Taking a closer look.

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"Influence of sampling on the variation and validity of analytical results"

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1. Abstract

Sampling is a crucial step for the quality and representativeness of the analytical results. Any mistakes at this level have a significant impact on the quality of the final result in a negative way.
In order to get a representative sample of high quality, many aspects have to be considered, like f. ex.:

- selection of an appropriate sampling standard, depending on the type of material and the purpose of the sampling;
- only qualified persons must perform the sampling using adequate devices; complete and correct documentation of the sampling; impartiality of the person as a basic requirement;
- property and homogeneity of the lot (related to the growers behind, field etc.) must be given;
- avoidance of any negative influence (cross-contamination, inadequate temperature during storage and transport, too long storage time, etc.).

Besides technical aspects, the factor "time" has to be considered when results for the same lot are compared: As pesticide compounds typically degrade over time, a reduction of pesticide levels must be considered for later analyses.

As a conclusion, the reliability of the analytical result is highly depending on the application of best practices during sampling.

2. Introduction

Reliable analytical results (from the statistical and analytical points of view) are one of the basic tools and requirements for global food trade. Consequently, obviously differing results can lead to troubles and cause serious financial damage.

As discussed in the relana® position paper 19-03[1], differing results between two analyses of samples deriving from the same food population (field, batch, lot) might have several plausible causes, and only some are under control of the involved laboratories.

This relana® position paper focusses on the important step of sampling and its influence on the final analytical result. Additionally, recommendations for representative sampling are provided, in order to support the comparability of analytical results.

[1] To be published in July 2019

3. Why is the sampling step so important?

Analytical results are often one of the major aspects for important decisions in the world-wide food business and the official control of food and feed.

While validation procedures and analytical competence schemes focus on the analytical measurement, further important steps such as sampling, sample transport and sample preparation (choosing sample parts to be analysed, cutting, discarding kernels where appropriate etc., see relana® position paper 18-01) are often undervalued.

Nevertheless, the influence of the sampling step on the variation of the final analytical result is massive – more massive than one might expect. As figure 1 (general example of analytical uncertainties; uncertainties of sample clean up steps and instrumental analysis will differ depending on the applied techniques) presents, the influence on the result decreases from sampling across sample preparation to the analytical measurement. Any mistake made during sampling will always produce a questionable or even valueless result.

