QUALITY ASSURANCE IN PESTICIDE RESIDUE ANALYSES
with emphasis on Codex guidelines

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INTRODUCTION

In the last 10-15 years the aspect of quality has become more and more important, not only when producing something but also when delivering services, or delivering information, and that is what the analytical laboratory is doing. Buyers and users had, in the time, more and more expectations with respect of the quality of the delivered product and service. To maintain or to improve their position in the market, with an increasing competition, providers of products and services became more and more conscious of this situation and behaved accordingly. Also analytical laboratories are increasingly being confronted with quality requirements.

Quality Assurance (QA), Quality Control (QC), Good Laboratory Practice (GLP), Laboratory Accreditation are terms very often met in European and American literature. It is a major issue in laboratories involved in regulatory investigations, like Food Inspection Services and private contract laboratories. Recently the WHO/FAO Codex Alimentarius Commission published an updated document entitled: «Codex Guidelines on Good Practice in Pesticide Residue Analysis».

Quality assurance: activities to provide a degree of confidence that QC systems are utilized in a manner to ensure work products are of the highest quality.

Quality control: combination of systems, procedures, instructions and activities that are performed to control and maintain work quality. Quality control is what analysts do to insure their results are correct.

Quality assurance is what the organisation does to insure that the analyst is correctly carrying out the quality control procedures. Quality Assurance programmes or systems includes both QA and QC. There is a difference in the meaning of QA/QC systems on the one hand and GLP on the other hand.

GLP is a retrospective quality approving system: This means that GLP compliance confirms the performance of the work after inspection, proving then that a quality job has been performed. *QA/QC* systems are prospective quality approving systems. They confirm competence after inspection. The laboratory is competent to do a quality job.

In arbitrage situations, for instance, one can wonder if a QA/QC system alone is enough, or that afterwards the quality of the product or service should be confirmed according to GLP

guidelines. *Laboratory Accreditation* is the formal recognition of a laboratory by an independent science-based organisation that the laboratory is competent to perform specific tests. Laboratory accreditation assures customers that a laboratory has well trained, competent personnel, well maintained instruments, and standard operating procedures that will produce consistent, accurate data.

Quality is a relative notion. It is in an absolute sense never high or low, but rather adequate or inadequate in terms of the extent to which an analytical result meets the requirements specified beforehand, dedicated by an objective or a customer. The availability of an operational QA/QC or GLP system satisfies customers in advance, as the chemical information that will be produced meets certain minimum quality requirements. It also forms a good basis for continuous improvement of the quality of your laboratory work.

STANDARDIZATION

It will be clear that, when many persons and laboratories are involved with QA/QC, at all kind of levels standardisation is important and has resulted in may documents. In several of these documents, like EN45001 or ISO/IEC Guide 25, general principles are established, but each document has its own problems that require special consideration and treatment. In the Netherlands recently a report has been presented in which the results of a comparison of several quality assurance systems, as used in the Netherlands, are reported. All these systems are based on standards formulated by CEN (Comité Europeén de Normalisation); ISO (International Organisation for standardisation); OECD (Organisation for Economic Cooperation and Development), but also include FDA (Food & Drug Administration) and EPA (Environmental Protection Agency) Quality assurance systems.

No real differences between EN 45001 and ISO/IEC Guide 25 do exist. However the Guide is more clearly formulated. The Guide asks for detailed description of applied methods, including statistical analysis of the results, asks for characterisation of calibration procedures. EN 45001 is primarily meant for measurements of a recurring nature; additional rules are required for non recurring measurements (more or less unique research) and are given in the Dutch NEN 3417 and together these documents meet almost all items mentioned in the OECD-GLP principles. ISO 9002 for quality systems describes requirements for a laboratory in a larger company or organisation with only an internal control function and therefore only reporting, internally.

