

9. HOW PESTICIDES ARE ANALYSED

Analytical chemistry has made extraordinary progress in its ability to identify and measure substances present in food at extremely low concentrations. Today, highly specialised laboratory instruments can test a single food sample for hundreds of pesticides at the same time. This capability represents an important advance for food safety and for our knowledge of the chemical composition of food products.

However, an analysis is not simply a matter of placing food in an instrument and obtaining a list of every substance it contains. Its outcome depends on many factors: how representative the sample is, how it is prepared, which substances are sought, the techniques employed, method sensitivity, and the quality of laboratory procedures.

Chemical analysis does not automatically reveal everything present in a food. It reveals what the method has been designed and validated to look for, within its measurement limits.

Understanding how these techniques work helps us interpret results correctly and distinguish regulatory testing from a more extensive analytical programme.

9.1 From a food product to a laboratory sample

The first stage of chemical analysis does not take place inside an instrument, but when the sample is collected. Suppose we want to determine whether pesticides are present in a batch of 1,000 kilograms of flour. It would not be practical to analyse every gram. We must therefore collect a limited amount of material that represents the characteristics of the batch as reliably as possible. This operation is called sampling.

When a product is sufficiently homogeneous, a properly designed sampling procedure can produce a representative sample. However, contaminants are not always uniformly distributed. Within a cereal batch, for instance, different residues may be associated with different agricultural consignments, uneven treatments or different storage conditions. A sample collected at a single location might therefore fail to describe the entire batch. To reduce this risk, several incremental samples may be collected and then combined and prepared according to an appropriate sampling plan.

The material is then mixed, homogenised and reduced to the amount required for analysis. Commission Implementing Regulation (EU) 2026/765 governs sampling and analytical procedures used in EU official controls on pesticide residues. Sampling methods depend on the nature of the product and the purpose of the controls.

The quality of an analytical result therefore begins before the analysis itself, with the selection and preparation of the sample. A laboratory may measure a substance with extraordinary precision, but it cannot correct a sampling error that excluded the contaminated part of a batch.

9.2 Preparing the sample: extracting pesticides from food

Once a laboratory receives a sample, it must prepare it for instrumental analysis. Foods are chemically complex materials. An apple contains water, sugars, fibre, organic acids and many other substances. Flour mainly contains starch, proteins, lipids and mineral components. Oil consists predominantly of lipids. Any pesticides present are therefore embedded in very different matrices. Before analysis, they often need to be at least partially separated from the other components of the product.

One widely used technique is QuEChERS, an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe. Its name highlights the characteristics that helped the procedure become widespread: speed, relative simplicity, low cost, effectiveness, robustness and operational safety.

The general principle is to extract numerous pesticides from the sample using a solvent, usually acetonitrile, under suitable separation conditions. The food sample is homogenised, weighed and mixed with the solvent. Salts are subsequently added to promote phase separation and transfer many pesticides into the extract. Many applications also include a purification step, called clean-up, to reduce matrix compounds that could interfere with measurement. The resulting extract can then undergo instrumental analysis.

QuEChERS is particularly common in multiresidue testing of fruit, vegetables and many other foods, but it is not universally suitable for every pesticide or matrix. Some substances require different extraction and preparation procedures. Foods rich in fat, pigments or other components may also need specific adaptations. Whether a pesticide can be measured depends partly on the method's ability to extract it from the food without losing it or transforming it in an uncontrolled manner.

9.3 Chromatography: separating substances before identifying them



Illustrative workflow. Appropriate methods and controls depend on the analytes and food matrix.

Once an extract has been prepared, the laboratory must distinguish the many molecules it may contain. A technique called chromatography is often used for this purpose.

Consider a mixture containing numerous chemical substances. If they all reached the detector simultaneously, distinguishing them could be very difficult. Chromatography separates compounds by exploiting differences in how they interact with a moving phase and a separation material. The substances travel through a chromatographic column and tend to emerge at different times. The interval is called the retention time.

Separation is not necessarily perfect: different substances can have similar or overlapping retention times. This is why modern chromatography is frequently combined with mass spectrometry, which provides additional information about molecular identity.

The two main chromatographic techniques used in pesticide multiresidue testing are gas chromatography and liquid chromatography. The choice depends on the properties of the compounds being investigated.