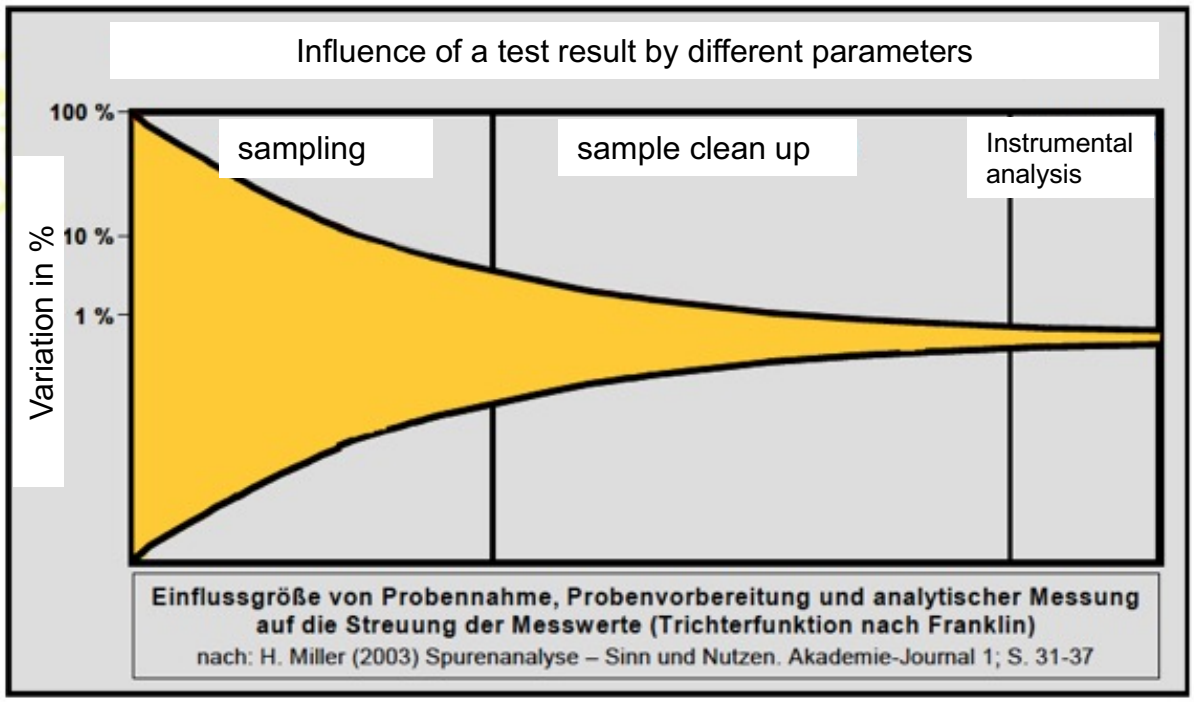

Figure 1. General influence of "sampling", "sample clean up" and "Instrumental analysis" on the variation of results

4. Parameters influencing the sampling

4.1. Choice of sampling procedure

Depending on the analyte and the aim of the sampling, an appropriate sampling procedure must be carefully chosen, if possible according to the latest technical and scientific findings.

For samples to be tested on pesticides, the EU directive 2002/63/EC sets the rules for official controls, but it is advisable to follow this directive for samples taken by food business operators as well.

Certain EU regulations relate to the sampling for the official control of **contaminants**, such as nitrate (reg. (EC) 1882/2006), mycotoxins (reg. (EC) 401/2006), and heavy metals (reg. (EC) 333/2007). For the sampling and analysis of **feeding stuff**, reg. (EC) 152/2009 applies. Consequently, it is important to choose an appropriate method for the planned sampling, in order to meet the relevant requirements and to achieve a meaningful analytical result.

4.2. Practical implementation of sampling scheme

Depending on the analyte and the overall sampling purpose, an adequate sampling scheme must be chosen:

- number and exact localisation of subsamples,
- scheme of sampling (z-scheme, start-middle-end, just one randomly picked sample etc.).

Nevertheless, external factors may limit the possibility to execute a representative sampling:

- available time (before loading, packing, processing etc.)
- technical possibilities (availability of sampling devices, appropriate sample bags, protection clothing when sampling in refrigerated or frozen environments etc.)
- lack of trained staff
- accessibility of the lot (fully-packed containers, pallets in high racks, sterilised vessels - which would become unsterile during sampling, freezer storage etc.)
- bad weather conditions when sampling outside (on the field, in the plantation etc.).

Generally speaking, an appropriate effort should be made to gain a representative sample. Any deviations from pre-settings should be carefully documented and justified in the sampling protocol and should be considered when evaluating the analytical results.

4. 3 Location of sampling

Sampling can be carried out at different locations across the entire food chain. The locations may vary depending on their accessibility, temperature, humidity etc.:

- on the field or in the plantation
- in packhouses (unpacked, packaged, uncooled, refrigerated or frozen)
- at retail level
- in big bags, containers, silos, etc.
- in transport vessels (trucks, tank trucks etc.).

Sampling may not be limited only to food and feed, but can also include water, soil, surfaces of machineries, inputs (fertilizers, plant protection products etc.), personnel (like hygiene checks, search for contamination sources).

4.4. Experience, impartiality, and training of sampling staff

Following official standards, samples must be taken by persons trained in sampling procedures. In some areas, sampling staff needs an authorisation, for example for drinking water or for official sampling empowerment.

As the number of possible mistakes during sampling is high and some standards have to be met, sampling should be carried out by **qualified staff** experienced in sampling in any case – not by someone who was “just around”.

