codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00100 ROME Tel.: +39(06)57051 Telex: 625825-625853 FAO I E-mail: Codex@fao.org Facsimile: +39(06)5705.4593

Agenda Item 7(a) **CX/PR 99/9**

February 1999

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON PESTICIDE RESIDUES
Thirty-first Session
The Hague, The Netherlands, 12 - 17 April 1999

RECOMMENDATIONS FOR METHODS OF ANALYSIS AND SAMPLING

IN-HOUSE VALIDATION OF METHODS OF ANALYSIS FOR PESTICIDE RESIDUES

(Prepared by the United Kingdom)

1. The Codex Committee on Pesticide Residues at its 29th Session supported the proposal of the Codex Committee on Residues of Veterinary Drugs in Foods that the Commission request FAO and WHO to give consideration to convening an expert consultation on method validation for food control purposes in relation to the lack of validation data for methods of analysis for veterinary drug residues and, to an extent, pesticide residues. A Joint FAO/IAEA Expert Consultation on Validation of Analytical Methods for Food Control was held from 2-4 December 1997.

2. At its 30th session, the Committee noted that due to accreditation requirements, in-house validation had gained great importance. Regarding the Working Group's recommendation that a section on validation of methods in the Guidelines on Good Laboratory Practice in Pesticide Residue Analysis should be revised, it requested preparation of a discussion paper on in-house validation, taking account of the need for harmonization between the CCPR and other Codex Committees (ALINORM 99/24, para. 99). In-house validation was considered by the CCMAS at its 22nd Session (November 1998; CX/MAS 98/8, CX/MAS 98/8-Add.1 and paras 47-51 of ALINORM 99/23[1]). The approach proposed by the CCMAS may be broadly applicable to many analytical methods supporting Codex standards. However, in the context of pesticide residues analysis, the CCMAS approach does not address sample preparation procedures, it does not provide assessment criteria, and the requirements would be costly to implement.

3. Table 1 summarizes an alternative approach to validation, in which the procedures comprising a method can be addressed separately or in combination, as required. Of course, if a method does not involve certain procedures (for example, sample processing or recovery determination), they are not involved in the validation.

4. In-house validation of methods does not replace the need for routine analytical quality control (AQC), intended to check on-going performance. The requirements in Table 1 are based on the expectation that routine AQC data (a sub-set of method validation data) will be used to determine the validity of minor extensions of methods (for example, to analysis of new products within a group of commodities).

5. Certain procedures are difficult to validate and may have to be omitted from validation of the complete method. For example, validation of extraction may be impractical for analytes which rarely

---

[1] Appendix 1 of this paper. Appendix II contains an extract of the Report of the Joint FAO/IAEA Expert Consultation on Validation of Analytical Methods for Food Control (Whole report is available at the URL: http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/validate.pdf)

occur as measurable incurred residues. In these cases, the method may have to be used without full validation.

6. The proposed flexible approach places considerable responsibility upon the analyst or laboratory manager, in deciding whether additional method validation or performance validation is required. A prescriptive approach is not practical for pesticide residues analysis.

7. The proposed flexible approach places considerable responsibility upon the analyst or laboratory manager, in deciding whether additional method validation or performance validation is required. A prescriptive approach is not practical for pesticide residues analysis.

8. The Committee is invited to consider recommendations for in-house validation of analytical methods and to consider appropriate ways to incorporate them into the existing Guidelines on Good Laboratory Practice (*Codex Alimentarius*, Volume 2, section 4.2), specifically in the section on "Validation of Methods".