9.4 GC-MS/MS: gas chromatography and tandem mass spectrometry

GC-MS/MS combines gas chromatography with tandem mass spectrometry. GC stands for Gas Chromatography; MS/MS refers to two-stage mass spectrometry.

In gas chromatography, a sample enters a system in which substances suitable for this technique are vaporised and carried through a column by a gas. As they travel through the column, molecules separate according to their chemical and physical characteristics. On leaving the column they reach the mass spectrometer. This instrument produces electrically charged particles, called ions, and distinguishes them

according to their mass-to-charge ratio.

In MS/MS systems, a particular ion is selected and may be fragmented further. The resulting fragments are then analysed. Combining retention time with specific ion transitions allows many compounds to be identified with high selectivity.

GC-MS/MS is especially useful for many pesticides that are sufficiently volatile and thermally stable, or otherwise compatible with gas-chromatographic conditions. It is frequently used for several families of insecticides and fungicides. It is not, however, suitable for every substance. Some pesticides are too polar, insufficiently volatile or unstable at the required temperatures and need different techniques.

GC-MS/MS is a very powerful tool, but it is not a universal detector of every molecule present in food.

9.5 LC-MS/MS: liquid chromatography and tandem mass spectrometry

The other major technique employed in pesticide analysis is LC-MS/MS. LC stands for Liquid Chromatography. The underlying principle resembles gas chromatography, but substances are transported through the column by a liquid mobile phase. Compounds therefore do not have to be vaporised under GC conditions.

This makes liquid chromatography particularly useful for many polar, poorly volatile or thermally unstable pesticides. After chromatographic separation, the molecules enter the mass spectrometer. Here too, tandem mass spectrometry makes it possible to select particular ions and examine their fragments, improving the ability to recognise substances.

LC-MS/MS is used for many families of modern pesticides, including several neonicotinoids, fungicides and other compounds. When a method is appropriately developed and validated, its high sensitivity enables very low concentrations to be quantified.

GC-MS/MS and LC-MS/MS are therefore complementary techniques. Many laboratories use both to increase the range of pesticides they can test for. No single technology can determine all pesticides equally effectively: the scope of testing depends on the combination of methods employed.

9.6 What multiresidue analysis means

Multiresidue analysis is a procedure designed to search for numerous substances in a single sample. Developments in chromatography and mass spectrometry now make it possible to include hundreds of pesticides in analytical protocols. The exact number depends on the laboratory, its instruments, the food matrix and the methods used.

For example, a laboratory may declare a multiresidue panel covering more than 500 substances. This does not mean that 500 pesticides are necessarily present in the food. It means that the laboratory has a list of substances it intends to search for using one or more analytical methods.

To interpret the result correctly, it is important to know which substances are actually covered and for which matrices the methods have been validated. It is also necessary to establish whether the declared number includes only active substances or also metabolites and degradation products. This matters because a pesticide may be transformed into compounds that will not necessarily be found by testing only for its parent molecule.

An extensive multiresidue analysis can provide much greater knowledge than testing for a few selected substances. Nevertheless, the stated number of pesticides is not, by itself, a guarantee of analytical completeness or quality. Sensitivity, validation and the suitability of the methods must also be considered.

9.7 Why some pesticides require specific analyses

Not all pesticides can be measured effectively by standard multiresidue protocols. Certain compounds have chemical properties that make extraction, separation or identification difficult with common methods.

Glyphosate is an instructive example. This very polar molecule has chemical characteristics that differ from those of many pesticides normally included in multiresidue panels and may require specific analytical procedures. Its main degradation product, AMPA, may also need dedicated testing. Methods are available for these compounds using, for instance, liquid chromatography and mass spectrometry, sometimes with special preliminary treatment.

The key point is not that glyphosate is impossible to analyse. On the contrary, validated methods exist that can measure it at low concentrations. The problem is that a standard multiresidue panel, even a very extensive one, does not necessarily include glyphosate. Its inclusion must be checked against the actual list of target substances.

The same principle applies to other pesticides or metabolites that require dedicated procedures. A protocol claiming to test for hundreds of pesticides does not automatically include every substance of particular interest for a given food matrix. A serious assessment must begin with the actual analyte list.

9.8 How a laboratory identifies a substance

Mass spectrometry is an extremely powerful tool, but identifying a compound should not be regarded as an automatic and infallible process. In targeted analyses, laboratories use known information about the molecules being sought, including retention times and the characteristics of the signals produced by the mass spectrometer. Tandem systems can monitor specific transitions between a selected ion and its fragments. Identification involves comparing observed signals with those obtained from reference substances.