Another relevant factor is the **impartiality of the sampling staff**, as he/she must be free from any doubt about the correctness of the sampling process which has to be done. Therefore, the production manager might not be the right person to carry out the sampling. In certain circumstance, it might be advisable to employ a third party for the sampling step, in order to ensure and stress impartiality of the sampling.

4.5. Purpose of sampling

The procedures fixed in official standards usually relate to the monitoring of residues and contaminants, testing if the lot meets legal requirements and specifications.

If the sampling is carried out for other purposes, it may be necessary to adjust the sampling scheme. Possible purposes:

- check if illegal pesticides have been used,
- check if the requirements of organic production are met (e.g. to identify fraud, commingling, etc.),
- check for contamination of soil with persistent pesticides (DDT, lindane, HCB etc.) or contaminants (dioxins, heavy metals etc.),
- check of microbiological quality,
- check for hotspots of a contamination,
- field studies to check for variability.

Depending on the purpose, the sampling scheme varies and might include not only food or feed, but also packing material, plants, soil, fertilisers etc. (see 4.2.).

4.6. Date of sampling: Time matters

As the most analytes are not stable in nature and also not in their concentration levels over time, the date of sampling is of high importance.
Due to the time gaps between samplings, pesticide levels can change significantly.
A certain degradation/disappearance, whose extent depends on the properties of the analyte and the environment, is typical and must be expected. As a consequence, products arriving after a long travel will show different (for the most part lower) pesticide levels than in the place of origin (earlier sampling). The speed of degradation depends on the molecule's properties, the conservation conditions (temperture, humidity, illumination, ...) and the product properties (water content, acidity, sugar content, enzyme presence, …).

4.7. Homogeneity

4.7.1. Definition of “lot”

Sampling procedures are usually designed to gain a representative sample, i.e. representing a well-defined property of the entire lot.
Nevertheless, it must be questioned if the “lot” always meets the definition of a “lot” (Reg. (EC) 401/2006 on sampling procedures for mycotoxins; see also Art. 1 (2) Directive 2011/91/EU):
“‘lot’ means an identifiable quantity of a food commodity delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor or markings;”

In practice, loads designated to form one lot may vary concerning:

- variety,
- growers and/or vendors (especially in case of cooperatives consisting of a high number of small enterprises),
- different fields or plantations,
- applied pesticides,
- the pesticides are likely to be distributed unequally across the lot, especially non-systemic ones.

This leads to more or less variability within one “lot”.

Fig. 2 shows a pesticide spray application in an apple plantation. Apples hanging on the outer branches close to the tractor are likely to receive a higher pesticide load than those ones hanging close to the stem or on the opposite side of the crown. In toxicological evaluations, this topic is covered by the application of variability factors gained by supervised field trials.

⇒ **In real-life lots, inhomogeneities are the rule, not the exception.**

Figure 2. Pesticide application in plantation

4.7.2. Homogeneity of samples

The degree of homogeneity of relevant properties in food and feed stuff and the samples derived from them can differ distinctly.