Table 1. Parameters to Be Tested and Criteria to Be Met for In-House Validation of Analytical Methods for Pesticide Residues

Procedure in method	Parameter to test and method of test	Analyte level(s)	Minimum number of replicate analyses required	Criteria to be met			Limits to validation ***(See notes 2 & 3)***
				Quantitative method	Semi-quantitative method	Screening method	
1.Sample processing	<u>Analyte stability and homogeneity.</u> Add analyte pre-processing and determine percentage remaining post-processing.	≈ 5 x LCL *(note 1)*.	≥5 replicate sub-samples from one sample of each representative commodity *(note 2)* post-processing.	Stability: no significant loss of analyte during processing (P = 0.05). Homogeneity: RSD ≤15% (not including analytical contribution, see 4.3).	Stability: no significant loss of analyte during processing (P = 0.05). Homogeneity: RSD ≤15% (not including analytical contribution, see 4.3).	Stability: analyte added at LCL remains detectable after processing. Homogeneity: no false negatives.	Stability data should be valid for any subsequent extraction, etc. Homogeneity data may be applicable to other analytes or commodities if they have similar physical properties.
2.Sample storage	<u>Analyte stability.</u> Determine analyte levels before, during and after storage for the maximum required time.	≈ 5 x LCL	≥5 replicates at each time point, including time zero. Time zero data may be obtained from 1, above, if the sample used is common to both series of experiments.	No significant loss of analyte during storage period (P = 0.05).	No significant loss of analyte during storage period (P = 0.05).	Analyte added at LCL remains detectable after storage period.	Storage data should be valid for any subsequent extraction, etc.
3.Extraction	<u>Extraction efficiency.</u> Analyse a reference material *(note 4)* containing incurred residues.	A readily measurable level, preferably between the LCL and the MRL.	≥5 replicates by the method under test and, for in-house reference materials *(note 4)*, ≥5 replicates by the alternative extraction procedure.	Mean from test procedure within 95% confidence intervals of the reference material, or of the alternative extraction procedure.	Mean from test procedure within 99% confidence intervals of the reference material, or of the alternative extraction procedure.	Analyte detected.	Extraction data should be valid for any subsequent clean-up or determination.

Note 1 LCL: the Lowest Calibrated Level adopted for the intended application. MRL: Maximum Residue Limit. The MRL and LCL may be similar where the MRL is set "at or about the limit of determination".

Note 2 Representative commodity: a commodity considered appropriate to represent other similar commodities (to limit the cost of method validation). The commodity may be chosen by the analyst or laboratory manager as being representative on the basis of water, fat, acid, solids content, etc., and/or biological relationships. Examples of limits to representative commodities are as follows:-

- validation data for wheat grain may be applicable to any grain or flour, but should not be taken to apply to bran or beer;
- validation data for chicken fat may be applicable to any animal fat, but should not be taken to apply to muscle, offal or eggs;
- validation data for a fresh product may be applicable to the corresponding cooked product, but the reverse may not apply;
- validation data for one brassica vegetable or citrus fruit, etc., may be applicable to similar products from the same broad group, but should not be taken to apply to all vegetables or fruit.

Use of procedures for analysis of "new" commodities requires some form of validation. The analyst or laboratory manager is responsible for deciding whether this can be achieved through routine performance validation or requires validation as described in the Table, but a cautious approach is advised.

Note 3 Representative analyte: an analyte which may be chosen by the analyst or laboratory manager as being representative of others, on the basis of similarity of its physical, chemical and biochemical characteristics. Extension of procedures to the determination of related analytes requires careful validation and cautious consideration. The analyst or laboratory manager is responsible for deciding whether this can be achieved through routine performance validation or requires validation as described in the Table, but it should be remembered that even closely related analytes may differ significantly in their behaviour.

Note 4 Reference material: a certified reference material or an in-house reference material containing incurred residues, for which the mean and confidence intervals of analyte concentration have been determined using an extraction procedure (or procedures) other than that under test. Alternative extraction procedures may involve a different solvent, etc., but must be at least as rigorous, in principle, as that under test. In-house reference materials may be prepared at the time of validating the procedure, from any suitable commodity containing appropriate incurred residues.