Appropriate criteria are needed to confirm a molecule's identity. An isolated instrumental signal may not be enough. Different compounds may interfere with each other or produce partially overlapping signals. The properties of the food matrix can also alter the instrument's response.

Validated methods therefore use identification criteria, controls and checks designed to reduce false-positive and false-negative results. A false positive reports a substance as present when it is not. A false negative occurs when a substance that is present is not correctly detected.

A result's reliability depends not only on instrument sophistication, but also on the quality of the procedures used to interpret its signals.

9.9 How sensitive are modern analyses?

Modern analytical techniques can achieve extremely high sensitivity. In many applications, residues can be quantified at concentrations of micrograms per kilogram of food. A microgram is one millionth of a gram. A concentration of 1 microgram per kilogram corresponds to 0.001 mg/kg. The following equivalences illustrate the scale.

CONCENTRATION	EQUIVALENT
1 mg/kg	1,000 µg/kg

0.1 mg/kg	100 µg/kg
0.01 mg/kg	10 µg/kg
0.001 mg/kg	1 µg/kg

These concentrations are extremely small, yet suitable analytical techniques can determine them. Sensitivity, however, is not the same for all substances. A laboratory might attain an LOQ of 0.001 mg/kg for one pesticide and a higher value for another. The food matrix can also significantly affect method performance.

A pesticide that is comparatively easy to quantify in an aqueous extract may be harder to determine in a food rich in fat or pigments. A laboratory's ability to measure very low residues does not imply identical sensitivity for every substance and food. The limit of quantification must therefore be specified for the individual substance and the method applied to the matrix examined.

9.10 The matrix effect: when food interferes with measurement

One of the most important challenges in pesticide testing is the so-called matrix effect. This term describes the influence that other food components can exert on the analytical response of the target substance. Foods contain numerous molecules that may be extracted alongside pesticides. These compounds may interfere with chromatographic separation or change the response of the mass spectrometer. In some cases, other substances suppress a pesticide's signal; in others, they enhance it. Unless properly corrected, the resulting concentration measurement may be inaccurate.

Laboratories use several techniques to address this problem. One approach involves preparing calibration solutions that reproduce the characteristics of the food matrix as closely as possible. Another uses internal standards: reference compounds added in known quantities to monitor or correct certain variations in the procedure. Isotopically labelled internal standards can be particularly useful when available. They consist of molecules that are chemically very similar to the target compounds but can be distinguished by the mass spectrometer.

Control procedures are selected and validated for the method concerned. The matrix effect explains why a procedure that performs well with apples cannot automatically be assumed to perform equally well with oil or flour without appropriate verification. Method validation must consider not only the pesticide but also the type of food in which it is being tested.

9.11 Recovery, precision and accuracy: assessing analytical quality

To demonstrate that a method is reliable, a laboratory must verify its performance. One key characteristic is recovery. During extraction and sample preparation, some of the pesticide may be lost. To assess this, the laboratory can add a known amount of a substance to a sample and determine how much is subsequently measured. If 100 micrograms of a pesticide are added and the analysis recovers 90, recovery is 90%.

That result is not necessarily unacceptable; it must be assessed under the applicable method criteria and guidelines. Another important characteristic is precision, which describes how closely repeated measurements agree under specified conditions. A method can produce very similar results while still showing a systematic error.

Accuracy must also be considered: the closeness of a result to a reference value, taking account of precision and possible systematic deviations. Selectivity, linearity of instrumental response and the stability of substances during preparation and analysis are also important.

The European SANTE guidelines establish criteria for method validation and quality control in pesticide residue analysis. The 2026 version includes, among other aspects, specific attention to variability arising from sample preparation and subdivision.

A trustworthy analytical certificate does not arise from a measurement alone, but from a set of checks demonstrating the quality of that measurement.

9.12 Measurement uncertainty

Every experimental measurement is associated with some uncertainty. This is true for measurements of length, mass, temperature and, of course, chemical concentrations. An analytical result should therefore not be interpreted as a mathematically exact number.

Suppose, for example, that a laboratory measures a pesticide concentration of 0.10 mg/kg. The result has an associated uncertainty depending on the method, sample preparation and analytical performance. If, purely for illustration, the expanded uncertainty were 20%, the result could be reported as 0.10 ± 0.02 mg/kg, together with the associated coverage level.