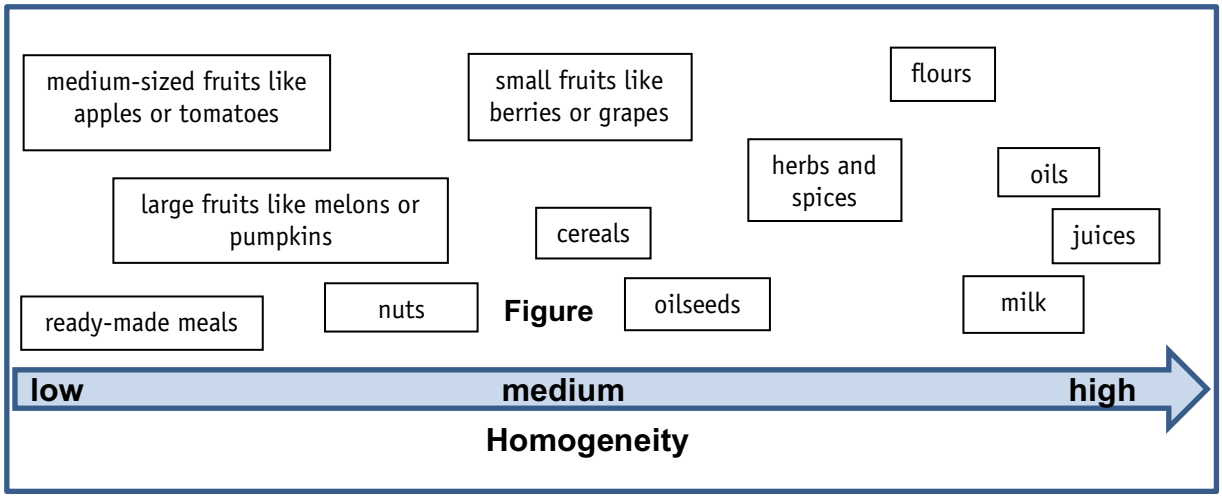

Figure 3. Rough classification of product according to their homogeneity of relevant properties

Generally speaking, samples are more homogeneous the lower the size of the population units. Liquids are in general much more homogenous than solids.
Please note that some major exceptions apply: For example, mycotoxins in nuts are usually not distributed equally, as the toxins are produced by fungi growing in so-called nests, resulting in so called “hot-spot contaminations”.

At this step it should be noted that the mass reduction during sampling, sample preparation and analysis is massive:

Lot: 20 metric tons
Lab sample: 1 kg
Weighted sample: 5 g (until this step: Factor 4 million)
Amount of sample injected in analytical system: 0,5 mg or even less

In the end, only 0,5 mg of sample represents 20 tons of material. This high reduction rate underlines the importance of high homogeneity of the material and a high-quality sampling in order to achieve a representative result.

5. Shortcomings during sampling

Beside the described unavoidable deviations occurring “by nature”, several sources for shortcomings and contaminations can devaluate the sampling carried out.

5.1. Technical mistakes

- sampled quantity too small
- number of single samples too small
- samples taken at one spot only
- lacking sampling devices (all-layer samplers, adequate sample bags etc.)

5.2. Contamination of samples due to carry-over from sampling staff

Staff carrying out the sampling can be a cause for cross-contamination, for example:

- carry-over of repellents against mosquitos, containing DEET or Icaridin (fig. 4)
- contamination with nicotine (smoking, chewing tobacco)
- contamination from cosmetics (MOSH/MOAH, 2-phenylphenol etc.)
- contamination with surface treatments, such as imazalil, thiabendazole or 2-phenylphenol, after consuming treated citrus fruits, mangoes etc.
- use of lab gloves or other single-use gloves which contain Sulfur compounds that lead to false positive dithiocarbamates (DTC) results or presence of other unsuitable chemicals

Figure 4. Application of a mosquito repellent as a source of an external contamination

5.3. Storage conditions after sampling / during transport

Samples must be stored immediately after sampling (also in the car of the sampling person) by applying appropriate conditions, not only considering the aspect of contamination (see point 5.3.).

The samples should be stored preferably cooled (f. ex. stored in a cooled box with thermal packs during transport). Usually, storage at room temperature is not suitable, as the degradation of pesticides might be much faster than under cooled conditions.

Furthermore, samples should be stored outside the influence of light, especially from sun light (UV) by means of an appropriate storage room or sampling bags.

5.4. Contamination during storage of the sample

A careful look at the storage conditions is of high importance, as many sources for cross-contamination may become visible:

- contamination with repellents, when applied indoors or when persons touching samples have used repellents prior to the sample contact (see 5.1.)
- use of insecticides and other biocides (for example, pyrethroids, pyrethrins, organochlorine pesticides, etc.) against bugs, moths, cockroaches etc.
- cross-contaminations with pesticides, surface treatments, disinfectants (quarternary ammonium compounds, chlorate derived from hypochlorite) via water, surfaces (like conveyor belts) etc.
- contamination with PCP, PCBs, lead etc. from building material, paintings, etc.
- contamination with plastic additives, monomers and polymers (phthalates, POSH, etc.) from surfaces.

