codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 5 **CX/MAS 01/6**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
Twenty-Third Session
Budapest, Hungary, 26 February - 2 March 2001

CONSIDERATION OF HARMONIZED GUIDELINES FOR THE USE OF RECOVERY INFORMATION ON ANALYTICAL MEASUREMENT

Background

The Committee on Methods of Analysis and Sampling has been considering the issue of recovery factors since its 19th Session and in particular the development of IUPAC Guidelines in this area. At the 22nd Session the Delegation of the United Kingdom presented a progress report on the IUPAC *Harmonized Guidelines for the Use of Recovery Information in Analytical Measurement*, to be published shortly afterwards. It was noted that differences between countries in the application of correction factors might lead to trade disputes. For example, the corrected and uncorrected results of an analysis of the same sample could indicate that the product analyzed was in conformity with the specification in one analysis report while not in conformity in another.

The Committee was generally of the view that there were differences in the use of recovery factors in the food analytical community and that it would be extremely difficult to reach consensus. There was an exchange of view on the use of recovery factors but no conclusion was reached at that stage. The Committee decided to postpone further discussion pending the publication of the IUPAC Harmonized Guidelines. When published, the Guidelines would be circulated for comments in a Circular Letter including pertinent elements of CRD 8 (presenting the position of the United States on this issue). The Committee would consider the published Guidelines and the comments received to decide whether the document should be adopted by reference (ALINORM 99/23, paras 32-35).

The main points raised by the United States in their comments were the following.
The United States pointed out that the document was being considered for the third time by the CCMAS and remained essentially unchanged although several critical issues had been raised which made the document impractical for use with foodstuffs. The premise that food laboratories wish to achieve “the best estimate of the true result” was questioned, as standards and specifications in many cases are not set up in terms of “the true result” defined as a specific chemical entity or group of distinct entities, but rather in terms of practical specifications.

As regards limits involving food safety (residues of pesticides and veterinary drugs) there are large uncertainties involved in establishing the limit, and the additional application of analytical correction factors would not be useful. Losses entailed in establishing the analytical limit were inherently included in the limit. In this respect, the Guidelines did not take into account the potential for double counting of correction factors and did not address the problem in a simple manner for Codex purposes.

There was a need for a guideline statement specifying that results should be reported in the same manner as the specification, limit or tolerance. If two countries with different requirements were involved a report in either system should be made together with the recovery factor used in the calculation.

The United States recommended that the CCMAS adopt a statement on recovery to the effect that :

> "With respect to specific chemical entities, results may be reported corrected or non corrected as stated or implied in the Codex standard. The report of analysis should give information as to use of a correction factor. If a correction is required or permitted, the calculation should be part of the method of analysis. If recovery is calculated and the directions are not present in the protocol, the report should state how the correction was derived."

The final version of the IUPAC *Harmonized Guidelines for the Use of Recovery Information in Analytical Measurement*, published since the last session of the CCMAS, is attached for consideration. The Delegation of the United Kingdom prepared a Progress Report introducing the final version of the Guidelines in order to facilitate the discussion in the Committee, as Dr. Roger Wood is one of the authors of the Technical Report on the IUPAC Guidelines. This is attached as Annex I and the Guidelines are attached as Annex II.

In conformity with the decision of the Committee, governments and international organizations are invited to consider the published IUPAC Guidelines, taking into account the proposals of the United States as presented above, and to submit comments especially on the action the Committee may wish to take in this respect. In particular, the Committee will decide whether it would be appropriate to recommend that the Commission adopt the Guidelines by reference for Codex purposes.

Governments and international organizations wishing to submit comments should do so in writing (if possible by Email) to the Secretary, Joint FAO/WHO Food Standards Programme, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy, with a copy to Dr. Mária Váradi, Central Food Research Institute (KÉKI), Herman Ottó út 15, H-1022 Budapest, Fax : +361 212 9853 or 361 355 8928, Email : m.varadi@mail.cfri.hu, **before 30 January 2000**

ANNEX I

PROGRESS REPORT ON THE DEVELOPMENT OF AN IUPAC/ISO/AOAC HARMONISED PROTOCOL FOR THE USE OF RECOVERY FACTORS

(Prepared by the United Kingdom)