One of the conclusions was that several existing GLP protocals only differ in minor details and that GLP on the one hand and that EN 45001 complemented by requirements for R & D laboratories (such as the Dutch standard NEN 3417) agree on almost all items!. That being so, the only difference between GLP and a QA/QC system will be the moment of inspection: retrospective or prospective.

Another conclusion of the Dutch comparison was that existing differences between several quality assurance systems were not insurmountable to come to one consistent worldwide system. The future will learn in how far this is realistic and attainable, as the situation is rather complex. Many international expert groups are promoting quality assurance and improvement in analytical

chemistry such as: AOAC International, CEN/CENELEC, CITAC, EURACHEM, EUROLAB, EOTC, ILAC, OECD panel on GLP, NELAC, WECC, WEA

ELEMENTS FOR QA/QC

The tools for a successful quality assurance system are given by the so called «M factors»: Menpower (staff and technicians), Method (analytical procedures), Machines (equipment and reagents), Materials (sample, reference materials, and standards) and last but not least Manipulation (data handling and interpretation). These M factors are of course also interrelated.

To control these «M factors» a Quality Assurance Committee or Quality Assurance Unit should be established, chaired by the Quality Assurance Coordinator. The Quality Assurance Unit is composed of representatives from the supervisory and scientific staff. This is important as it seems to reduce staff resistance and negativism toward the concept of a mandated quality assurance program. A main task of this Unit is to prepare the manual for the Quality Assurance Programme and to revise the manual in the future. It must be clear that the chairman of this unit, but also the unit self must operate independent of the laboratory management; it should conduct full quality assurance audits, evaluate procedures and report to a higher level of the management. Together this forms the basis of Quality Assurance. It is an activity of providing evidence that results obtained in an investigation are reliable through documentation of practices, conditions and controls in the laboratory.

Introduction of a QA system into an institute is not something that can be realized from one day into the other. It involves all persons working in the institute. Therefore it must be a careful planned proces. It is important that at least the chairman of the QA Unit, is educated and trained adequately for his task. A next step is the writing of Standard Operating Procedures, usually called SOPs. These SOPs form the laboratory's handbook and should be available to the staff. They cover the whole chain of events - sampling, transport, storage, analysis, use or maintenance of equipment, safety and quality control, calibration, reporting of results and so on and so on. Lets consider now the five «M» factors.

1) Menpower (staff and technicians)

Even when from the theoretical point the quality system is as good as possible, the efficiency is for a major part related to the person who should carry out the activities. The technician shall at the end deliver the quality at the laboratory bench!! Motivation of the person is the keyword.

It is very important that coworkers have been involved from the start when incorporating a quality system in the institute. It is important that the QA manager or QA committee introduces planning in such a way that the operating personnel immediately will see the advantages. Of course the quality system must be checked regularly on efficientness and progress. It is important to write down conditions and recommendations and to make them available to whom it concerns. It is very important for the person to know where and how he or she fits into the

organisation. An organisational structure chart and a job description, including duties and responsibilities of the person, are important in this respect.

Of course the person must be experienced and have certain skills before he can do the job. In case that somebody has no relevant experience, the aspect of training and education is of utmost importance. But education and training should be an on-going activity for all grades of staff, and should take account on both short-term and on the long term needs.

2) Machines (equipment and reagents)

Measuring equipment like GCs, HPLCs, spectrophotometers, pH meters, pipettes, balances etc, must be serviced and their performance checked regularly and a record of all services/ repairs must be maintained for every such item of equipment.

Calibration is essential for equipment used for performing measurements. Balances, performing absolute measurements, must be recalibrated regularly and again records should be kept. Also processing equipment like macerators, centrifuges, ovens, furnaces, refrigerators etc. need some degree of maintenance and record keeping.

All reagents must be clearly labelled with an expiry date and stored under proper conditions. Of course reagents and equipment should not interfere with the final determination (blank should be zero). Of course the used equipment must be adequit, able to perform their task, only used for procedures for which it is suitable.