5.5. Choice of sample packaging

The choice of sample packaging being inert to the compounds to be analysed later on, and matching the properties of the sample is of high importance.

Typical troubles include

- contamination with plastic additives, monomers and polymers (phthalates, POSH, etc.) from sampling bags,
- contamination with anthraquinone from paper bags (should be of less relevance by now),
- contamination of sampling bags/vessels with biocides, as they might have been stored in a location, which was treated with biocides before,
- use of glass vessels for pesticide analyses (some compounds stick to glass surfaces),
- improper sealing.

5.6. Cutting of large samples

Large units such as pumpkins, water melons or cabbage are not easy to sample and transport, especially when considering that 5 units are required (Dir. 2002/63/EC) to make up one laboratory sample. The idea to cut samples and send only parts to the lab is self-evident, but the cited regulation indicates:

"Individual eggs, fresh fruit or vegetables must not be cut or broken to produce units." (Annex No. 3 of Dir. 2002/63)

If units are cut, some unfavourable processes may start, which can change the pesticide content, such as:

- degradation of sensitive analytes, like dithiocarbamates
- enzymatic degradation
- microbiological growth, influencing the pesticides content
- loss of water.

5.7. Missing, incomplete or erroneous documentation

If the sampling protocol lacks important information (description of the product, batch number, date, temperature (where necessary) etc.), or the information contains errors (such as mixed-up numbers), the sample and the related analytical results can become useless.

6. Influence of sampling on the comparability of results

Most types of ring tests are carried out by making use of homogenised test material, thereby not covering the important step of sampling, sample transport and sample pre-preparation (choice of parts, cutting. grinding etc.).
Ring tests carried out on real samples show an increased variability, and it can be shown that analytical results vary to a high degree when the sampled lot is inhomogeneous, as it consists of fruits from different parts of a plantation, different growers, or similar (see figure 5 below).

Pesticide	PVT 85	PVT 86	PVT 87	PVT 88	PVT 89	PVT 90	PVT 91	PVT 92	PVT 93	PVT 94	PVT 95	PVT 96	max	min	Median	mixed sample
Pyrimethanil	0,002	0,002	0,000	0,000	0,002	0,001	0,003	0,001	0,001	0,001	0,003	0,002	**0,003**	**0,000**	**0,002**	**0,001**
Chlorthalonil	0,057	0,029	0,142	0,158	0,057	0,088	0,018	0,024	0,086	0,028	0,030	0,023	**0,158**	**0,018**	**0,062**	**0,088**
Chlorpyrifos-methyl	0,003	0,002	0,002	0,002	0,003	0,003	0,010	0,004	0,004	0,002	0,009	0,004	**0,010**	**0,002**	**0,004**	**0,003**
Cyprodinil	0,007	0,004	0,003	0,003	0,007	0,004	0,005	0,003	0,004	0,002	0,019	0,005	**0,019**	**0,002**	**0,006**	**0,004**
Mepanipyrim	0,016	0,024	0,011	0,011	0,009	0,013	0,000	0,034	0,002	0,009	0,008	0,011	**0,034**	**0,000**	**0,012**	**0,013**
Fludioxonil	0,007	0,002	0,012	0,010	0,015	0,007	0,006	0,000	0,005	0,007	0,035	0,012	**0,035**	**0,000**	**0,010**	**0,007**
Benalaxyl	0,000	0,002	0,001	0,003	0,002	0,001	0,002	0,001	0,001	0,000	0,000	0,000	**0,003**	**0,000**	**0,001**	**0,001**
Tebuconazol	0,000	0,000	0,003	0,000	0,000	0,000	0,000	0,000	0,007	0,000	0,000	0,000	**0,007**	**0,000**	**0,001**	**0,000**
Spiromesifen	0,000	0,005	0,000	0,000	0,000	0,000	0,000	0,007	0,000	0,000	0,000	0,001	**0,007**	**0,000**	**0,001**	**0,000**
Bifenthrin	0,003	0,003	0,006	0,003	0,001	0,002	0,006	0,002	0,008	0,001	0,006	0,003	**0,008**	**0,001**	**0,004**	**0,002**
Etofenprox	0,000	0,033	0,011	0,028	0,019	0,017	0,003	0,024	0,007	0,014	0,024	0,018	**0,033**	**0,000**	**0,017**	**0,017**
Difenoconazol	0,000	0,029	0,010	0,003	0,003	0,009	0,000	0,051	0,013	0,012	0,012	0,015	**0,051**	**0,000**	**0,013**	**0,009**

