electrochemically generated bromine or with pyridinium hydrobromide perbromide (PBPB), or by derivatisation with iodine.

Many laboratories use a device known as a Kobra cell for the electrochemical generation of bromine.

An alternative to chemical derivatisation is provided by the use of a photochemical reactor. Again the use of a photochemical reactor, post column, enhances the fluorescence of aflatoxin B1 and G1 by altering their chemical structure.

For specific types of food the following standards provide detailed methodologies:

- **EN 12955:1999** – Describes a method for the determination of aflatoxin B1, and total aflatoxins (B1 + B2 + G1 + G2) in cereals, shell-fruits and derived products. The method is based on the use of high performance liquid chromatography with post column derivatisation and immunoaffinity column clean up. The aflatoxins are quantified by reverse phase HPLC with fluorescence detection and post column derivatisation with iodine.
- **EN 14123** – Describes a method for the determination of aflatoxin B1, and total aflatoxins (B1 + B2 + G1 + G2) in hazelnuts, peanuts, pistachios, figs, and paprika powder. The method is based on the use of high performance liquid chromatography with post column derivatisation and immunoaffinity column clean up. The aflatoxins are quantified by reverse phase HPLC with fluorescence detection but in this method, post column derivatisation is carried out either by bromination, using electrochemically generated bromine or with pyridinium hydrobromide perbromide (PBPB).
- **ISO 16050:2003** – Describes a reverse phase HPLC method, with immunoaffinity column clean-up and post column derivatisation, for the determination of aflatoxins in cereals, nuts and derived products. The limit of quantification for aflatoxin B1, and for the sum of aflatoxins B1, B2, G1 and G2, is 8 micrograms per kilogram. The method has been validated for:
  - maize containing 24,5 µg/kg total aflatoxins;
  - peanut butter containing 8,4 µg/kg total aflatoxins;
  - raw peanuts containing 16 µg/kg total aflatoxins.

  It has also been shown that this method can be used for oilseed products, dried fruits and derived products.
- **EN 15851:2010** – Describes a method for the determination of aflatoxin B1 in cereal based foods for infants and young children. Again the method is based on the use of HPLC with immunoaffinity column clean-up and fluorescence detection

Aflatoxin M1

Aflatoxin M1 is a metabolite of aflatoxin B1 and can occur in the milk of animals fed with feedstuffs containing high levels of aflatoxin B1. Aflatoxin M1 is really only of concern for milk and dairy products.

The method described in EN ISO 14501:2007 – Milk and milk powder – Determination of aflatoxin M1 content – Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography.

The limit of detection of the method is 0.08 µg/kg for whole milk powder, *i.e.* 0.008 µg/L for reconstituted liquid milk. The method is also applicable to low fat milk, skimmed milk, low fat milk powder, and skimmed milk powder. The immunoaffinity column used for the extraction of aflatoxin M1 and clean-up of the sample contains antibodies against aflatoxin M1.

Aflatoxin M1 is extracted by passing the test portion through an immunoaffinity column that contains specific antibodies bound onto a solid support material. As the sample passes through the column, the antibodies are selectively bound with any aflatoxin M1 (antigen) present and form an antibody-antigen complex. All other components of the sample matrix are washed off the column with water. Aflatoxin M1 is eluted from the column and the eluate is collected. The amount of aflatoxin M1 present in this eluate is determined by means of HPLC coupled with fluorimetric detection. Since the native fluorescence of aflatoxin M1 is relatively high, there is no need for post column derivatisation.

Methods for determination of ochratoxin A

There are a number of ISO / EN methods for the determination of ochratoxin A (OTA) in different types of food. In all cases, methods are based on the use of HPLC with fluorescence detection. Standard EN ISO 15141-1:1998 – specifies a method for the determination of ochratoxin A in cereals and cereal products by high performance liquid chromatography with silica gel clean up.

Ochratoxin A is extracted from the sample with toluene after acidification with hydrochloric acid and after the ionic strength has been increased by adding magnesium chloride. The extract is purified using a mini silica gel column and ochratoxin A is determined by reverse phase – high performance liquid chromatography with fluorimetric detection.

The method is suitable for the determination of ochratoxin A at levels greater than 0.4 µg/kg and has been validated in 2 interlaboratory studies on wheat whole meal containing 0.4 µg/kg and 1.2 µg/kg of ochratoxin A.

A number of laboratories have shown that this method is also applicable to cereals, dried fruits, oilseeds, pulses, wine, beer, fruit juices and raw coffee.

EN ISO 15141-2:1998 – specifies a method for the determination of Ochratoxin A in cereals and cereal products by high performance liquid chromatography with bicarbonate clean up. Ochratoxin A is extracted from grains with chloroform-aqueous phosphoric acid and isolated by liquid-liquid partitioning into aqueous bicarbonate solution. The solution is applied to a C18 cartridge, and ochratoxin A is eluted with ethyl acetate-methanol-acetic acid. Ochratoxin A is separated by reversed phase

HPLC with fluorimetric detection. The method is suitable for the determination of ochratoxin A at levels greater than 3 µg/kg and has been validated in interlaboratory studies on:

- whole barley containing 2.9 µg/kg, 3.0 µg/kg, 7.4 µg/kg and 14.4 µg/kg of ochratoxin A,
- whole maize containing 8.2 µg/kg and 16.3 µg/kg of ochratoxin A,
- wheat bran containing 3.8 µg/kg and 4.5 µg/kg of ochratoxin A.

NOTE: Numerous laboratory experiences have shown that this method is also applicable to wheat flour.

A number of methods have been published for the determination of ochratoxin A, based on the use of reverse phase HPLC with fluorimetric detection and immunoaffinity column clean-up. Such methods include:

- **EN 14133:2009** – Foodstuffs – Determination of ochratoxin A in wine and beer – HPLC method with immunoaffinity column clean-up;
- **EN 14132:2009** – Foodstuffs – Determination of ochratoxin A in barley and roasted coffee – HPLC method with immunoaffinity column clean-up;
- **EN 15829:2010** – Foodstuffs – Determination of ochratoxin A in currants, raisins, sultanas, mixed dried fruit and dried figs – HPLC method with immunoaffinity column clean-up and fluorescence detection;
- **EN 15835:2010** – Foodstuffs – Determination of ochratoxin A in cereal based foods for infants and young children – HPLC method with immunoaffinity column clean-up and fluorescence detection.

For each of these methods an immunoaffinity column with antibodies against ochratoxin A is required.

Methods for determination of patulin

- BS EN 14177:2003 specifies a method for the determination of patulin in clear and cloudy apple juice and puree. This is an HPLC method with liquid-liquid partition clean-up. Detection and quantification of patulin is by means of a fluorimetric detector.
- AOAC Method 2000.02 specifies an alternative HPLC method for the determination of patulin in clear and cloudy apple juices and apple puree. In this method apple juice or puree is extracted with ethyl acetate and then cleaned up by extraction with sodium carbonate solution. The ethyl acetate extract is dried with anhydrous sodium sulphate. After evaporation of the solvent, patulin is determined quantitatively by reverse phase HPLC with UV detection.

Methods for determination of deoxynivalenol

BS EN 15891:2010 describes a method for the determination of deoxynivalenol (DON) in cereals, cereal products and cereal based foods for infants and young children. Again, this is an HPLC method with immunoaffinity column clean-up and UV detection. The immunoaffinity column contains antibodies raised against deoxynivalenol. A portion of the sample extract is applied to the column and then washed through with water. The deoxynivalenol is the stripped off by using methanol as the eluting solvent. The eluate is then analysed by reverse phase HPLC.

Methods for determination of zearalenone

EN 15850 describes a method for the determination of zearalenone in maize based baby food, barley flour, maize flour, polenta, wheat flour and cereal based foods for infants and young children. The method is based on the use an immunoaffinity column clean-up, followed by HPLC with fluorimetric detection.

Methods for determination of fumonisins

Methods are currently being developed for the determination of fumonisin B1 (FB1) and fumonisin B2 (FB2) in processed maize-containing foods for infants and young children by high performance liquid chromatography (HPLC) with immunoaffinity clean-up and fluorescence detection. The principle of the method is as follows.

FB1 and FB2 are extracted from the test material with a solution of methanol in phosphate buffered saline (PBS). Then the extract is diluted with PBS and cleaned up using an immunoaffinity column (IAC). FB1 and FB2 are eluted from the IAC using methanol and then water. After adjusting the volume the eluate is directly injected into the HPLC and FB1 and FB2 are detected by their fluorescence after either pre- or post column derivatisation.

Pre-column derivatisation does have disadvantages related to more demanding chromatography and the instability of the derivatives. Strict time control of all processes is required to obtain adequate repeatability which necessitates the use of programmable auto liquid samplers (ALS). This may be overcome by using post column derivatisation instead.

A report on a collaborative study "Validation of an analytical method to determine the content of fumonisins in baby food, breakfast cereals and animal feed" has been published.[191]

191 irmm.jrc.ec.europa.eu/interlaboratory_comparisons/Documents/jrc_44148_(food).pdf.