3) Materials (sample, reference materials, standards)

Pre-knowledge on the sample is in the pesticide residue lab in general not available. Samples are very often taken at random. There is no special reason, that this particular sample, is taken. In environmental samples (soil, sludge etc.) this can be a different situation very often. When in the recent past many samples from the same location have been analysed, the lab is completely informed. When sampling is not carried out by the lab itself, but through the customer, then the customer himself is responsible for a correct sampling. Responsibility of the laboratory starts in that situation at the moment of receipt of the sample.

In the EU and in the CCPR sampling protocols are available for all kind of foods. In the CCPR last year an updated sampling protocol was proposed, and that protocol will be finalised in next years CCPR meeting. It contains also all definitions and procedures to come to sub samples and the analytical portion etc. The value of this document lies also in the fact that it tries to unite and to uniform and standardize all other sampling protocols as developped through IUPAC, IDF, ISO, Code Committee on Methods of Analysis and Sampling etc.

The laboratory should check that samples are transported in clean containers and robust packaging. Samples of prepacked commodities should not have been removed from their original packing. Fresh meat, fresh fish and other perishable animal products should be transported in a chilled state, or better, in a frozen state. Samples must be identified clearly and logged in as soon as possible on receipt and get a unique identification code. Sub samples must also be

solution is added. The result of the recovery should be between 80 - 110%, but the question is what this result exactly means. Finding a good recovery of a spiked sample does only prove that the manipulations and techniques used after the addition step seem to be O.K. It does not say anything on the extraction efficiency. The addition of a spike solution does not resemble the way of binding to the matrix as an incurred pesticide residue does!. When the recovery is too low, one knows that first the extraction, clean up and/or determination steps should be improved.

A Reference Material is a material or substance of which one or more properties are well established for it, to be used for calibration of an apparatus, the assessment of a measurement method. It is a Certified Reference Material when the properties values are certified by a

technical valid procedure and when the material is accompanied by, or traceable to, a certificate or other documentation issued by a certifying body. In the USA reference materials are sold by NIST (National Institute for Standards and Technology). In Europe the Standard Measurements and Testing Programme (the former BCR) is a producer of materials. They are sold by Promochem in Germany.

4) Methods

Methods have to be chosen on the basis of their performance in relation to the agreed requirements. The most important technical ones are:

Scope	The range of matrices to which the performance characteristics apply.
Specificity	The extent to which other (known) substances may give rise to an interfering signal.
Limit of Detection (LOD)	May be defined as the minimum concentration of a pesticide in the matrix that just can be qualitatively detected, but not quantitatively determined. Very often the LOD = 3 times the noise.
Limit of Quantification (LOQ)	Also named Limit of Determination in CCPR documents, is the minimum concentration of the pesticide in the matrix that can be determined quantitatively. Very often the LOQ = 10 times the noise.
Accuracy	The lack of systematic error, how near the mean results from several determinations would be to the true value.
Precision	The closeness of agreement between repeated independent determinations on the same test material.
Sensitivity	The change in response per unit change in concentration.
Practicality	Usage range, relevance.
Reliability	Ruggedness, relative non-dependence on operator skill.

Other characteristics which may need to be considered are simplicity, speed and cost. The methods must be described in a SOP, a standard operating system.

Methods can have a different status, such as: Official methods for reference purposes; Official methods for routine analyses; Routine in-house-methods and Screening methods. Both of the official methods are collaboratively tested and of course accepted. The method for reference purposes is less a practical method, in contrary to the routine method. Maybe the official routine method is not giving the same high quality of performance characteristics as the reference method but they are quicker, less expensive. The in-house methods have been developed by the lab themselves, based on literature information or own inventions and, of course, it is very often thought that this is the best method in the world. Very often they are not

collaboratively tested and accepted. Sometimes they are tested with 2 or 3 other labs and gave acceptable results. Especially results obtained with certified reference samples can give an idea how good the method is. Results obtained in proficiency testing also are very useful. Nevertheless there must be available a validation report on that method wherein is described all tests and results that led to the conclusion that the method is good enough for the job.