Figure 5. Variance of pesticide results (mg/kg) for single packages of tomatoes from one lot
(Data provided by Analytica Alimentaria GmbH, Almería, Spain)

In fig. 5, analytical results for single packages of tomatoes analysed for pesticides are presented, with “PVT 90” being the mixed sample. The table shows the large variety of pesticide concentrations, even when taking into consideration the analytic variance. For example, the concentration of difenoconazole ranges from “zero” (<0.001 mg/kg) up to 0.051 mg/kg.

Consequently, it is likely that variations between two pesticide analyses of the same lot appear, depending on the randomly chosen samples and considering the inhomogeneous distribution of some compounds.

6.1. Unit-to-unit variability

As described above, the unit-to-unit variability can be massive (although the sample origin is of the same lot). The influence of the variability on the overall analytical result of the analyses will be shown with the help of a fictitious example:

- One plum sample “A” is containing 10 plums with a total sample weight of 1 kg;
- A level of a particular pesticide at 1 mg/kg is measured (in this entire 1 kg).

To make sure that the dietary consumer risk is not underestimated by relying on residue data for composite samples, the analytical results are multiplied by a variability factor (according to the German Federal Institute for Risk Assessment, BfR). Usually, the default variability factor of 5 or 7, respectively, is used (depending on the unit weight). In the case of plums, a variability factor of 5 is recommended. Taking this factor into account and calculating a worst-case scenario, one plum out of the ten plums of the entire sample might have a pesticide level of 5 mg/kg, while the other 9 plums are below 1 mg/kg.

If a new sample with 11 plums of 1,1 kg weight also contains one plum with such a high concentration (5 mg/kg), whereas the other 10 plums contain 1 mg/kg, the mean level of the entire sample would be calculated to (1 mg/kg × 10/11) + (5 mg/kg × 1/11) = 1,36 mg/kg in 1,1 kg sample material. As a conclusion, having that one plum in or out of the laboratory sample makes a difference of 36%.

As a consequence, the variability on the result caused by unit-to-unit variability can be huge, even if the correct sample quantity is provided.

7. Conclusion

Sampling, sample storage and sample transport are crucial steps, which must be carried out with diligence and accuracy in order to avoid that the results of the subsequent analysis becomes useless for the evaluation of the corresponding population (field, lot, etc.).

Prior to the sampling, the following points should be considered:

- aim of the sampling (monitoring, risk assessment etc.)
- kind of sample (food, feed, water, soil, fertilisers etc.)
- location of sampling (field, river, silo, pallets, etc.),
- precise definition and traceability of the “lot”
- expected homogeneity of the lot (growers, product sizes etc.)
- accessibility of the lot (packaging, storage conditions, etc.)
- analytes to be tested (for example, pesticides, nitrate, microbiology)
- adequate resources and conditions (trained person, documentation, suitable sampling devices, sample vessels etc.)
- thorough look at possible sources for cross-contamination: gloves, repellents etc.

In other words: The unexpected must be expected, considering obstacles during sampling and possible sources for cross-contamination. Only a doubtless sampling is a correct sampling.

Literature

Named legal regulations in current version as valid on December 3rd, 2018, especially:
Commission Directive 2002/63/EC of 11 July 2002 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, OJ L 187 dated 16.7.2002, p. 30
[1] University of Hertfordshire: PPDB – Pesticides Properties Database, available online: https://sitem.herts.ac.uk/aeru/ppdb/ (accessed on November 16th, 2018)

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