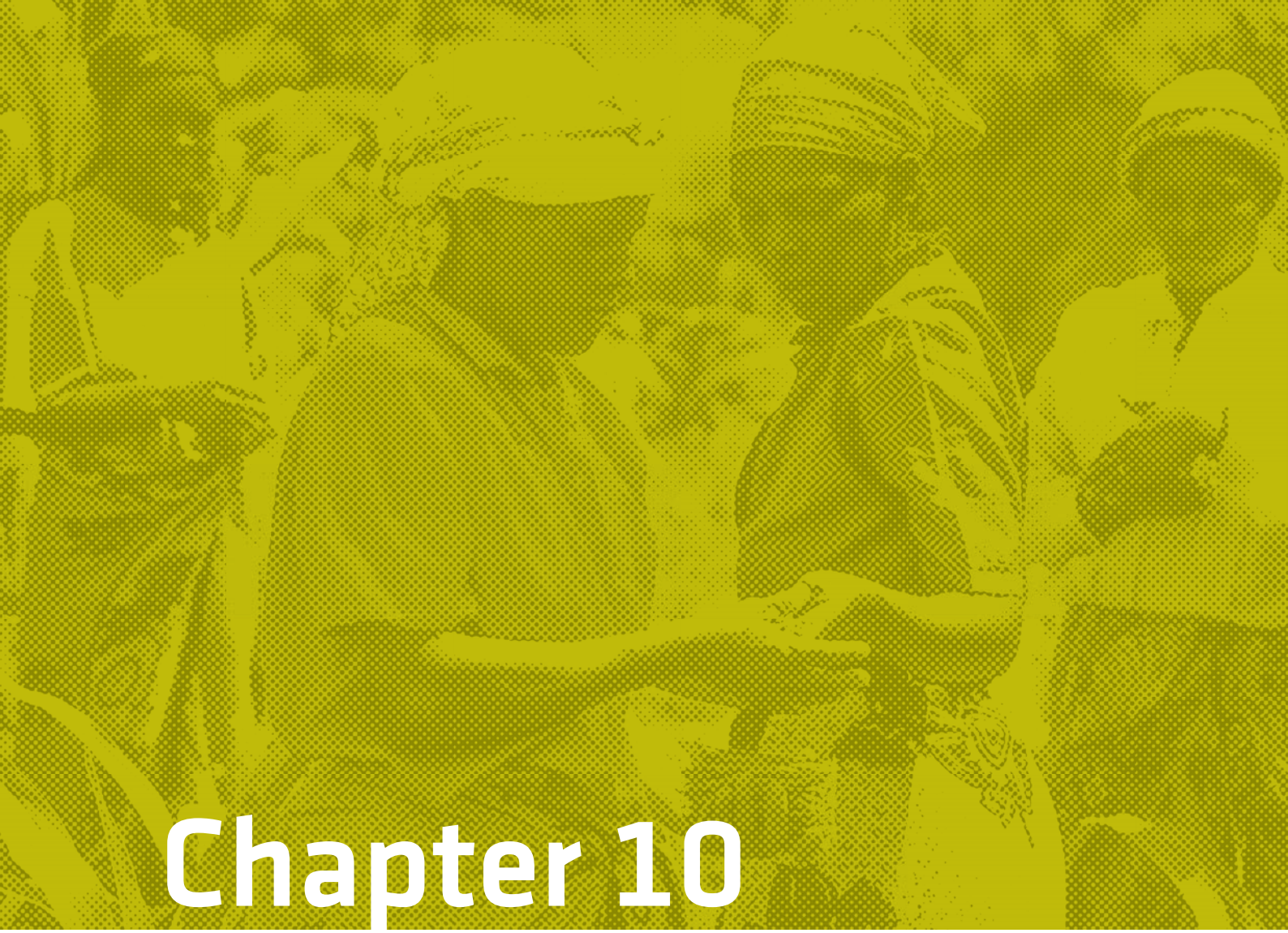
Le lien ne fonctionne pas

Methods for determination of T-2 and HT-2 toxin

Methods have been collaboratively developed for the determination of T2 and HT2 toxin in cereals and baby food and in animal feeds.

The method is as follows. An aliquot of the sample is extracted with a mixture of methanol/water (80/20, v/v). The sample extract is then diluted, filtered, and applied to an immunoaffinity column. After washing and elution with acetonitrile the eluate is evaporated to dryness. T-2 and HT-2 toxins in the dry residue are then derivatised with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA)/ trimethylchlorosilane (TMCS) (99/1, v/v) and injected into a gas chromatograph. T2 and HT2 toxin are detected and quantified by mass spectrometry.[192]

192 publications.jrc.ec.europa.eu/repository/bitstream/111111111/4409/1/final%20report%20baby%20food.pdf.



Chapter 10

Writing analytical reports

10.1. GENERAL INTRODUCTION

10.1.1. Context

This chapter provides guidance on the reporting of results obtained from the chemical analysis and microbiological examination of food and feedstuffs. It is aimed at helping laboratory analysts and managers in Competent Authorities to provide clear and concise reports and that may be used in legal proceedings, or other purposes as part of a Competent Authority's responsibilities for the official control of food and feedstuffs.

Article 12 of Regulation (EC) No. 882/2004 "on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules" requires that 'Competent Authorities' may only designate laboratories that operate and are assessed and accredited in accordance with the following European accreditation standards:

a. EN ISO/IEC 17025 on "General requirements for the competence of testing and calibration laboratories";

b. EN ISO/IEC 17011 on "General requirements for accreditation bodies accrediting conformity assessment bodies".

It is important that third country laboratories carrying out chemical analysis or microbiological examination of samples as part of the official control of food and feedstuffs for export to the EU be accredited. Their legislation regarding official controls for domestic markets may also require accreditation. It is also increasingly the case that laboratories undertaking analysis of food and feed for purposes other than official control are also required to be accredited.

One of the main advantages for a laboratory of accreditation is that it is an indication to the laboratory's customers, that the laboratory is technically competent in those

analyses that are included in their scope of accreditation. The results reported by an accredited laboratory are generally accepted without question, minimising dispute and the need for re-testing. Within the EU test certificates issued by an official laboratory in one Member state are accepted by the Competent Authorities in another Member State, thus allowing for the free movement of goods.

ISO 17025 covers both management and technical requirements for accredited testing laboratories. The technical requirements are covered in Section 5 of the standard and these include a number of requirements related to the reporting of results, presented in greater detail in the following sections.

10.2. DATA TO BE PRESENTED IN THE REPORTING OF RESULTS

10.2.1. General requirements for presentation of results

It is important to be able to write analytical reports to communicate technical work performed in the laboratory. Writing good analytical reports are essential to avoid misleading conclusions or incorrect managerial decisions. After validation of an analytical method the data should be presented in a validation report hereby showing what the method is capable to.

English is today the most recognized international language, and most scientific communication such as reports, articles and books are performed in English, but dependent on the target group it can be relevant to write in other language. For example in the local language to be sure that all details are understood by everyone. It is recommended to discuss what is most appropriate in your laboratory. If the laboratory is international orientated it will be appropriate to use the English Language.

Good recommendations for writing analytical reports:

- write in short and precise sentences;
- write in sentences and not phrases;
- be objective and honest;
- use punctuations and abbreviations when relevant;
- be consistent with chemical names through the report;
- minimise misinterpretation and unintended meanings;
- once completed read the report thoroughly several times for assessing content and organization;
- let a second person proof-read the report.

The following headings should be considered to be included in any reports:

- Title page;
- Abstract;
- Table of content;
- Introduction;
- Material and methods;
- Results;
- Discussion;
- Conclusion;
- References;
- Appendix(ces).

Some recommendations are provided in the Annex.

The results of each test or series of tests or calibrations carried out by the laboratory must be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the test method. It is important that laboratory reports are prepared in an impartial manner. The results must be traceable, such that the data presented must properly identify the sample, the analyst, and other relevant factors which may retrospectively need checking.

The results must be reported, usually in a test report and must include all the information requested by the customer and necessary for the interpretation of the test results and all information required by the method used.

In the case of tests performed for internal customers, or in the case of a written agreement with the customer, the results may be reported in a simplified way.

The test reports may be issued as hard copies or by electronic data transfer provided that **the requirements of ISO 17025** are met. Electronic transmission of results includes transmission by telephone, telex, facsimile or other electronic or electromagnetic means. Care must also be taken to maintain the confidentiality of the results.

10.2.2. Content of test reports

Clause 5.10.2 concerns "Test reports and calibration certificates".

Each test report must include at least the following information, unless the laboratory has valid reasons for not doing so.

a. A title (e.g. "Test Report");
b. The name and address of the laboratory, and the location where the tests were carried out, if different from the address of the laboratory;
c. Unique identification of the test report (such as the serial number), and on each page an identification in order to ensure that the page is recognized as a part of the test report and a clear identification of the end of the test report;
d. The name and address of the customer;
e. Identification of the method used;
f. A description of, the condition of, and unambiguous identification of the item(s) tested;
g. The date of receipt of the test item(s) where this is critical to the validity and application of the results, and the date(s) of performance of the test;
h. Reference to the sampling plan (if this is known) and sample preparation procedures used by the laboratory or other bodies where these are relevant to the validity or application of the results;
i. The test or calibration results with, where appropriate, the units of measurement;
j. The name(s), function(s) and signature(s) or equivalent identification of person(s) authorising the test report;
k. Where relevant, a statement to the effect that the results relate only to the items tested.
l. Hard copies of test reports should also include the page number and total number of pages.
m. It is recommended that laboratories include a statement specifying that the test report or calibration certificate shall not be reproduced except in full, without written approval of the laboratory.

Clause 5.10.3.1 specifies the additional information that must be included in test reports and that are necessary for the interpretation of the test results

a. deviations from, additions to, or exclusions from the test method, and information on specific test conditions, such as environmental conditions;
b. where relevant, a statement of compliance/non-compliance with requirements and/or specifications;
c. where applicable, a statement on the estimated uncertainty of measurement; information on uncertainty is needed in test reports when it is relevant to the validity or application of the test results, when a customer's instruction so requires, or when the uncertainty affects compliance to a specification limit;

d. where appropriate and needed, opinions and interpretations;
e. additional information which may be required by specific methods, customers or groups of customers.

10.2.3. Additional information

Sometimes additional information may be included where necessary for the interpretation of test results. Note that laboratories should not normally be involved in sampling of products for the purposes of official control, since knowledge of the provenance of the sample this may impact on their impartiality. However, sometimes the laboratory may be provided with information regarding the sampling, for example where the analyst is requested to provide a professional opinion on the test results (see below), in terms of their interpretation. In such cases, the analyst may need to be informed of the circumstance of the sampling.

These additional requirements may include:

a. the date of sampling;
b. unambiguous identification of the substance, material or product sampled (including the name of the manufacturer, the model or type of designation and serial numbers as appropriate);
c. the location of sampling, including any diagrams, sketches or photographs;
d. a reference to the sampling plan and procedures used;
e. details of any environmental conditions during sampling that may affect the interpretation of the test results;
f. any standard or other specification for the sampling method or procedure, and deviations, additions to or exclusions from the specification concerned.

10.2.4. Opinions of the analyst

Normally the job of the laboratory is to report the test result. The analyst should only provide an opinion when specifically requested to do so.

Opinions and interpretations included in a test report may comprise, but not be limited to, the following:

1. An opinion on the statement of compliance/non-compliance of the results with requirements set out in a standard;
2. Fulfilment of contractual requirements;
3. Recommendations on how to use the results;
4. Guidance to be used for improvements.

Where this is the case, Clause 5.10.5 is applied, concerning "Opinions and Interpretations". This states that when opinions and interpretations are included, the laboratory must document the basis upon which the opinions and interpretations have been made.

Opinions and interpretations must be clearly marked as such in the test report.

10.2.5. Subcontracted analysis

Sometimes laboratories will sub-contract the testing to another laboratory, for example at times when they do not have sufficient capacity to meet demand, or where they do not have the scope to perform the test.

Clause 5.10.6 covers the reporting of test results that have been obtained by subcontracting of analysis to another laboratory and requires that where an accredited laboratory issues a test report that includes the results of tests carried out by another laboratory, those test results must be clearly identified as having been obtained from a subcontracted laboratory.

10.2.6. Amendments to test reports

Sometimes an analyst has to issue an amendment to a report, for example to correct an error in the reporting. Clause 5.10.9 of ISO 17025 requires that when it is necessary to amend a test report after it has been issued, a further test report must be issued that includes the following statement:

"Supplement to Test Report [or Calibration Certificate], serial number... [or as otherwise identified]", or an equivalent form of wording.

Any amendments to test reports must meet all the requirements of ISO 17025. If it is necessary to issue a complete new test report, this must be uniquely identified and must contain a reference to the original that it replaces.