Screening methods are very often based on principles not used in official methods. Think about Elisa tests. Here a rapid answer with a relative simple and practical test procedure is obtained. They can be used in GO/NOGO situations and that is very often our type of work.

To realise an acceptable level of the results of our analysis many technical possibilities are available and should be used.

Analytical blanks

This seems self evident! However in practice the question is how often these determinations are carried out. Blank values are (in general) always zero, so why perform them? It is not responsible to omit these determination. Each series of determination must include a blank determination, as it tells something on the quality of used chemicals, glassware etc., but also on the laboratory environment. Working with standards and standards solution, or samples with high residues can contaminate glassware, syringes etc. There is a big chance for cross contamination.

It has happened that paints used in the building or in the laboratory itself contained pesticides to combat insects in that laboratory, or that pentachlorophenol was used for wood preservation. Also known is the contamination of the lab through electrical equipment, like condensators from TL illumination. Chlorinated solvents can be present in the air of the laboratory and influence the analysis.

Repeated analysis: simplo/duplo

This is a controversial topic. It is dependent on the goals of the work. A Food Inspection Service is interested in GO/NOGO decisions, a single analysis should be enough. In general one expects with unknown samples, pesticide concentration below MRLs. Analysis are carried out conform QA/QC principles, repeatability is known! Everything is under control. As long as the result in the unknown sample is clearly below MRL, there is no reason to analyse in duplo. If you decide to choose for duplo analysis (institute policy, results close to or above MRL, suspected samples) you have several options:

-Repeated analysis by same technician after some time
-Repeated sampling, and analysis in simplo by one technician
-Repeated analysis of same sample by two technicians
-Etc. (different methods of analysis, contra expertise in a different laboratory.

The first possibility is very often used, but questionable. The duplo is not independent, it gives only information on random errors and not on systematic errors. On the other hand big errors can be detected. Think of cross contamination, serious losses during concentrating/clean up procedures. (see later). The most powerful (and most expensive) method is the analysis of different subsamples by two technicians on two different times using different equipment and different standard solutions! This technique will be used when economical interest is high.

Important human mistakes in residues analysis can be detected by using one or more internal standards. It is of course necessary that known amounts of this or these compounds are added to the sample or, less recommendable, to the extract. When at the end the results for the internal standards is as expected, it indicates that no mistakes have been made. It can also be a help to determine if homogenisation of the sample was in order, when you are analysing more subsamples. This technique of internal standards is only possible when the compound(s) do not interfere with the determination of other compounds. So in general internal standards can only be used in case of chromatographic determinations.

The use of internal standards offers some advantages. You can prove for yourself as technician and the customer that appropriate quality of the analysis is assured. Therefore lab management must enable the technician to prove for himself that he did a good job and that he can guarantee the results of all his analyses. Good results for control samples do only prove that that particular analysis was in order. Good results for internal standards in practice samples help assure quality of analysis!. The nicest method for the use of I.S. can be applied in the case that mass spectrometry, after GC separation is used as detection method. Labelled compounds (with 13 C), can be separated easily with the MS, while the retention time is very close or equal to to the native compound.

Standard addition

This is a technique not very often used in pesticide residue analysis. It is a well known technique in the determination of metals (or elements) with atomic adsorption. Here it is used to overcome influences of the matrix on the response of the detector which influence is not present in pure standard element solutions and therefore can cause wrong results. This phenomenon does also exist in pesticide residue analysis, but is very often not recognised. When a calibration curve of pure pesticide solutions is made and compared with the one obtained for the same pesticides in presence of matrix, very often rather big differences will be found!. These matrix effects should get enough attention in case of positive samples, recovery experiments with spiked blanks etc.