Table 1. Parameters to Be Tested and Criteria to Be Met for In-House Validation of Analytical Methods for Pesticide Residues

Procedure in method	Parameter to test and method of test	Analyte level(s)	Minimum number of replicate analyses required	Criteria to be met			Limits to validation ***(See notes 2 & 3)***
				Quantitative method	Semi-quantitative method	Screening method	
4.Clean-up and determination	4.1 Specificity. Check detected response is due to the analyte and that appropriate blanks are free of analyte. Identify response by mass spectrometry or most specific technique available.	LCL *(note 1)*.	Single check of analyte identity. Analyse ≥5 independent blank samples of each representative commodity. Where analyte-free samples are not available, analyse 5 reagent/procedural blanks.	Response measured shown to derive from the analyte. No response (≤ one-third x LCL) from matrix or reagent blanks.	Response measured shown to derive from the analyte. No response (≤ one-third x LCL) from matrix or reagent blanks.	Response measured derives from the analyte. Preferably no response (≤ LCL) from matrix or reagent blanks but a small proportion of "false positives" may be tolerated.	If the specificity achievable differs between the highest and lowest levels tested, appropriate "cut-off" levels should be identified.
	4.2 Calibration and range Determine the shape, practical range and repeatability of the calibration curve.	From the LCL to the required maximum.	≥3 replicates at ≥3 levels (≥5 levels for non-linear detection systems), before and after a series of "sample analyses", on a minimum of 2 occasions. Incorporate this test into any other test.	Sufficiently repeatable response, and closeness of fit of calibration line, to enable accuracy and precision criteria to be met.	Sufficiently repeatable response, and closeness of fit of calibration line, to enable accuracy and precision criteria to be met.	Sufficiently repeatable response, and closeness of fit of calibration line, to enable accuracy and precision criteria to be met.	Changes of volume (dilution or concentration) are acceptable if accuracy and precision of calibration are not affected.
	4.3 Accuracy and precision Determine recovery of added analyte.	≈ LCL and at the MRL.	≥5 replicates at each level for each analyte/representative commodity combination.	Recovery: mean 70-110% with RSD ≤10%. Reference materials: all results within 99% confidence intervals.	Recovery: either mean 50-120% with RSD ≤25%, or mean recovery 20-150% with RSD ≤10%. Reference materials: mean result within 99% confidence intervals.	Recovery: all detectable at LCL. Reference materials: analyte detected. No "false negatives".	Where recovery cannot be determined (for example in direct analysis of liquids, SPME or in some headspace methods), the precision criteria should be applied to the calibration data and the accuracy may be assumed to be 100%.
	4.4 Analyte stability in extracts and standard solutions Determine stability over an appropriate time period, comparing "old" with freshly prepared solutions.	≈ LCL and at the MRL.	≥5 replicates at each level and at each appropriate point in time (including time zero), for each solvent used for standard solutions and each extract of a representative commodity.	No significant (P = 0.05) change in analyte concentration.	No significant (P = 0.05) change in analyte concentration.	No significant (P = 0.05) change in analyte concentration in solvent only. Analyte added at LCL remains detectable in extracts.	Storage should include the maximum time likely to be required.

APPENDIX I

EXTRACT FROM ALINORM 99/23
(Report of the 22nd Session of the Codex Committee on Methods of Analysis and Sampling)

IN-HOUSE METHOD VALIDATION (Agenda Item 9) [2]

47. The Committee recalled that at its last Session it had considered a paper on establishing routine methods, which had been referred to it by the Codex Committee on Residues of Veterinary Drugs in Foods. The paper explained the difficulties encountered in the area of veterinary drug residue analysis in performing large scale method validation and finding appropriate validated methods. The Committee had proposed to initiate work on in-house method validation, which was approved by the Commission at its 22nd Session. The Delegations of the Netherlands and the United Kingdom had prepared a paper.

48. The Delegation of the Netherlands, in introducing the paper, stated that in the cases of analyses of food moving in trade, inter-laboratory recognition was important. However, where no collaboratively studied methods were available, an in-house method validation could be utilized. Among validation routes, it might be possible to utilize the following routes in an in-house validation scheme and then obtain an externally referenced method yielding acceptable results: (1) calibration using reference materials; and (2) comparison of results achieved with reference methods. It was further stated that an appropriate inter-laboratory study would give important information that might be extrapolated to other analytes and matrices using an in-house validation protocol. However, criteria to be established for such an in-house validation would be different from those for the normal method validation.