10.2.7. Validation reports

A larger part of the reports written in an accredited analytical laboratory are validations reports. During a validation some documentation are needed. A validation plan is prepared before the experimental part of the validation starts, and it is included in the validation report in an appendix. The following should be included in a validation report:

- **Validation plan:** This plan shall include an overview of what the method should be used for. The needs of the customer and the control authorities should be considered, as well as the internal conditions in the laboratory, such as working environmental legislation, equipment and resources.
- **Materials and methods:** A description of the sample preparation, reagents and analysis should be mentioned in the validation report. The report can otherwise include a reference to a protocol containing the experimental data.
- **Raw data:** All raw data including calibration curves or a reference to where the raw data may be found should appear from the validation report.
- **Validation data:** Obtained results and how these have been calculated should be specified in the validation report. It is especially important to include which matrices and at which concentrations there are for a specific precision, trueness, detection limit and quantification limit. The validation data should be evaluated in relation to the validation plan.

- **Conclusion:** The validation report should end up with an unambiguous conclusion concerning which analytical tasks the examined method is suitable for. If the method is found to be unsuitable for some matrices or concentrations, this must be evident in the report.

Method validation is further described in the chapter 8 on Method Validation. More information about reporting during validation can be found in the following documents:

- "AOAC guidelines for Single Laboratory Validation".[193] Section 4 contains a comprehensive description of what the validation report should contain.
- "NMKL procedure for Validation of chemical analytical methods".[194] Section 4 describes documentation during validation.

10.3. FORMAT OF REPORT

10.3.1. General approach to report format

The format of reports and certificates must be designed to accommodate each type of test or calibration carried out and to minimize the possibility of misunderstanding or misuse.

The lay-out of the test report or calibration certificate should be such that the test data reported can be easily understood and interpreted by the recipient of the test report.

As far as possible, the headings included in test reports should be standardized across different tests. Some of these general requirements are set out in clause 5.10.8 of ISO17025 concerning the "Format of reports and certificates". However additional specific requirements may be included in the methodologies.

193 AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals 2002. AOAC INTERNATIONAL; Gaithersburg.

194 Validation of Chemical Analytical Methods, NMKL Secretariat, Finland, NMKL Procedure No. 4 (1996).

10.3.2. Requirements of specific analytical standards

In addition to the technical requirements of ISO17025, many ISO and EN standards for analytical methodologies include requirements for calculation of results, expression of results and test reports. Some typical examples of reporting of specific analyses are provided in the following subsections.

10.3.2.1. Reporting heavy metal analysis by AAS

EN 14083 describes a method for the determination of lead, cadmium, chromium and molybdenum by in foodstuffs, by graphite furnace atomic absorption spectrometry. It requires the analyst to report the content of each element as mass fraction, of the element to be determined, in milligram per kilogram of the sample. It also sets out that the report shall specify at least the following:

- all information necessary for the complete identification of the sample;
- the test method used, with reference to the European Standard;
- the test results obtained and the units in which they are specified;
- date of sampling and sampling procedure (if known);
- date when the analysis was finished;
- whether the requirement of the repeatability limit has been fulfilled;
- all operating details not specified in the European Standard, or regarded as optional, together with details of any incidents that occurred when performing the method, which might have influenced the test result(s).

10.3.2.2. Reporting aflatoxin analysis

EN 14123 describes a method for the determination of aflatoxin B1 and the sum of aflatoxin B1, B2, G1 and G2 in hazelnuts, peanuts, pistachios, figs, and paprika powder, and requires that the test report shall contain the following data:

- all information necessary for the identification of the sample (kind of sample, origin of sample, designation);
- all information necessary for the identification of the calibrant;
- a reference to the European Standard;
- date and type of sampling procedure (if known);
- the date of receipt;
- the date of test;
- the test results and the units in which they have been expressed;
- any particular points observed in the course of the test;
- any operations not specified in the method or regarded as optional, which might have affected the results.

10.3.2.3. Reporting nitrogen content in milk

For example, EN ISO 8968 concerning a method for the determination of the nitrogen content of milk includes clauses concerning the calculation and expression of test results and format of the test report. In this case it specifies the method of calculation and expression of results. Obtained results must be expressed to four decimal places if needed for further calculations. End results, should be expressed to three decimal places (for the nitrogen content) and two decimal places (for the protein content). It also states that the Test Reports shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this part of ISO 8968 / IDF 20;
- all operating details not specified in this part of ISO 8968 / IDF 20, or regarded as optional, together with details of any incident which may have influenced the result(s);
- the test result(s) obtained; if the repeatability has been checked, the final quoted result obtained; if the recovery has been checked, the final quoted result obtained.

10.3.2.4. Reporting results of microbiological examination

Methods for the microbiological examination of food and feed also include clauses related to the expression and reporting of results. For example ISO 21528-2:2004 describing a colony count method for the enumeration of *Enterobacteriaceae* includes the following requirements in the test report.

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this part of ISO 21528;
- the incubation temperature used;
- all operating details not specified in this part of ISO 21528, or regarded as optional, together with details of any incidents which may have influenced the test results;
- the test results obtained.

10.4. INTERPRETATION OF ANALYTICAL RESULTS

10.4.1. Need for standard approaches to interpretation

Some laboratories adjust their results in different ways. This is done to express the correct number of significant figures, or to take into account measurement uncertainty and recover. Some laboratories may not make such adjustments, or may make them in different ways. Consequently, different decisions could be taken after analysis of the "same" sample. For example, material for which there is a statutory limit of, say, 4µg/kg for a contaminant, may be interpreted as containing 3µg/kg on analysis in one laboratory but 10 µg/kg in another, on the basis that some laboratories correct their analytical results for recovery whereas others do not.

Differences in the reporting and treatment of analytical results can therefore lead to differences in the implementation of legislative standards by different competent authorities. These differences in implementation of legislative standards are most apparent for those regulations concerning the occurrence of contaminants in food and in animal feeds.

According to a report[195] published in 2004, before 2003 there was no common interpretation of analytical results across the EU. The EU has since taken steps to ensure a uniform interpretation of EU food and feed legislation across the EU, by adopting certain provisions in EU Directives in order to ensure a uniform interpretation of analytical results.

The development of international guidelines on the use of recovery factors when reporting analytical results and other guides dealing with measurement uncertainty has sought to address the problems regarding the interpretation of analytical results and the consequent differences in implementation of legislative standards. The analyst should always consult the relevant standard to ensure that the correct approach is applied.

In considering the reporting of results it is also necessary for the analyst to consider the potential for different interpretations of the result, and to seek to ensure that reporting approaches minimise the scope for differences. The main issues associated with interpretation of results of analysis for contaminants lie with:

a. the number of significant figures taken into account when reporting results and interpreting them in relation to statutory limits;
b. rounding up or down of results obtained to results expressed
c. the treatment of analytical variability (or "measurement uncertainty") in the interpretation of a specification;
d. the use of corrections for recovery when calculating and reporting an analytical result.

10.4.2. Number of significant figures reported

There are no hard and fast rules governing the number of significant figures to be used when reporting analytical results. In some cases, guidance may be provided in the ISO or EN methodology standards as described previously. However in many cases, there is no such guidance.

This can have a significant impact on interpretation. For example the impact on interpretation of compliance of the number of significant figures specified in a statutory limit is shown in Table 1.

195 Report on the Relationship Between Analytical Results, Measurement Uncertainty, Recovery Factors and the Provisions of EU Food and Feed Legislation, with Particular Reference to Community Legislation Concerning Contaminants in Food (Council Regulation (EEC) No 315/93 of 8 February 1993 Laying Down Community Procedures For Contaminants In Food) and Undesirable Substances in Feed (Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on Undesirable Substances in Animal Feed)'; see: http://ec.europa.eu/food/food/chemicalsafety/contaminants/report-sampling_analysis_2004_en.pdf.

Table 1: Interpretation of compliance and the number of significant figures in a standard

Specification (independent of units)	Range within which a "satisfactory" result will lie
1	0 to 1.4
1.0	0 to 1.04
1.00	0 to 1.004

Source: 'Report on the Relationship Between Analytical Results, Measurement Uncertainty, Recovery Factors and the Provisions of EU Food and Feed Legislation"
http://ec.europa.eu/food/food/chemicalsafety/contaminants/report-sampling_analysis_2004_en.pdf

The values given in the above table clearly show that there are significant differences in interpretation between maximum levels when expressed as 1 mg/kg, 1.0 mg/kg and 1.00 mg/kg.

In order to avoid situations where analytical results are interpreted differently when compared against corresponding statutory limits, it is therefore important to ensure that the statutory limits are expressed in a uniform and consistent manner. This problem is not just the responsibility of the analyst reporting results but also of the legislators. Officials involved in setting maximum levels may not be aware of the consequences of the form in which maximum levels are expressed.

Therefore as a basic minimum, the following should be stated or considered when developing food and feed legislation:

- the units in which the results are to be expressed;
- the number of significant figures to be included in the reported result;
- the interpretation of an analytical result in relation to a statutory limit;
- the expected precision of the method of analysis likely to be used for the determination, and thus whether the number of significant figures being specified in legislation is 'realistic'.

10.4.3. Rounding of test results

On a related issue, test results may be rounded up or down to the number of significant figures specified in a legal standard.

In general, when considering rounding of test results, the obtained test results should not be rounded further until the final use of the test value is made. This is particularly true when the results are going to be used for further calculations.

One example is when individual test values obtained from the analysis of many sample materials are used to calculate method performance statistics for within and between laboratory variations. Another example is when the values are used as a reference for instrument calibration (e.g. an infrared milk analyser) where the values from many samples will be used in a simple or multiple regression calculation. In such cases, the obtained results should not be rounded before they are used for further calculations.

10.4.4. Reporting of results with respect to their measurement uncertainty

The treatment of measurement uncertainty is another way in which interpretation approach can impact on the reported result. Measurement uncertainty is an expressed of the estimated range within which the result has a given probability of falling.

To explain in more detail, all analytical results expressed take the form of:

$a \pm 2u$ or $a \pm U$

where:

a is the best estimate of the true value of the concentration of the measure and (the analytical result); *u* is the standard uncertainty; *U* (equal to 2u) is the expanded uncertainty.

As a consequence 4u is the range within which the true value is estimated, with a high probability, to fall. The value of U or 2u is the value which is normally used and reported by analysts and is generally referred to as 'measurement uncertainty'. The measurement uncertainty of an analytical result may be estimated and expressed by the analyst in a number of different ways.

In those cases where the statutory limit is a maximum permissible concentration, the procedure adopted by some analysts is to report samples as containing:

"not less than a – 2u".

Consequently, enforcement action is only taken when the analyst is sure that the specification has been exceeded. This is consistent with the requirement to prove beyond reasonable doubt that a limit has been exceeded if the case should come to court. This means that in those countries that adopt this approach, the effective enforcement level is not identical to the numerical value given in the legislation. Instead the enforcement level is the maximum permissible level plus the expanded uncertainty *i.e.* a higher level to take into account the measurement uncertainty.

On the other hand, other control analysts may report and use the value *'a'* without taking account of any measurement uncertainty. There are potentially important consequences of reporting results in different ways. The following example may be used to illustrate the consequences of either taking account of measurement uncertainty or not.