This does not mean that the true value has the same probability of occurring at every point in that interval. Rather, the laboratory reports an uncertainty interval established according to defined metrological principles.

Uncertainty is particularly important when a result is close to a regulatory limit. EU rules provide for the interpretation of official-control results with measurement uncertainty taken into account. In 2026, these procedures were updated by Regulation (EU) 2026/765.

Uncertainty is not a defect or evidence of poor quality. Declaring it means properly acknowledging the limitations of measurement and making the result scientifically interpretable.

9.13 What it means when a pesticide is not found

When a laboratory reports that a pesticide was not quantified, the meaning depends on the method. Suppose the limit of quantification is 0.01 mg/kg. A result reported as below 0.01 mg/kg means that the laboratory did not quantify the substance at or above that level. It does not necessarily mean that its concentration is zero. A smaller amount might be present. Depending on reporting procedures, it may also be necessary to distinguish a detected but non-quantifiable signal from one that was not detected.

The statement must therefore be interpreted using the analytical documentation. There is a second limitation: a negative result concerns only the substances actually tested for. If a protocol covers 500 pesticides and all results are below their respective LOQs, this does not demonstrate the absence of every other chemical substance. Nor does it establish the absence of all possible metabolites of the pesticides tested for if those metabolites were not included in the analyte list.

A scientifically accurate statement would therefore be: "In the sample analysed, no residues of the target substances were quantified at concentrations equal to or above their respective limits of quantification."

This is less striking than "zero pesticides", but much more precise. Above all, it tells consumers what was actually verified.

9.14 Targeted analysis versus the search for unknown substances

GC-MS/MS and LC-MS/MS techniques used in multiresidue protocols are generally configured to look for already known substances. The laboratory has a list of molecules and uses methods developed to identify and quantify them. This approach is called targeted analysis. It is extremely effective when we know which substances we want to find.

But what happens if food contains a molecule that is not covered by the method? In many cases, a standard targeted protocol cannot identify it or does not produce a result for that substance.

To broaden the scope of investigation, high-resolution mass spectrometry techniques such as LC-HRMS are also used. These instruments can enable extensive screening and retrospective searches of previously acquired data.

Different approaches can be distinguished. Suspect screening searches for substances whose presence is considered plausible, without necessarily having every reference standard available. Non-target analysis searches for chemical signals not previously assigned to a narrowly defined list of compounds.

These approaches can be particularly useful for identifying emerging contaminants or poorly studied transformation products. However, confident identification and reliable quantification of unknown molecules may require additional checks and suitable analytical standards.

The ability to detect an unknown substance does not mean a laboratory can automatically identify every molecule present in food. Even the most advanced techniques have limitations in sensitivity, separation and identification. Nevertheless, the search for unknown contaminants represents one of the most interesting frontiers in food analytical chemistry.

9.15 How much can we trust an analytical certificate?

A certificate of analysis can provide very important information, but not all certificates offer the same level of detail. Several elements should be checked to interpret it correctly.

First, the sample analysed must be identifiable, preferably with a clear link to its production batch. The substances tested for and their respective results must also be specified. The documentation should allow readers to identify the methods employed, measurement units and limits of quantification.

Laboratory competence is another important factor. Accreditation under ISO/IEC 17025 is one of the principal international means of demonstrating the competence of testing laboratories. However, it is important to check that the analyses of interest fall within the applicable scope of accreditation. An accredited laboratory is not automatically accredited for every substance, method or food matrix.

Sampling must also be considered. A certificate can document with great precision what was measured in the sample received, without necessarily establishing on its own that the sample correctly represents the entire batch.

The quality of analytical information depends on the entire chain: sampling, preparation, measurement, validation and interpretation. Together these elements make a testing protocol credible.

9.16 From regulatory compliance to analytical excellence

The European food safety system uses inspections, sampling and analyses to verify compliance with legislation and assess risks to the population. These activities are essential to consumer protection. Their purpose is not, however, to subject every individual food batch to an extensive programme covering every family of contaminants. Official analyses are selected according to regulatory priorities, risk assessments and monitoring programme objectives.

A voluntary protocol may adopt different criteria. It may choose to test for more substances, use particularly low limits of quantification and apply acceptance conditions more stringent than regulatory limits. For example, it may exclude a batch when one of the substances subject to a non-quantifiability criterion is measured at a concentration equal to or above the LOQ specified by the protocol.