49. The Delegation of the United Kingdom reported that IUPAC had initiated work on the development of the *Harmonized Guidelines for the In-house Validation of Methods of Analysis* last year by the same working group that had finalized a number of protocols and guidelines such as those for collaborative studies and recovery factors. The text contained in Annex 1 of the referenced document was its first draft. He invited participants to comment on the IUPAC Guidelines. He also informed the Committee that there would be an FAO/IAEA/IUPAC Workshop on Method Validation scheduled to be held from 27-29 October 1999 in Budapest, where the Guidelines on In-House Validation would also be considered.

50. The Committee welcomed the paper. However, some delegations stressed that the paper did not and should not discourage performing collaborative studies. The Delegation of France informed the Committee that AFNOR VO3-110 containing an intra-laboratory validation protocol had just been revised and would be published and sent to IUPAC.

51. The Committee **decided** to request the Netherlands, together with France and the United States, to prepare a paper on the use of information from the proficiency testing studies for the elaboration of characteristics of in-house validated methods for consideration by the Committee at its next session. The Committee **agreed** that when the next draft of the Harmonized Guidelines became available, it would consider the text to determine if it would be appropriate to recommend it to the Commission for adoption by reference for Codex purposes.

---

[2] CX/MAS 98/9, CX/MAS 98/8-Add.1 (recommendations of the Joint FAO/IAEA Expert Consultation on Validation of Analytical Methods for Food Control (December 1997)), CRD 16 (comments from Argentina).

APPENDIX II

EXTRACT FROM REPORT "VALIDATION OF ANALYTICAL METHODS FOR FOOD CONTROL" PREPARED BY FAO/IAEA[3]

The following is reproduced from the above Report:

"6. ALTERNATIVE PROCEDURES FOR ESTIMATING PERFORMANCE CHARACTERISTICS

Introduction

The Consultation reviewed current procedures for assessing method validation criteria. It was noted that in general, it is required that before Codex methods are accepted, they are tested in inter-laboratory methods performance (collaborative) studies in order to obtain a reliable estimation of the performance characteristics of the method. Such studies should be designed, conducted and its results interpreted and reported in accordance with internationally recognized protocols or standards.

As noted above, the Consultation acknowledged that in recent years it has become evident that it is neither practical nor always possible to estimate the performance characteristics of methods for the analysis of residues of veterinary drugs or pesticides using internationally accepted protocols for inter-laboratory studies. The recently adopted increased requirements on internal and external laboratory quality control measures (12) adds an extra level of confidence to chemical measurements. This Consultation agreed that the increase in such confidence permits alternative approaches to method validation to be considered.

Alternative Procedures

The Consultation affirmed that the preferred validation procedure is a collaborative study carried out according to generally accepted international protocols. In those instances when for practical reasons that procedure is not feasible or suitable, a three or more laboratory validation protocol may be used as a second option. The third option would be a two laboratory validation protocol (e.g. similar to the AOAC International Peer Verified Method Protocol) and the final option would be a single laboratory validation protocol. With any of the alternative validation schemes, the Consultation strongly encourages that the validation work be conducted according to the five principles outlined below:

1. The laboratories carrying out the method validation operate under an appropriate, quality system based on internationally recognized principles.
2. The laboratories have in operation a periodic, independent, third party assessment mechanism of their quality system and validation work, carried out by, e.g. an accreditation agency, a GLP authority, or one or more collaborating laboratories. Alternatively, the laboratory carrying out the validation may submit the validation work for peer review to be assessed by an appropriate, professional organization. Such an independent assessment and review helps to ensure the transferability of the validated method from the originating laboratory to other laboratories.
3. The analytical method is assessed according to the criteria noted above, using the definitions that have been adopted by the Commission (3). In those instances where the CAC has not adopted a definition, the definitions given in part 4 of this report may be used.
4. The validation work should be carefully documented in a validation report in which it is unambiguously stated for which purposes (matrices and analyte levels) the method has been found to perform in a satisfactory manner.
5. Evidence of transferability.