Example of different treatments of measurement uncertainty

The analysis of three different batches of paprika gave the following results for aflatoxin B1 (analytical results already corrected for recovery):

1. 3.0 µg/kg (40 % expanded MU) = 3.0 ± 1.2 µg/kg
   The true value for the aflatoxin B1 content of the sample lies in the range 1.8 – 4.2 µg/kg
2. 6.0 µg/kg (40 % expanded MU) = 6.0 ± 2.4 µg/kg
   The true value for the aflatoxin B1 content of the sample lies in the range 3.6 – 8.4 µg/kg
3. 9.0 µg/kg (40 % expanded MU) = 9.0 ± 3.6 µg/kg
   The true value for the aflatoxin B1 content of the sample lies in the range 5.4 – 12.6 µg/kg

The result for Sample 1 is below the limit (5.0 µg/kg aflatoxin B1) both with and without expanded measurement uncertainty being taken into account. **This sample is therefore compliant** with the maximum limit.

The reported result for Sample 2 is above the statutory limit, but the true value for this analysis lays in the range 3.6 – 8.4 µg/kg. **This sample is considered compliant**, as it is not beyond reasonable doubt that the maximum limit has actually been exceeded.

The reported result for Sample 3 is once again above the statutory limit and the range of values obtained, taking into account the expanded measurement uncertainty is also above the limit. **This sample is therefore non-compliant.**

10.4.5. Use of recovery information in analytical measurement

All laboratories undertaking the analysis of food and feed samples for the purposes of official control are required to use validated methods and to document the procedures used and the results obtained for the validation of such methods. For some methods, as part of the validation process, it is necessary to determine the ability of the method to recover the analyte to be determined. Recovery can be defined as the amount of material extracted for analysis as a fraction of the amount present.

Many of the methods used for the analysis of contaminants are known to give less than 100 % recovery for the analyte being determined. The question arises as to whether or not the results obtained for a particular analysis, should be corrected for recovery. Again some official analysts may report results that are corrected for recovery and others may not. This leads to different interpretations when results are compared against regulatory limits. The following example shows the result of taking account of the measurement uncertainty together with the recovery.

Correction of analytical results for recovery needs to be approached with caution. If the recovery of a method is too low then the method must be considered to be not fit for purpose and methods that are capable of recovering higher amounts of the analyte must be used instead.

In some cases the minimum recovery rates of the methods are set by legislation to ensure that the method performance is adequate. For example, Regulation (EC) No. 401/2006 "laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs" specifies performance criteria that apply to analytical methods used for the determination of mycotoxins. These performance criteria include requirements for method recovery. Examples of the recommended values for recovery of aflatoxins and of ochratoxin A are given below.

Example of different treatments of measurement uncertainty and recovery

Analysis of different batches of paprika gave the following results for Aflatoxin B1 (analytical results still to be corrected for recovery):

1. 3.0 µg/kg (40 % expanded MU, 75 % recovery) = 4.0 ± 1.6 µg/kg
   Taking account both of recovery and uncertainty, the true value for the aflatoxin B1 content of the sample lies in the range 2.4 – 5.6 µg/kg
2. 3.0 µg/kg (40 % expanded MU, 110 % recovery) = 2.7 ± 1.1 µg/kg
   Taking account both of recovery and uncertainty, the true value for the aflatoxin B1 content of the sample lies in the range 1.6 – 3.8 µg/kg
3. 6.0 µg/kg (40 % expanded MU, 75 % recovery) = 8.0 ± 3.2 µg/kg
   Taking account both of recovery and uncertainty, the true value for the aflatoxin B1 content of the sample lies in the range 4.8 – 11.2 µg/kg
4. 6.0 µg/kg (40 % expanded MU, 110 % recovery) = 5.5 ± 2.2 µg/kg
   Taking account both of recovery and uncertainty, the true value for the aflatoxin B1 content of the sample lies in the range 3.3 – 7.7 µg/kg.
5. 9.0 µg/kg (40 % expanded MU, 75 % recovery) = 12.0 ± 4.8 µg/kg
   Taking account both of recovery and uncertainty, the true value for the aflatoxin B1 content of the sample lies in the range 7.2 – 16.8 µg/kg
6. 9.0 µg/kg (40 % expanded MU, 110 % recovery) = 8.2 ± 3.3 µg/kg
   Taking account both of recovery and uncertainty, the true value for the aflatoxin B1 content of the sample lies in the range 4.9 – 11.5 µg/kg.

Given that for paprika, the limit for aflatoxin B1 is 5.0 µg/kg –

- samples 1, 2, 3, 4 and 6, are considered to be compliant with the maximum limit;
- sample 5 is considered to be non-compliant with the maximum limit.

Criterion	Concentration range	Recommended value
Recovery – Aflatoxins B1, B2, G1, G2	< 1,0 µg/kg	50 to 120 %
	1-10 µg/kg	70 to 110 %
	> 10 µg/kg	80 to 110 %
Ochratoxin A	< 1 µg/kg	50 to 120 %
	1-10 µg/kg	70 to 110 %

10.5. REPORTING OF RESULTS BY OFFICIAL ANALYSTS AND FOOD EXAMINERS

10.5.1. Requirements for official certificates

In many countries regulations require that an 'official' food analyst or food examiner, issues a certificate for each sample that is submitted to the laboratory and that the certificate must provide the results obtained for the chemical analysis or microbiological examination of the submitted samples. Note therefore that there is a difference between an official certificate from the laboratory and an analytical report. The certificate is essentially a legal document that may be produced in court and can be sufficient evidence of the facts stated therein.

Whilst there may be a requirement for the certificate to be signed by the official food analyst or food examiner, the actual analysis or microbiological examination may be carried out by any suitably qualified and competent person acting under the direction of the official food analyst or food examiner. Where it is necessary, one or more people can carry out all or part of the required analysis or microbiological examination, provided that this has been done by a person acting under proper direction.

The form of the certificate to be issued by a food analyst or food examiner should be prescribed by regulations and this should be followed by the analyst. The certificate provided by the food analyst or examiner should then normally be accepted by the court and all parties as evidence of the facts stated. The law may even indicate a presumption of veracity. However, it should be considered that the truth of the certificate may be challenged in court, and that the food analyst or food examiner

may be called as a witness in legal proceedings where he/she may be cross examined on any aspect of the analysis, including his/her competence.

10.5.2. Content of certificates

It is most important therefore that the certificate be drawn up in a manner such as to offer the defence no chance of questioning its validity. Care is therefore required in the wording and preparation of certificates. The UK's Association of Public Analysts' training guide on certificate and report writing advises that the wording of the certificate should be direct, factual and concise, but must always provide sufficient information such that the court may be enabled to come to its own conclusions regarding an alleged offence.

The overall test of a good certificate is that it satisfies all of the following criteria, namely that it is:

- targeted;
- concise;
- understandable;
- professional (in stature, legality and value).

The certificate issued by the food analyst or food examiner must provide the following three categories of information to the court in order to establish whether an offence has been committed:

- the facts in respect of the sample itself, including administrative information and analytical results as appropriate;
- the standard against which the sample is to be judged;
- the contravention, if any, against the standard and the extent of it.

In many ways the requirements of the factual part of a food analyst's or food examiner's certificate are the same as those required by ISO 17025.

For example, the certificate is likely to include:

- a record of the person delivering the sample to the laboratory;
- the time and date of receipt of the sample;
- the sample reference number or other identification;
- an accurate description of the sample.

In reporting the results of chemical analysis or microbiological examination, only the results obtained should be reported.

The certificate makes provision in other parts for the expression of opinions on the basis of the results obtained for chemical analysis or microbiological examination of the sample.

The UK's Association of Public Analysts' training guide[196] also includes advice on the number of significant figures to be used when reporting results. In particular the training guide includes advice on the way in which values derived for meat or fish content, where these values are based on calculation factors for naturally occurring constituents, are reported. Here it is emphasised that unrealistic significant figures should not be used and that when reporting fish or meat content, these values should only ever be reported to the nearest 1%.

With regard to the use of units for reporting analytical data it is best to use exactly the same units in any standard to be quoted later in the certificate. Thus if a regulation specifies 'mg/kg' it is potentially confusing to quote 'parts per million' in the data section.

With regard to uncertainty and accuracy of results, the APA guide advises that "The data reported must be justified in terms of 'uncertainty' or 'accuracy'. In effect a Public Analyst must be prepared to argue that the chances of being wrong are sufficiently low as to be 'beyond reasonable doubt'".

The point is made that in most cases the figure quoted will be accurate to within two standard deviations for the method at the 95% confidence level. It is important that the Public Analyst is aware of the uncertainty in any particular result reported, particularly if the sample is reported as failing to meet a standard.

Finally and very importantly, the food analyst or food examiner is required to certify that the sample has undergone no changes that would affect the results of analysis or microbiological examination. Obviously this is particularly relevant where samples are submitted for microbiological examination but there are also many cases where delays in commencing analysis or incorrect storage of samples prior to analysis, can adversely affect the results obtained.

The certificate must also be signed.

196 Association of Public Analysts – Training Guide – Certificate and Report Writing – Issue: 2 – 2005.

10.6. ANNEXES

A.1. Report organization and content

Title page

The first page in a formal report is the title page. The title should be short and completely describe the subject of the report. The following should appear from the title page:

- for whom was the report prepared (name, organization or person);
- the name or organization submitting the report;
- the date.

Short reports do not require a separate title page. In these cases the title, including the information mentioned above, can appear on the first page together with the abstract.

Abstract

An abstract is a very important part of the report and is defined as a summary of the information in the report. A well-prepared abstract enables readers to identify the basic content of the report quickly and accurately, and to determine its relevance to their interests. Therefore it should provide a very brief summary of the report sections with aim, methods and most important results. The abstract is commonly not more than 100-200 words in total and definitely not more than one page.

For writing the abstract consider the following points:

- identify the most important goals;
- identify the most important results;
- formulate your hypothesis and methods in the first sentence;
- write the abstract as one part;
- write it more than once. Remove unnecessary wording and sentences.

It is possible to write the abstract as the last part of the whole report. Make sure that there is time to go through the whole abstract more than once.

Table of contents

Larger and formal reports require a table of contents, while shorter reports do not. Many reports will fall somewhere between these two extremes, and then it will be a matter of judgment if a table of contents should be included. It might be relevant to include the table, if it will clarify and demonstrate the continuity of the report, help the reader to understand the scope or make it more convenient for the reader to find any point of interest.

It is recommended to use numbers for sections; it keeps the reader on the track while going through the report, and it is recommended not to use more than 4 digits.

Table of contents should list the title of the sections including page number in the order they appear, including all appendices found last in the report. Tables

and figures can be identified by number, title and page in separate lists immediately following the table of contents. Remember to double-check the list as a last thing in the proof-reading against title, page and so on, where the item occurs in the report. With modern word processing programs a table of contents can be automatically generated.

Introduction

The purpose of the introduction is to give the reader the minimum amount of information to understand the results, conclusions and recommendations. The introduction should include the purpose of the study (the problem statement) and typical also the scope. In larger reports the introduction will also contain a description of the background. In general, theoretical principles related to the subject are to be explained and briefly discussed. This will typically include a survey of relevant literature, and the author should remember to make appropriate references. For small reports a formal introduction might not be necessary, and a simple introductory statement will be sufficient.

Materials and methods

The aim of this section is for other scientists to be able to repeat the work and to be able to evaluate the quality of the practical work and methods used.