Also at the first European Pesticides Workshop two posters were presented concerning the influence of matrix extracts and effects on the detector response. In the one study a comparison was made for 15 N/P and 17 ECD compounds. Responses of pure standard solutions were compared with responses of standards dissolved in a cleaned matrix extract. In general it appeared that:

- response is enhanced due to the matrix
- there is some influence depending on the stationary phase (DB 5 & DB 1701)
- sometimes a strong increase in response (azinphos-methyl; dichlorofluanide) is obtained
- matrix effect decreases sometimes with compound concentration

The other study indicated no influence on the response of the compounds in relation to the number of pesticides in the solution. Also noted was an increased response in matrix extracts. Matrix effect is mostly independent of commodity. It is therefore strongly advised to use matrix standards!

Recovery experiments

Spiking of samples with a pesticide standard mixture is a recovery experiment. Recovery should be between 80-110%. But also the use of internal standards (IS) is in fact a recovery experiment, under the condition that the IS is added to the sample as early as possible. When during the performance of the analysis an evaporation step is included, before or after a cleanup step, it is a good idea to use two compounds as internal standard, the one being rather volatile the other less volatile. In case of GC amenable pesticides the one component elutes at a rather short retention time, the other at the end of the chromatogram. When the ratio of the responses of the two compounds in the standard solution is equal to the ratio in the sample, no losses due to evaporation have occurred.

Second level control

When an institute has a quality system operating this means that a quality assurance group (QAG) or committee is installed. The idea of second level control is following: one of the tasks of the QAG can be, that they provide control samples with known concentration of pesticides to the laboratory for examination, without the performing technician knowing that this is a control sample. The aim of this control is to see how well the technician is working. The frequency of this exercise is of course much lower than the frequency of using control samples by the technician himself. Only when important deviations are obtained in comparison to the expected values, of course immediate action is obliged. However this type of control is different to realise without knowledge or suspicion of the technician. Maybe only in case of a big sample throughput, more or less of the same type as the control sample, this is realisable. Remember that this control test does not detect problems with standards, equipment, reagents and environmental factors.

Third level control

This is more important. Here is meant the participating in collaborative studies, proficiency tests or ringtests. At national levels, but also internationally, participation is possible but costs in general money. Results of this participation indicate where the lab stands with regard to other labs. It is perhaps the ultimate measure of the effectiveness of the laboratory's QA/QC system.

It should be realised that first and second level are not always conclusive. Only when certified reference materials are used a good indication can be obtained. Good repeatability of results of internal control samples can be, in an absolute sense, completely wrong!!

Confirmation

In case of a positive sample, with a pesticide exceeding the MRL there is a need to confirm the result. In the guidelines on GPRA (Good Practice in Pesticide Residue Analysis) from the Codex Alimentarius Commission (CAC) several confirmatory tests are mentioned. The GPRA understands under confirmation not only the confirmation of the identity but also the confirmation of the quantitative result. A first step is quantitative confirmation by repeating the analysis. Qualitative confirmation includes some very evident possibilities like the use of HPLC or TLC.

Derivatisation, especially the chemical reaction, knows many examples and applications. Physical reactions can be a photochemical alteration of a pesticide residue, to give one or more products with a reproducible chromatographic pattern. Under «other methods» the GPRA document refers to pesticides that are susceptible to degradation or transformation by enzymes. These reactions are very specific and generally consists of oxidation, hydrolysis or de-alkylation. The products possess different (gas)chromatographic characteristics.

Mass spectrometry is nowadays one of the first choices. For GC amenable pesticides the qualitative confirmation can be combined with the quantitative determination. The ion trap is the first choice and it is expected that also HPLC determinations with electrospray or atmospheric pressure interface in the near future are possible with ion trap detection.

Bio analytical techniques are also mentioned and should be understood as fungal spores bio assays, cholinesterase inhibition, immunological techniques.