This choice does not mean that every quantifiable residue constitutes a health hazard. It means adopting a quality criterion aimed at minimising the measurable presence of selected substances.

The distinction is substantial. A food may comply with legislation and nevertheless fail to meet a stricter voluntary protocol. That does not automatically make the legally compliant food dangerous. Instead, it makes a difference in analytical composition verifiable against declared criteria.

For the distinction to be meaningful, the protocol must be transparent. The substances tested for, the methods, the limits of quantification and the acceptance criteria must be available. Even an exceptionally thorough protocol has limits and cannot guarantee the universal absence of every contaminant. But it can provide consumers with additional knowledge beyond simple regulatory compliance.

Analytical quality does not come from declaring a food pure. It comes from demonstrating what was sought, what was measured and what the results were.

9.17 Current limitations of analytical chemistry and future prospects

The ability to analyse food has advanced enormously over recent decades. Increasingly sensitive instruments can test for a growing range of substances at concentrations that were previously difficult to measure.

Technological progress brings an important consequence: as testing capabilities improve, contamination that once remained unknown can become detectable. The absence of a measured signal in the past does not necessarily mean the substance was absent. It may simply mean it was not sought, or that available technology lacked sufficient sensitivity.

This applies to pesticides as well as many other types of contaminants. Better analytical techniques also enable researchers to investigate metabolites and degradation products that may have properties different from those of the parent substances. Studying these compounds is a major challenge.

Another prospect is closer integration of chemical analysis and toxicological assessment. Knowing that a substance is present is essential information. Understanding what that presence may mean for health requires additional knowledge of toxicity, dose and exposure. The two activities are complementary.

Analytical chemistry allows us to see further and further. Toxicology must help us understand the significance of what we can see. Both will continue to evolve.

Conclusion to Chapter 9

Modern pesticide analytical techniques allow many substances to be identified and quantified in foods with extraordinarily high sensitivity. The combination of chromatography and mass spectrometry is now one of the principal tools for residue monitoring. Multiresidue analyses can examine hundreds of substances, while dedicated methods can detect compounds that are not covered by routine protocols.

Analytical capabilities, however, are not unlimited. Results depend on how representative the sample is, which substances are sought, matrix characteristics, method sensitivity and the quality of laboratory procedures. A negative result does not necessarily mean the substance is absolutely absent. Likewise, a substance that was not tested for cannot be declared absent on the basis of that analysis.

These considerations do not diminish the value of chemical analysis. On the contrary, they help us understand its power and its limitations correctly. The difference between a food that merely complies with the law and one subjected to an extensive analytical protocol lies in the amount and quality of information available about its composition.

Regulatory compliance is an indispensable condition. Analytical knowledge provides an additional opportunity. It cannot eliminate every possible risk, but it can reveal what would normally remain unknown.

This possibility opens up a new perspective on food choices: relying not merely on generic declarations of quality, but on measurable, documented and verifiable information.

Science cannot promise to know everything a food contains. But it can enable us to know far more than we are accustomed to knowing today.

Scientific and regulatory references

European Commission — Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. SANTE/11312/2021, 2026 version.

https://food.ec.europa.eu/plants/pesticides/maximum-residue-levels/guidelines-maximum-residue-levels_en

Commission Implementing Regulation (EU) 2026/765 of 1 April 2026 — Sampling, analysis and interpretation of results in official pesticide residue controls.

https://eur-lex.europa.eu/eli/reg_impl/2026/765/oj

European Union Reference Laboratories for Pesticide Residues (EURL) — Analytical methods, quality assurance and validation procedures.

<https://www.eurl-pesticides.eu/>

EURL — Single Residue Methods, including methods for pesticides and metabolites not necessarily covered by multiresidue testing.

<https://www.eurl-pesticides.eu/docs/public/tmplArticle.asp?CntID=1302&LabID=200&Lang=EN>

QuEChERS — Method description and EN 15662 reference.

<https://www.quechers.eu/method>

Regulation (EC) No 396/2005 — Maximum residue levels in food and feed.

<https://eur-lex.europa.eu/legal-content/IT/TXT/?uri=CELEX:32005R0396>

ISO/IEC 17025 — General requirements for the competence of testing and calibration laboratories.