This section typical includes a description of:

- chemicals:
  - reagents – reagents usually present in a laboratory need not to be listed;
  - reference standards including identity, source and purity;
- instruments – including operating conditions;
- software used for sample processing;
- experimental methods.

The experimental methods should be clearly described, with the purpose that the reader should be able to understand how the raw data were collected. If the experimental method has been reported earlier it is sufficient to refer to these articles, methods or reports and explain modifications, if such are included. It is however important that the reader has access to the reports.

Use of flow-charts often makes method description more clear (see Figure 1 for illustration of a method). Remember to include references if illustrations, flow-charts or methods are borrowed from others (see Figure 1).

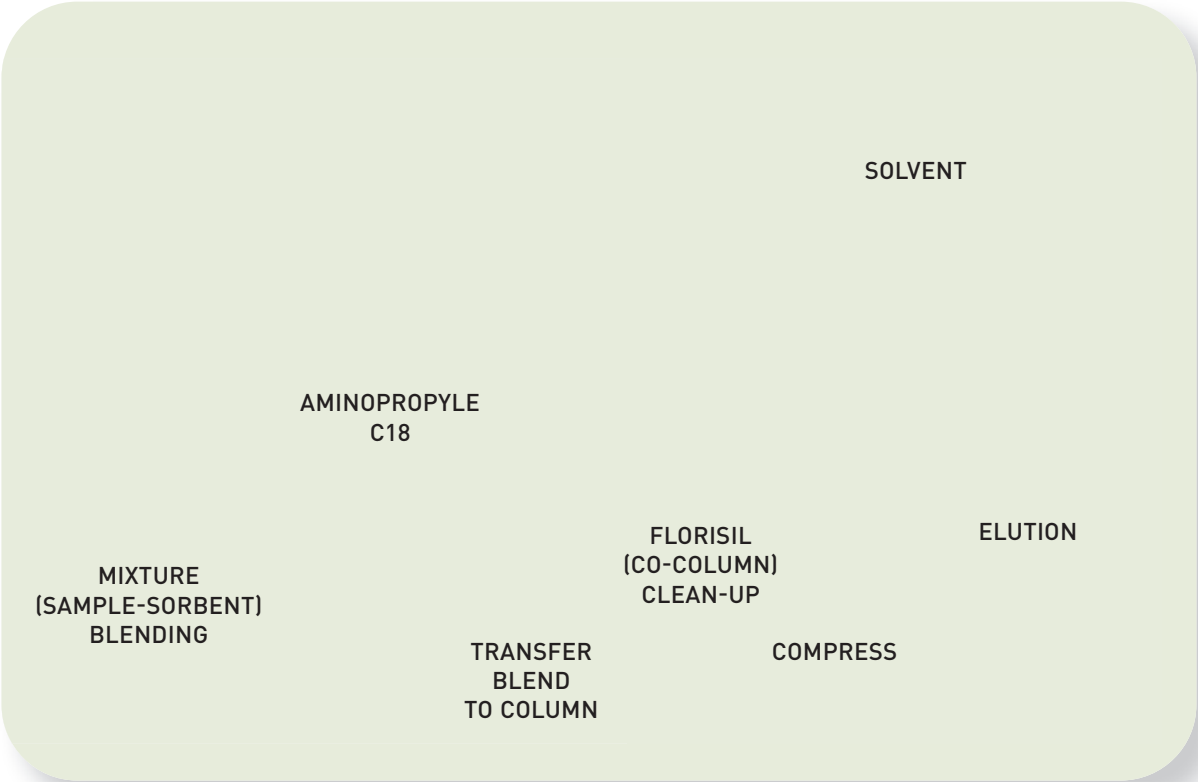


Figure 1 - Schematic presentation of the matrix solid-phase dispersion (MSPD) procedure *(in C. Ferrer, M.J. Gómez, J.F. García-Reyes, I. Ferrer, E.M. Thurman, A.R. Fernández-Alba, J. Chromatogr. A.1069 (2005), 183).*

Results

In this section results should be presented. It should however be noticed that it is not the raw data collected from the method, but the final values resulting from computational analysis of the raw data, which should be presented in this section. In large reports raw data can be shown in appendices. When relevant, statistical analysis of the data should be performed, and the results should be presented in this section.

The results should be presented in a logic way either in the sequence the experiments were carried out, the results easiest to discuss first followed by the more difficult ones or the most important results first and less important results last.

Consider carefully how the results can be presented. Use relevant figures or tables for illustration or include the results in the text. Figures and tables should be supplied with a text that makes it possible to understand the results presented without reading the rest of the text. For tables, text must be placed above the table (see table 1 below) and for figures text should be placed below the figure (see Figure 1 above). Remember to use a systematic numbering of the tables and figures. Carefully consider the number of digits for presentation of results and remember to include the units. All figures and tables should be mentioned in the text to lead the reader through the results. Be honest, clear and precise in describing results.

Write the section in past tense (the experiments are done before the results can be written).

Table 1: Average and ranges of total sum of PAHs, sum of genotoxic PAHs and sums of light and heavy PAHs in µg kg-1 for vegetable oils analysed. N is the number of samples.[197]

Vegetable oil	N	Total PAHs Average	Genotoxic PAHsa) Average	Light PAHsb) Average	Heavy PAHsc) Average
Olive oil, extra virgin	46	16.0	2.7	15	1.6
Olive oil	6	8.1	1.9	6.8	1.3
Rapeseed oil	8	5.5	1.3	4.0	1.5
Sunflower oil	3	7.4	3.7	3.8	3.7
Sunflower oil	1	172.0	93	97	75
Grape seed oil	4	42.0	11	35	7.2
Sesame oil	1	11.0	1.6	9.8	1.2

a. *Genotoxic PAHs include the sum of benzo[a]anthracene, chrysene, benzo[b+j] fluoranthene, benzo[k]-fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene.*

b. *Light PAHs (4 or less aromatic rings) include the sum of acenaphthylene, acenaphthene, fluorene, anthracene, fluoranthene, pyrene, benzo[a]anthracene and chrysene*

c. *Heavy PAHs (5 or more aromatic rings) include the sum of benzo[b+j]fluoranthene, benzo[k]fluor-anthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-c,d]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene.*

Discussion

This section is possibly the most difficult and very important part of the report. The aim of the discussion is to explain the meaning of your results and put them into context to the topics dealt with in the introduction.

The discussion starts with specific topics and ends up in general terms. Therefore begin relating your results to your hypothesis and answer questions raised in the introduction. Use your results to confirm your thoughts. Explain clearly why your results confirm or do not confirm the hypothesis. Relate your results to results found in literature and how they can contribute to the field. If results can be understood in more than one way, discuss it. Round of with as broad a perspective as possible for your results. This section again includes use of references.

Discussions can also be applied after each result section, if so, an overall discussion should then be included. Use terms from the introduction and write in present tense.

Sometimes it is difficult to divide results and discussion, and in these cases it might be relevant to combine the sections into one "Results and discussion" section.

197 Fromberg., A., Højgård, A and Duedahl-Olesen, L. (2007).Analysis of polycyclic aromatic hydrocarbons in vegetable oils combining gel permeation chromatography with solid-phase extraction clean-up. Food Additives & Contaminants: Part A, 24: 7, 758-767.

Conclusion

In this section significant elements of the report should be highlighted with a link to the rest of the report. It is important not to introduce new material in the conclusion.

In many reports it might be relevant to write the conclusion as the last thing in the discussion.

References and literature cited

It is very important to list all cited references. The references should be listed so detailed that an interested reader will be able to find the article.

Different methods can be used to cite literature in the report. The list of references includes references cited the same way for all references. If cited in the report as numbers the list of references should be listed in numerical order, with the same number through the whole report. If references is given as author and year in the report (e.g. Pinsky *et al.*, 1971) the references should be listed alphabetically in the list of references. All references cited should be included and no more. Be consistent and aware of the differences.

Think carefully on the use of references e.g. use books and articles for theory and background information and not homepages.

Examples of citations for the list of references:

- **Article**

  Pinsky, A., Grossman, S. and Trap, M.. "Lipoxygenase content and antioxidant activity of some fruits and vegetables", *Journal of Food Science*, 35, 1971pp. 571-572. (Journal name can be abbreviated to official short form [3], example above: *J. Food Sci.*).

- **Book chapters**

  Danzart, M., "Evaluation of the performance of panel judges", in *Food research and data analysis* (Martens, H. and Russwurm, H. eds.), London/New York, Applied Science Publ., 1983, pp 305-319 (an ISBN number can be included)

- **Report**

  Fromberg, A., Larsen, E.H., Hartkopp, H.B., Larsen, J.C., Granby, K., Jørgensen. K., Rasmussen, P.H., Cederberg, T.L. and Christensen, T., *Chemical contaminants. Food monitoring 1998-2003: Part 1*, Danish Veterinary and Food Administration, 2005.

- **Patents**

  Inventor. Patent number. Title. Year. Place

- **Homepages**

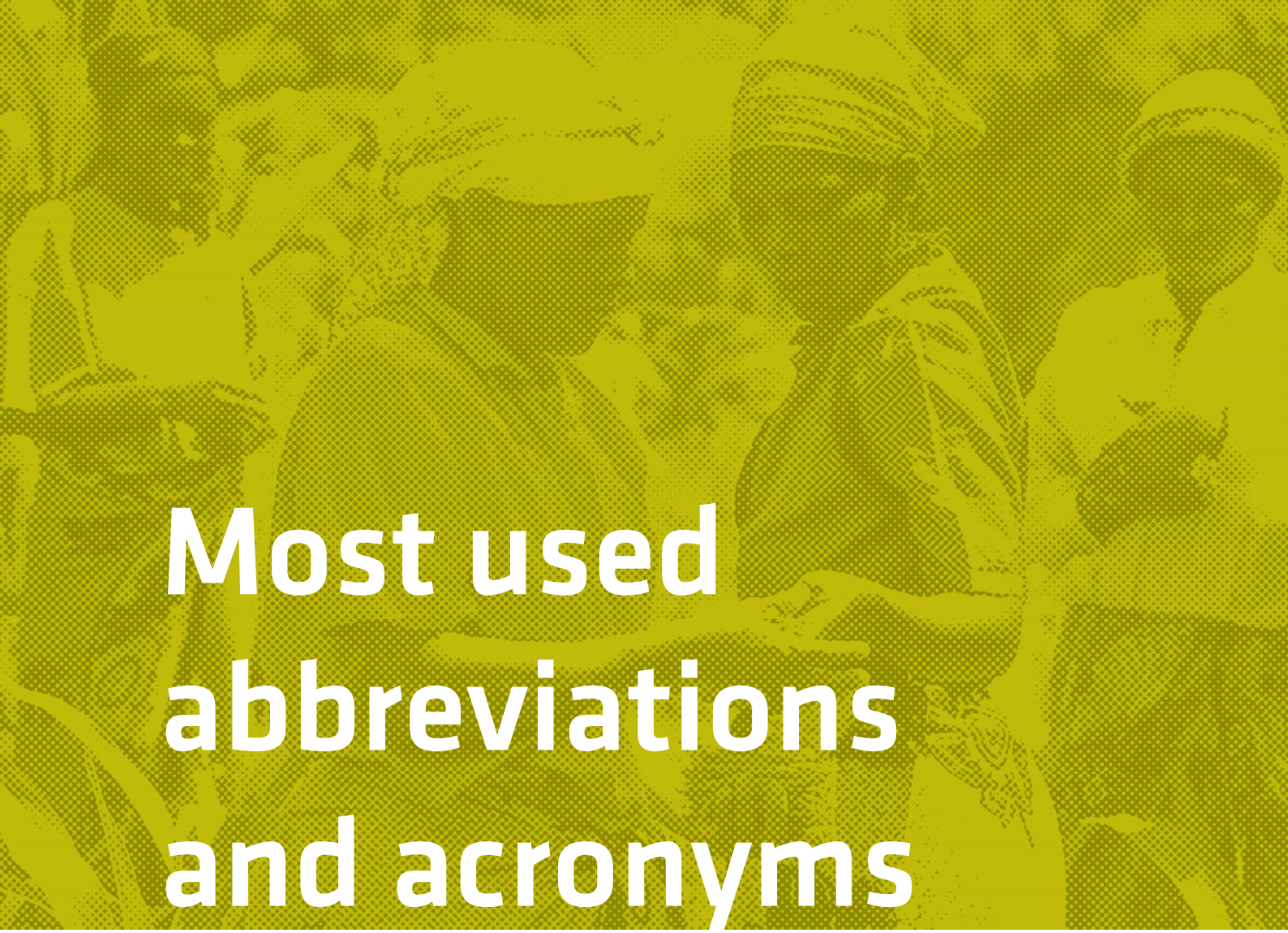
  Author if available, company, title of the page, assessing date, address