5) Manipulations (data, handling and interpretation)

A useful tool in the QA programme is control charting. It is a graphical plot of test results with respect to time or sequence of measurements with limits drawn within which results are expected to lie. There are two types of control limits, a warning limit corresponding to ± 2 standard deviations from the mean and an action limit set at ± 3 standard deviation from the mean. Stewhart control charts are easy to construct, use and understand. Indication for interpreting these charts are:

- one point outside the 3 sd limit
- 2 or more consecutive points outside the 2 sd limit
- a series of 7 or more points above or below the mean
- an increasing or decreasing trend

REPORTING

When all tests and checks are in good order and within the criteria, calculation of the contents of the pesticide in the sample are carried out and expressed in mg/kg or µg/kg, not in ppm or ppb. Residue data should not be corrected for recovery or «blank» values. Especially when the result of the measurement can have a legal consequence: do not correct. You have never measured the corrected part. But correction for recovery is still a discussion point. In september 1996 at the AOAC International meeting in the USA a workshop will be held on this topic.

When replicate results are obtained, report their mean. Rejecting of values must be reported. Rules for rounding of data are given by CCPR. Interpretation of the data is not always the task of the institute or organisation. However Food Inspection Service should interpret the data, as in the Dutch situation, the service herself takes, or advises to take, measures. But it also can be the task and responsibility of the client.

Pesticide residues causing a recognisable GLC, or HPLC, peak below the LOQ may still be within the LOD though not within the LOQ. These findings are reported as «trace». Below that level residues whose peaks cannot be distinguished from the baseline are considered as «not detected». In reporting this, it is clear that the accompanying LOD are reported. When reporting «trace» it must be clarified what is meant.

Reporting to your customer should meet certain criteria and goes in writing or by electronic data transmission. This report must be clearly identified, page by page. It should contain at least: name and address of the laboratoria; name and address of the client; name of the person who is responsible for the work in the lab; period taken for carrying out the work; identification and description of the material; goal of the work; date of the report; the analytical procedure and of course the results.

Additional information should be: information on work that has been put out to other laboratories, including the names of these labs; names and functions of involved staff, technicians; kind and identification of means of research; date of material reception; description of sampling and sample treatment; raw data and manipulation of these; description of standard conditions and deviations; reference to the statistical method; accuracy, and how it was calculated; circumstances of storage the material and a declaration that the results only refer to that part of the material that has been analysed. Signature should be by the person, who has final responsibility for the various activities.

CCPR RECOMMENDATIONS

When the above items are compared with the compact CCPR recommendations with respect of Good Practice in Residue Analysis, than only the concept of Lower Practical Levels for the determination of pesticides is not mentioned. It is a CCPR definition. It has to do with the fact that labs are able to measure less and less residues due to improved techniques. On the other hand CCPR is only involved in measuring residues in samples moving in international trade. Of course one must use methods sensitive enough to establish and monitor against the MRL, but not necessarily be able to measure two orders of magnitude lower than the MRL. These methods are also very expensive. Therefore a lower practical level (LPL) to be determined in any sample was defined. When the MRL is set at the LOQ, the LPL is also set at that value.

MRL	LPL
5 mg/kg	0,5 mg/kg
0,5 - 5	0,1 - 0,5
0,05 - 0,5	0,02 - 0,1
less than 0,05	0,5 x MRL

CONCLUSION

QA/QC systems for use in the laboratory environment are based on internationally accepted documents, like EN 45001 and/or ISO/Guide 25. Worldwide many organisations are working on further standardisation of these documents and hope for one final general accepted document. This means on the other hand that for a specific analytical environment the institute itself must make a tailor made QA/QC system. The Codex Alimentarius Commission and in particular the Codex Committee on Pesticide Residues has recently updated a compact document titled: «Good Practice in Pesticide Residue Analysis», which, as the title indicates, is more appropriate for labs involved with pesticide residue analyses.

In this publication items of interest for pesticide residue analysts (taken from literature that contains of course more detailed information) have been combined and at the end compared with the CCPR document.

LITERATURE

General reading

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