The Consultation noted that those participants from developing countries found these alternative validation schemes, with the principles listed above, acceptable to their needs and their resources.

---

[3] For Conclusions and Recommendations see CX/MAS 98/8-Add.1.

7. CONCLUSIONS

The Consultation **CONCLUDED** that:

- There is a continuing need for analytical methods to be used in determining compliance with international standards and the identification of appropriate and reliable methods is an integral part of decision making in a risk analysis framework.
- The preferred means to validate an analytical chemical method used to determine compliance with Codex limits is a full collaborative study using internationally accepted protocols and in which all the participating laboratories operate under internationally accepted principles of quality assurance. However, due to decreasing resources available for such studies at the national level and other factors including but not limited to, insufficient numbers of qualified laboratories to participate and increased costs, full collaborative studies are less frequently undertaken.
- Due to the above conditions, some Codex Committees have developed criteria which vary in their rigour for the identification of methods which can be recommended for determining compliance with Codex standards.
- Chemical analytical methods used in veterinary drug residue depletion studies in target animals constitute a potential source of suitable methods for determining compliance of tissue residues with established MRLs. In some situations these methods may have been used in several laboratories conducting depletion studies in the same analyte/tissue combination. Similar considerations may be available for pesticide residue methods. Often, however, the information on these analytical methods may not have been studied or processed any further for their suitability as regulatory methods.
- There should be analytical chemical methods available to governments for use in determining if veterinary drug and pesticide residues or traces of food contaminants comply with MRLs or other requirements. With regard to developing countries, participants from those countries noted that the criteria for method validation proposed by the Consultation are suitable for their needs.
- The purpose of use of analytical chemical methods, such as screening, quantitation and confirmation, is an issue to be decided by national food control authorities.

8. RECOMMENDATIONS

The Consultation **RECOMMENDS** that:

1. All methods used for determining compliance with international or other standards which have not been subjected to a full collaborative study should be subject to a form of independent review, which may include a multi-laboratory study involving a smaller number of laboratories, second laboratory verification, validation in a laboratory operating under GLP or validation in a laboratory which has been recognized under ISO/IEC Guide 25, or equivalent.
2. Analytical methods for veterinary drug and pesticide residues should be selected on the basis of their suitability to determine compliance with MRLs.
3. The evaluation of methods should form an integral part of the evaluation of substances carried out by the JECFA and JMPR. These expert committees should also establish procedures to evaluate and recommend methods for the analysis of residues, for consideration by the competent Codex Committees as Codex methods. In doing so, the expert committees would be guided by the established procedures and stated needs of the Codex committees.
4. Sponsors or other parties submitting data for evaluation and determination of an MRL, should provide an expert report on analytical methods that are used in studies (e.g. drug residue depletion) including their performance characteristics. This would enable a review of these methods for their suitability for determining compliance with recommended MRLs.
5. In those cases where collaborative studies or other inter-laboratory studies are impractical or impossible to carry out, evaluations of analytical methods could be done in one laboratory, provided that the validation work is conducted according to the five principles discussed in the body of this report. In brief, these principles are:
    - Laboratories carrying out the validation studies operate under a suitable quality system based upon internationally recognized principles;
    - Laboratories have in operation a third party review of the whole validation process (e.g. GLP registration, accreditation according to ISO/IEC Guide 25, or Peer Review);
    - Analytical methods are assessed in respect to the Codex general criteria for selection of methods of analysis (3), with emphasis on the assessment of the limit of quantitation rather than the limit of detection.
    - The validation work be carefully documented in an expert validation report in which it is unambiguously stated for which purposes (matrices and analyte levels) the method has been found to perform in a satisfactory manner; and
    - Evidence of transferability be provided for all methods intended for Codex use for food control purposes.
6. The CAC consider some means to provide access to otherwise unpublished analytical methods which have been accepted for Codex purposes to determine compliance with residue MRLs. If possible, this should include performance characteristics data."