- **Personal communication**

  Name and title (or position), company, place, time and duration.

A.2. References

1. Regulation (EC) No. 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.
2. EN ISO/IEC 17025:2005 – General requirements for the competence of testing and calibration laboratories.
3. EN ISO/IEC 17011 – General requirements for accreditation bodies accrediting conformity assessment bodies.
4. EN ISO 8968 – Milk – Method for the determination of the nitrogen content of milk.
5. EN 14083 – Foodstuffs – method for the determination of lead, cadmium, chromium and molybdenum by in foodstuffs, by graphite furnace atomic absorption spectrometry.
6. EN 14123 – Method for the determination of aflatoxin B1 and the sum of aflatoxin B1, B2, G1 and G2 in hazelnuts, peanuts, pistachios, figs, and paprika powder.
7. ISO 21528-2:2004 – Colony count method for the enumeration of Enterobacteriaceae.
8. Report on the Relationship between Analytical Results, Measurement Uncertainty, Recovery Factors and the Provisions of EU Food and Feed Legislation, with Particular Reference to Community Legislation Concerning – Contaminants in Food (Council Regulation (EEC) 315/93 of 8 February 1993 Laying Down Community Procedures For Contaminants In Food) – Undesirable Substances in Feed (Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on Undesirable Substances in Animal Feed).



Most used abbreviations and acronyms

MOST USEFUL ABBREVIATIONS AND ACRONYMS

2HCH	Hexachlorocyclohexane
3-MCPD	3-monochloropropane-1,2-diol
A	Ampere
AAS	*Atomic Absorption Spectrometry*
ACN	Acetonitrile
ACP	African, Caribbean and Pacific Group of States
ADI	Acceptable Daily Intake
AES	Atomic emission spectroscopy
AFSCA	*Agence fédérale pour la sécurité de la chaîne alimentaire* (Belgian Federal Agency for the Safety of the Food Chain)
AFRAC	African Accreditation Cooperation
AHD	1-aminohydantoin (metabolite of nitrofurantoin)
AML	Multilateral Agreement
AMOZ	3-amino-5-morpholinomethyl- 2-oxazolidinone (metabolite of Furaltadone)
ANSES	*Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail* (French Agency for Food, Environmental and Occupational Health & Safety)
AOAC	Association of Analytical Communities/Association of Official Analytical Chemists
AOZ	3- amino- 2-oxazolidinone (metabolite of Furazolidone)
APCI	Atmospheric pressure chemical ionisation
APLAC	Asia Pacific Laboratory Accreditation Cooperation
APLMF	Asia Pacific Legal Metrology Forum
APMP	Asia Pacific Metrology Programme
ARfD	Acute Reference Dose
a.s.	Active substance
ASE	Accelerated solvent extraction

BAM	Bacteriological Analytical Manual
BIML	*Bureau international de métrologie légale* (International Bureau of Legal Metrology, OIML)
BIPM	International Bureau of Weights and Measures
BRC	British Retail Consortium
BS	British Standard
bw	Body weight
C	Contribution
C^{14}	Chemical symbol of carbon 14
Ca	Chemical symbol of calcium
CA	Competent Authority
CAB	Conformity Assessment Body
CAC	*Codex Alimentarius* Commission
CAM	Chloramphenicol
CASCO	Committee on Conformity Assessment
CB	Certification Body
CC	Consultative Committee
CCAUV	Consultative Committee for Acoustics, Ultrasound and Vibration
CCEM	Consultative Committee for Electricity and Magnetism
CCL	Consultative Committee for Length
CCM	Consultative Committee for Mass and Related Quantities
CCPR	Codex Committee on Pesticide Residues
CCPR	Consultative Committee for Photometry and Radiometry
CCQA	*Comité consultatif pour les questions administratives* (Consultative Committee on Administrative Questions)
CCQM	Consultative Committee for Amount of Substance – Metrology in Chemistry
CCRI	Consultative Committee for Ionizing Radiation

CCT	Consultative Committee for Thermometry
CCTF	Consultative Committee for Time and Frequency
CCU	Consultative Committee for Units
Cd	Cadmium
cd	Candela
CEN	European Committee for Standardisation (*Comité européen de normalisation*)
CF	Correction factor
CFS	Critical Success Factors
CGPM	General Conference on Weights and Measures
CI	Confidence index
CIML	International Committee of Legal Metrology (*Comité international de métrologie légale*)
CIPM	International Committee for Weights and Measures
CO_2	Chemical symbol of carbon dioxide
COOH	Chemical symbol of carboxylic acid
COOMET	Euro-Asian Cooperation of National Metrological Institutions
CP	Control Plan
Cr	Criticity of risk
CRM	Certified reference material
CS	Collaborative Study
CS_2	Chemical symbol of carbon disulfide
CV	*Curriculum vitæ*
CVAAS	Cold-Vapour technique
CVMP	Committee for Medicinal Products for Veterinary Use
D	Degradation
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene

DDT	Dichlorodiphenyltrichloroethane
DG SANCO	Directorate General for Health and Consumer Affairs
DI	Designated Institute
DL	Designated laboratory
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DoMC	Declarations of Mutual Confidence
DON	Deoxynivalenol
DT_{50}	Half-life time
E	Estimation
EA	European co-operation for Accreditation
EC	European Commission / European Community
ECD	Electron capture dissociation
EDI	Estimated daily intake
EDL	Electrodeless discharge lamps
EE	European Community
EEC	European Economic Community
EFSA	European Food Safety Authority
EFTA	European Free Trade Area
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EN	Euro Norm/European Standard
EPAR	European Public Assessment Report
EPPO	European and Mediterranean Plant Protection Organization
EQA	External Quality Assessment
ESA	European Space Agency
ESI	Electrospray ionisation

EU	European Union
EURAMET	European Association of National Metrology Institutes
EU-RL	EU Reference Laboratories
EW	Emulsion in water
FAO	Food and Agriculture Organisation of the United Nations
FB1	Fumonisin B1
FB2	Fumonisin B2
FBO	Food business operators
FDA	Food and Drug Administration
FPD	Flame Photometric Detector
FSA	Food Standard Agency
FSMS	Food Safety Management System
FVO	Food and Veterinary Office
GAP	Good agricultural practices
GC	Gas chromatography
GC-EC	Gas Chromatography with Electron Capture Detection
GC-MS	Gas Chromatography-Mass Spectrometry
GC-MS-MS	Gas Chromatography-Mass Spectrometry-Mass Spectrometry
GCP	Good clinical practices
GEMS	Global Environment Monitoring System
GFAAS	Graphite furnace atomic absorption spectrometry
GLC	Gas Liquid Chromatography
GLP	Good laboratory practices
GMO	Genetically Modified Organism
GMP	Good manufacturing practices
GPC	Gel Permeation Chromatography
GSP	Good storage practices

GUM	Guide to the expression of uncertainty in measurement
HACCP	Hazard analysis and critical control point
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HCL	Hollow cathode lamps
HFBI	N-Heptafluoro-butyrylimidazole
Hg	Chemical symbol of mercury
HGAAS	Hybrid Generation Atomic Absorption Spectrometry
HLB	Hydrophilic-lipophilic balanced copolymer
HPGPC	High Performance Gel Permeation Chromatography
HPLC	High Performance Liquid Chromatography
IAAC	Inter-American Accreditation Cooperation
IAC	Immunoaffinity column
IAEA	International Atomic Energy Agency
IAF	International Accreditation Forum
IC	International Conference
ICAO	International Civil Aviation Organisation
ICH	International Conference on Harmonisation
ICP	Inductively coupled plasma
ICPMS	Inductively coupled plasma coupling to a mass spectrometer
ICPOES	Inductively coupled plasma coupling to an optical emission spectrometer
IDF	International Dairy Federation
IEC	International Electrotechnical Commission
IFCC	International Federation of Clinical Chemistry
IFS	International Food Standard
ILAC	International Laboratory Accreditation Cooperation

IMMR	*Institut des matériaux et mesures de référence* (Institute for Reference Materials and Measurementsi
IP	Identification points
IPPC	Internationale Plant Protection Convention
IQA	Internal Quality Assessment
IQC	Internal Quality Control
IS	Inter laboratory studies
ISO	International Organisation for Standardisation
IT	Import tolerance
IUPAP	Union of Pure and Applied Physics
IUPAC	International Union of Pure and Applied Chemistry
JCGM	Joint Committee for Guides in Metrology
JCRB	Joint Committee of the Regional Metrology Organisations and the BIPM
JCTLM	Joint Committee on Traceability in Laboratory Medicine
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K	Kelvin
KCDB	Key Comparisons Data Base
K_d	Partition coefficient of a molecule between the dissolved phase and the particulate phase
Kg	Kilogram
KPI	Key performance indicator
LC-MS-MS	Liquid Chromatography-Mass Spectrometry-Mass Spectrometry
LLE	Liquid-liquid extraction
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of detection
LoI	Letter of intent
LOQ	Limit of quantification
LP	Large portion

LT	Long term
m	Meter
M	Metabolite
MA	Marketing Authorisation
MAA	Mutual Acceptance Arrangement
MAE	Microwave-assisted extraction
$MgSO_4$	Magnesium sulfate
MIP	Molecular imprinted polymers
ML	maximum limit/level
MLA	Multilateral Arrangement
Mol	Mole
MP	Monitoring plan
MPCP	Monitoring plan and control plan
MPN	Most probable number
MRA	Mutual recognition agreement
MRL	Maximum Residue Level
MRM	Multi-Residue Method
MRPL	Minimum required performance limits
MS	M MS spectrométrie ass-spectrometry
MTE	Metal trace elements
MU	Measurement Uncertainty
n	Number of samples to be taken
N	Newton
NaCl	Chemical symbol of sodium chloride
NATA	National Association of Testing Authorities, Australia
NCP	National Control Plan
NEDI	Individual commodity National Estimates of Dietary Intakes

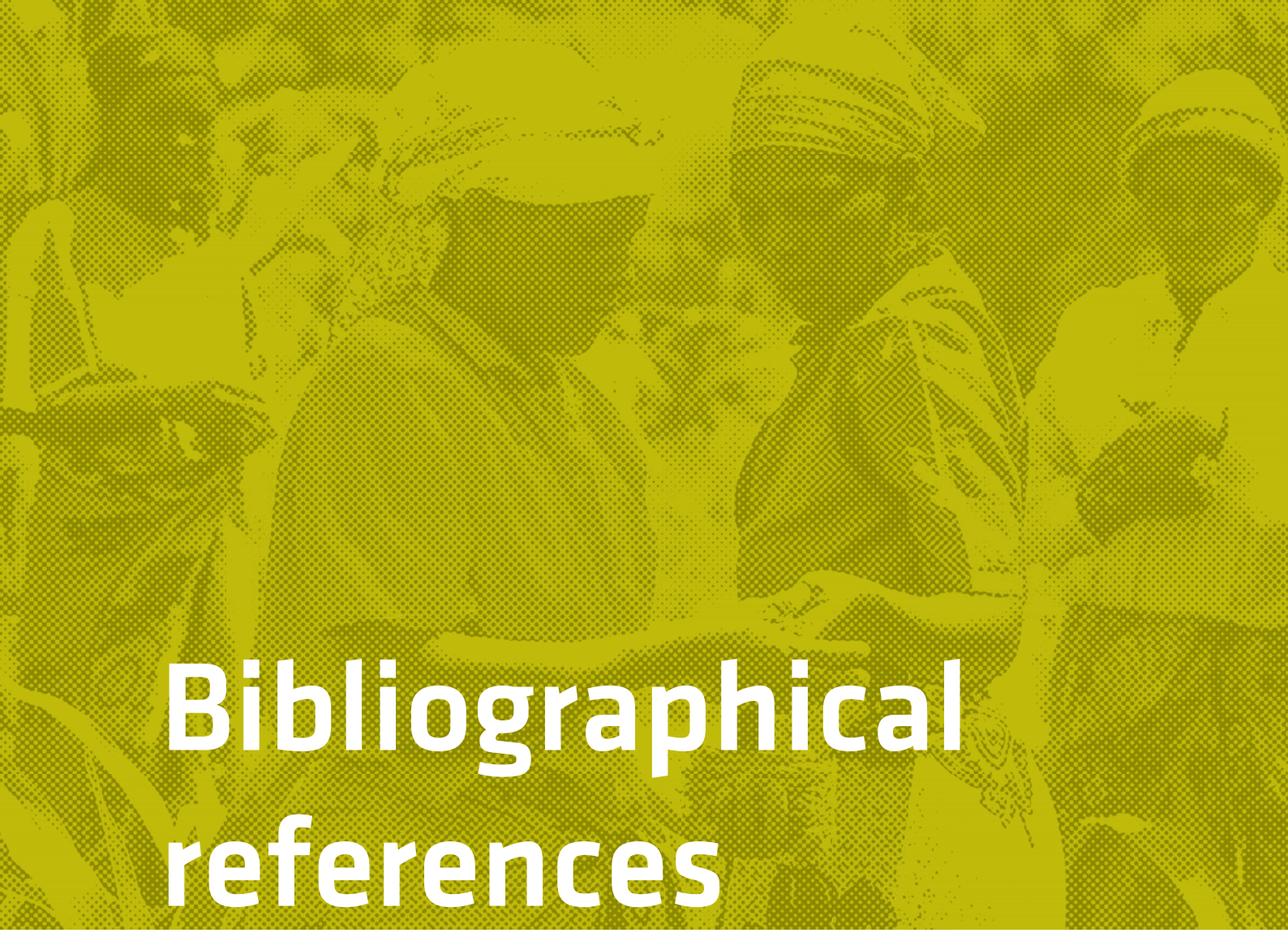
NESTI	National Estimates of Short Term Intakes
NGO	Non-governmental Organisation
NH_2	Primary amine
NH_4	Chemical symbol of ammonium
NIST	National Institute of Standards and Technology
Nm	Nanometre
NMI	National Metrology Institute
NMKL	Nordic Committee on Food Analysis
NML	National Metrology Laboratory
NO_2	Chemical symbol of dioxyde d'azote
NOAEL	No Observed Adverse Effect Level
NPD	Nitrogen phosphorus detector
NRCP	National residue control plan
NRL	National Reference Laboratory
NSAID	Non-steroidal anti-inflammatory drugs
NWA	National Works Agency
OCP	Organochlorine pesticide
OECD	Organisation for Economic Co-operation and Development
OES	Optic emission spectroscopy
OH	Hydroxyl radical
OIE	World Organisation for Animal Health (*Office international des épizooties*)
OIML	International Organization of Legal Metrology
OMA	Official Methods of Analysis
OTA	Ochratoxin A
P	Electrical power
P	Percentile

P	Prevalence
PAH	Polycyclic aromatic hydrocarbons
Pb	Lead
PBPB	Pyridine hydrobromide perbromide
PBS	Phosphate buffered saline
PC	Presidential Council
PCB	Polychlorinated biphenyl
PCDD	Dibenzo-p-dioxins
PCDF	Polychlorinated dibenzofuranes
PCR	Polymerase Chain Reaction
PCS	Pre-collaborative Study
Pf	*Processing factor*
PFA	Perfluroalcoxyfluorocarbons
pH	Potential of hydrogen
PHI	Pre-harvest interval
PLC	Prevalence level to be controlled
PLE	Pressurized liquid extraction
PNCP	Pluri-annual National Control Plan
POP	Persistent Organic Pollutants
Ppb	Parts per billion
PSA	Pressure Swing Adsorption
PSD	Pesticide Safety Directorate
PSTI	Predicted Short Term Intake
PT	Proficiency Tests
PTFE	Polytetrafluoroethylene
PT/ILC	Proficiency testing / inter laboratory comparison
PTM	Performance Tested Methods

PTV	Temperature-programmed-vaporising technique
PVM	Peer-Verified Methods
QA	Quality Assurance
QAU	Quality Assurance Unit
QC	Quality control
QM	Quality management
QMS	Quality management system
r	Repeatability
R	Reproductibility
R	Resistence
RASFF	Rapid Alert System for Food and Feed
RIA	Radio-immuno-assay
RM	Reference Material
RMO	Regional Metrology Organisation
Rpm	Revolutions per minute
s	Second
S	Severity
SADCA	Southern African Development Community in Accreditation
SBSE	Stir-bar sorptive extraction
SC	Suspension concentrate
SD	Study Director
SDME	Single-drop and liquid microextraction
SEM	Semicarbazide (nitrofurazone metabolite)
SF	Security factor
SFE	Supercritical fluid extraction
SI	International System of Units

SIM	*Sistema Interamericano de Metrología* (Inter-American Metrology System)
SMART	Specific, Measurable, Attainable, Relevant, Timely
SOP	Standard operations procedures
SPE	Solid phase extraction
SPME	Solid-phase microextraction
SPS	Sanitary and Phytosanitary (Agreement)
SQAM	Standardisation, quality management, accreditation and metrology
SRM	Standard reference material
ST	Short term
STMR	Supervised trials median residue
SWOT	Strengths, Weaknesses, Opportunities, Threats
T	Temperature
TBT	Technical Barriers to Trade
TCDD	Tetrachloro dibenzo-p-dioxins
TDE	Tetrachlorodiphenylethane
TEF	Toxicity equivalency factors
TLC	Thin-layer chromatography
TMDI	Theoretical Maximum Daily Intake
TRV	Toxicological Reference Value /*Benchmark Dose Limit*
ULV/UBV	Ultra low volume
UKAS	United Kingdom Accreditation Service
UPLC	Ultra performance liquid chromatography
USDA	United States Department of Agriculture
USDA FSIS	United States Department of Agriculture – Food Safety and Inspection Service
UV	Ultraviolet
V	Potential difference

V	Variance
VIM	International Vocabulary of Metrology (*Vocabulaire international de métrologie*)
VIML	Legal International Vocabulary of Metrology (*Vocabulaire international de métrologie légale*)
WELMEC	European Cooperation in Legal Metrology
WG	Wettable granules
WHO	World Health Organisation
WMO	World meteorological Organisation
WP	Wettable powder
WTO	World Trade Organisation
γ-HCH	Gamma-hexachlorocyclohexane / Lindane
YOPI	Young, old, pregnant and immunosuppressed



Bibliographical references

BIBLIOGRAPHICAL REFERENCES

Al-Sayeda, H., *Transfert d'un insecticide systémique, l'imidaclopride, chez la tomate: implication du transport phloémien*, Doctoral thesis, Toulouse, Institut national polytechnique, 2007.

BIPM, *Le système international d'unités*, Sèvres, BIPM, 1998, www.bipm.org/fr/publications/si-brochure.

Blanchard, R., *Guide Culture: Raisonner ses interventions d'automne pour allier production et performance. Résultats d'essais et préconisation*, Agricultures and Territories, Chamber of Agriculture of Nièvre, 2012-2013 campaign.

Bodin, L., "Principes de la détermination des valeurs toxiques de reference", Anses, 2010, www.sftox.com/congres/sft2010/Pres/Congres_Annuel_de_la_SFT_Paris_L_Bodin.pdf.

Capar, S.G. and Szefer, P., *Determination and Speciation of Trace Elements in Foods: Methods of Analysis of Food Components and Additives*, 2nd ed., Boca Raton, CRC Press, 2011, pp. 165-210.

Carlson, R., *Silent Spring*, Boston, Houghton Mifflin, 1962.

Caroli, S. (ed.), *The Determination of Chemical Elements in Food – Applications for Atomic and Mass Spectrometry*, Hoboken, Wiley, 2007.

CEN European standard EN13804, "Foodstuffs – Determination of trace elements – Performance criteria, general considerations and sample preparation", 2002.

Codex Alimentarius Commission, "Code of Hygienic Practice for Fresh Fruits and Vegetables", CAC/RCP 53-2003.

Codex Alimentarius Commission, "Recommended International Code of Practice – General Principles of Food Hygiene", CAC/RCP 1-1969, Rev. 4, 2003.

Codex Alimentarius Commission, "Code of Practice for the Packaging and Transport of Tropical Fresh Fruits and Vegetables", CAC/RCP 44-1995, AMD. 1-2004.

Codex Alimentarius Commission, "General guidelines on sampling", CAC/GL 50-2004.

Codex Alimentarius Commission, "Recommended Methods for Pesticide Residue Sampling for Compliance with MRLs", CAC/GL 33-1999.

Codex Alimentarius Commission, "Codex General Standard for Contaminants and Toxins in Foods", CODEX STAN 193-1995, Rév. 1, 1997.

Codex Alimentarius Commission, "Principles for Traceability / Product Tracing as a Tool in a Food Inspection and Certification System", CAC/GL 60-2006.

Codex Alimentarius Commission, "Principles for the Establishment and Application of Microbiological Criteria for Foods", CAC/GL 21-1997.

CRL guidance paper, "CRLs view on state of the art analytical methods for national residue control plans", 7 December 2007, www.bvl.bund.de/SharedDocs/Downloads/09_Untersuchungen/EURL_Empfehlungen_Konzentrationsauswahl_Methodenvalierungen.pdf?__blob=publicationFile.

Delcour, I., Rademaker, M., Jacxsens, L., De Win, J., De Baets, B. and Spanoghe, P., "A risk-based pesticide residue monitoring tool to prioritize the sampling of fresh produce", *Food Control*, 50, 2015, pp. 690-698.

Duffus, J.H., "'Heavy metals': a meaningless term?" (IUPAC technical report). *Pure Appl Chem*, vol. 74, 2002, pp. 793-807.

EC, "Guidance Document – Key questions related to import requirements and the new rules on food hygiene and official food controls", DG SANCO, 2005.

EC, "Guidance Document – Implementation of certain provisions of Regulation (EC) No 852/2004 on the hygiene of foodstuffs", DG SANCO, 2005.

EC, "Guidance Document – Implementation of procedures based on the HACCP principles, and facilitation of the implementation of the HACCP principles in certain food businesses", DG SANCO, 2005.

EC, Regulation (CE)° 333/2007 of the Commission of 28 March 2007 laying down the sampling methods and the methods of analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo (a) pyrene in foodstuffs, *OJEU*, n° L 88/29 of 29 March 2007.

EDES, "Manual for the Development of Official Control Programs. Guidance document", Brussels, COLEACP, 2015.

EFSA, "Standard sample description for food and feed", *EFSA Journal*, 2010, 8(1):1457, www.efsa.europa.eu/en/efsajournal/pub/1457.htm.

EFSA, "The 2009 European Union Report on Pesticide Residues in Food", *EFSA Journal*, 2011, 9(11):2430, www.efsa.europa.eu/en/efsajournal/pub/2430.htm.

EFSA, "Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment – Guidance document", *EFSA Journal*, 2011;9(3):2097.

Ellison, S.L.R. and Williams, A. (ed.), "Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement", 3rd ed., 2012, www.eurachem.org.

EU (2004), Regulation (EC) No. 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

EU (2005), Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin

EU (2009), Regulation (EC) No 669/2009 of 24 July 2009 implementing Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards the increased level of official controls on imports of certain feed and food of non-animal origin and amending Decision 2006/504/EC

EU (2015), Implementing Regulation (EU) 2015/595 of 15 April 2015 concerning a coordinated multiannual control programme of the Union for 2016, 2017 and 2018 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin

Eurachem, "Terminology in Analytical Measurement – Introduction to VIM 3", 1st ed., 2011, www.eurachem.org/index.php/publications/guides/48-gdtam11.

Euramet, "Metrology – In Short", 3rd edd., 2008, www.euramet.org/Media/docs/Publications/Metrology_in_short_3rd_ed.pdf.

FAO, "International Convention for the Protection of Plants", new revised text as approved by the FAO Conference at its 29th Session, November 1997.

FAO, "Ensuring food safety and quality: guidelines for strengthening national food control systems", www.fao.org/docrep/006/y8705e/y8705e00.htm.

Fastier, A. and Vergnet, Cl., "Exposition aux pesticides", Anses, 2010, www.sftox.com/congres/sft2010/Pres/Congres_Annuel_de_la_SFT_Paris_Fastier_Vergnet.pdf.

Focant, J.-F., Eppe, G., Pirard, C. and de Pauw, E., "Fast clean-up for polychlorodibenzo-p-dioxines, dibenzofurans and coplanar polychlorobiphenyls analysis of high-fat-content biological samples", *J. Chromatogr.*, A 925, 2001, pp. 207-221.

Fromberg., A., Højgård, A and Duedahl-Olesen, L., "Analyse d'hydrocarbures aromatiques polycycliques dans des huiles végétales combinant la chromatographie sur gel perméable avec nettoyage par extraction en phase solide", *Food Additives & Contaminants*, Part A, 2007, 24: 7, pp. 758-767.

Gerault, F. and Reulet, P., "Les résidus de pesticides: LMR, échantillons, laboratoires et résultats d'analyse. Les contaminations sur fruits et legumes", Anses, 2013, crdp.acbordeaux.fr/edd/academie/Formation_BX_30_012013_PH_Reulet.pdf.

IPPC, "International Standards for Phytosanitary Measures" (ISPM 1 to 31), Rome, FAO, 2008.

ISO, "ISO 3534-1. Statistics – Vocabulary and symbols", Part 1: "General statistical terms and terms used in calculating probabilities", Geneva, ISO, 1993.

ISO, "ISO 9001:2000, Quality Management Systems – Requirements", Geneva, ISO, 2000, www.iso.org/iso/fr/catalogue_detail?csnumber=21823.

ISO, "ISO 10012:2003 Measurement management systems – Requirements for processes and measuring equipment", Geneva, ISO, 2003, www.iso.org/iso/fr/catalogue_detail?csnumber=26033.

ISO, "ISO 22000:2004, Food safety management systems – Requirements for any organization in the food chain", Geneva, ISO, 2004.

ISO, "ISO/IEC 17025:2005 General Requirements for the Competence of Calibration and Testing Laboratories", Geneva, ISO, 2005, www.iso.org/iso/fr/catalogue_detail?csnumber=39883.

ISO 22005:2007, "Traceability of the food chain – General principles and basic requirements for system design and implementation", Geneva, ISO, 2007.

Kinsella, B. *et al.*, "Current trends in sample preparation for growth promoter and veterinary drug residue analysis", *J. Chrom. A*, 1216, 2009, pp. 7977-8015

Leslie, J.F., Bandyopadhyay, R. and Visconti, A. (ed.), "Reducing Impact of Mycotoxins in Tropical Agriculture" (Mycoglobe Conference, Accra, 2005, Mycotoxins: detection methods, management, public health, and agricultural trade, 2008.

Maudoux, J.P., Saegerman, C, Retigner, C, Houins, G, Van Huffel, X. and Berkvens, D., "Food Safety Surveillance through a risk-based control programme: Approach employed by the Belgian Federal Agency for the Safety of the Food Chain", *Veterinary Quaterly*, Vol. 28, No. 4, 2006, pp. 140-154.

Moreira, C., "Réglementation sur les pesticides et les biocides", in COLEACP-PIP, *Réglementation, normes et standards*, Brussels, COLEACP, 2011, pp. 124-151.

Nollet, L.M.L. and Ratore, H.S. (ed.), *Handbook of Pesticides: Methods of Pesticide Residues Analysis*, Chap. 2, "Methods of and Problems in Analyzing Pesticide Residues in the Environment", Boca Raton, CRC Press, 2010.

Thompson, M., Ellison, S.L.R. and Wood, R., "Harmonised guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report)", *Pure Appl. Chem.*, vol. 74, No. 5, 2002, pp. 835-855.

USDA, "Pesticide Data Program, Annual Summary, Calendar Year 2009", www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5091055.

US FDA, "Fish and fishery products hazards and control guidance", 4th ed., April 2011, www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Seafood/ucm2018426.htm.

Wagacha, J.M. and Muthomi, J.W., "Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies", *Int'l J. of Food Microbiology*, 124, 2008, pp. 1-12.

White, J.-C. *et al.*. "Tracking chlordane compositional and chiral profiles in soil and vegetation", *Chemosphere*, No. 47, 2002, pp. 639-646.

Wilson, J.S. and Abiola, V.O. (ed.), *Standards & Global Trade, A Voice for Africa*, World Bank, 2003.

WHO, "Strategies for Implementing HACCP in Small or Less Developed Businesses'', Geneva, WHO, 1999, www.who.int/foodsafety/publications/strategies-haccp/en.

WHO, "Ten chemicals of major public health concern", 2010, www.who.int/ipcs/assessment/public_health/chemicals_phc.

Useful Websites

USEFUL WEB SITES

AOAC
www.aoac.org

Australian Government – Australian Pesticides and Veterinary Medicines Authority
apvma.gov.au

BIPM
www.bipm.org/en/about-us/

CEN, European Committe for Standardisation
www.cen.eu

Codex Alimentarius
www.fao.org/fao-who-codexalimentarius/codex-home/en/ Le lien ne fonctionne pas

COLEACP
www.coleacp.org/en Le lien ne fonctionne pas

Department Health of Republic of South Africa
www.health.gov.za

EFSA
www.efsa.europa.eu

Eurachem
www.eurachem.org

EURAMET
www.euramet.org Le lien ne fonctionne pas

European Accreditation, EA
www.european-accreditation.org

European Commission
ec.europa.eu/commission/index_en

European Medicines Agency
www.ema.europa.eu/ema/index.jsp Le lien ne fonctionne pas

Eur-Lex
eur-lex.europa.eu/homepage.html?locale=en

FAO
www.fao.org/home/en

IFREMER
wwz.ifremer.fr

ILAC
www.ilac.org

INCHEM
www.inchem.org

IPPC
www.ippc.int/en

ISO
www.iso.org/home.html

Ministry for Primary Industries New Zealand
www.foodsafety.govt.nz

OECD
www.oecd.org

OEPP
www.eppo.org

Pesticides online
www.pesticides-online.com

QuEChERS
quechers.cvua-stuttgart.de

RASFF
ec.europa.eu/food/safety/rasff_en

The Japan Food Chemical Research Foundation
www.ffcr.or.jp/zaidan/ffcrhome.nsf/TrueMainE?OpenFrameSet

USDA
www.ams.usda.gov

U.S. Environmental Protection Agency
www3.epa.gov

U.S. Government Publishing Office
www.gpo.gov

WIN EPI
www.winepi.net/uk/index.htm

WHO
www.who.int/en

COLEACP E-LEARNING PLATFORM

...TFORM.
...OR IN AFRICAN,

AT YOUR OWN RHYTHM!

TOPICS

- FOOD SAFETY
- ENVIRONMENTAL MANAGEMENT
- PLANT HEALTH
- BUSINESS MANAGEMENT AND DEVELOPMENT
- SOCIAL ACCOUNTABILITY AND EMPOWERMENT
- SUSTAINABLE PRODUCTION AND TRADE
- AGRICULTURAL PRODUCTION AND PROCESSING

https://training.coleacp.org

REF. : 00104-03

SUSTAINABLE PRODUCTION
AND TRADE

PLANT HEALTH

FOOD SAFETY

AGRICULTURAL PRODUCTION
AND PROCESSING

SOCIAL ACCOUNTABILITY
AND EMPOWERMENT

ENVIRONMENTAL
MANAGEMENT

BUSINESS MANAGEMENT
AND DEVELOPMENT

TRAINING METHODOLOGIES

JUNE 2020

COLEACP