Introduction

1. The Codex Committee on Methods of Analysis Sampling (CCMAS) has primarily been concerned with the development and endorsement of methods of analysis and sampling contained or attached to Codex Standards. However, there has been a change in the terms of reference of the Committee so that it is now also concerned with how well its methods are being used by the laboratory. This is demonstrated by the adoption by the Codex Alimentarius Commission in June 1997 of the quality standards for laboratories involved in the import/export of foodstuffs. In essence the laboratories must:

1 Use validated methods of analysis
2 Participate in proficiency testing schemes
3 Introduce appropriate quality control procedures
4 Become accredited to ISO/IEC Guide 25

2. These principles are all aimed to enabling laboratories achieve the "best estimate of the true result". To this end the Codex Alimentarius Commission has adopted by reference a number of protocols/guidelines prepared by the IUPAC Interdivisional Working Party for Harmonisation of Quality Assurance Schemes for Analytical Laboratories and the IUPAC/ISO/AOAC INTERNSTIONAL harmonisation programme. To date the CAC has adopted Protocols/Guidelines on collaborative studies[1], proficiency testing[2] and internal quality control[3]. The Interdivisional Working Party has recently adopted and published Guidelines on for the use of recovery information in analytical measurement[4].

3. These last Guidelines were discussed when in their draft form at the 22nd Session of CCMAS. It was then recognised that this was an area of concern to analysts. The application of recovery factors in analysis could lead to the reporting of different data by analysts on nominally the same batch. This was considered by CCMAS at its 22nd Session.

4. Some delegations considered the use of recovery factors when calculating results to be routine. Others pointed out that some methods, such as those for residues of pesticides (which are not a direct concern of CCMS within the Codex system), did not require correction for recovery. The need for recovery should be established as part of method development and validation. The method clearly needs to describe the recovery procedure as part of the method and not a separate protocol. The United States, in particular, prepared a conference room document (CRD 8) outlining their opinions, and gave reference to an article dealing with the issue[5] from their perspective.

5. Nevertheless the Committee recognised that the use of recovery information to correct/adjust analytical results is a contentious one for analytical chemists and that different sectors of analytical chemistry have different practices. Formal legislative requirements with regard to the use of recovery factors also varies sector-to-sector.

6. Although not explicitly stated, it is assumed by most delegates to Codex Commodity Committees that the specifications are to be set and enforced on a corrected basis rather than on an unknown, variable unrecovered basis.

HARMONISED GUIDELINES FOR THE USE OF RECOVERY INFORMATION IN ANALYTICAL MEASUREMENT

7. IUPAC has prepared general Guidelines which may be seen to aid the reporting of the "best estimate of the true result" and to contribute to the comparability of the analytical results reported. The Guidelines are freely available and are reproduced as Annex I to this paper. However, it is essential that the following points are noted from the Guidelines:

Arguments for Correction

8. The following arguments may be made for correcting results for recovery:

- The purpose of analytical science is to obtain an estimate of the true concentration of the native analyse with an uncertainty that is fit for purpose.
- The true concentration can be estimated only if significantly low recoveries of analyse are corrected.
- An uncorrected bias due to low recovery means that results will not be universally comparable, not transportable and therefore unfit to support mutual recognition.
- Methods of correction advocated are isomorphic with perfectly acceptable analytical techniques such as internal standardisation and isotope dilution and therefore not suspect in principle.
- Although some uncertainty is inevitably associated with correction factors, that uncertainty can be estimated and incorporated into a combined uncertainty for the final result.

Arguments against Correction

9. The following arguments may be made against correcting results for recovery:

- Estimated recoveries based on a surrogate may be higher than the corresponding value for the native analyte. The resultant corrected result would still have a negative bias.
- Estimated correction factors may be of doubtful applicability because they may vary among different matrices and for different concentrations of analyte.
- Estimated correction factors often have a high relative uncertainty, whereas uncorrected results usually have the smaller relative uncertainty associated with volumetric and instrumental measurement alone. (However, the uncertainty is small only if no contribution from the bias is included). Therefore corrected results will have a high relative uncertainty, sufficiently high if made explicit to create an unfavourable impression among those unfamiliar with the problems of analysis. This in turn might affect the credibility of science in the enforcement of legislation.
- Relatively small deviations from unity in correction factors could arise largely through random errors rather than a systematic loss of analyte. In that circumstance correction could make the absolute uncertainty of the result greater.
- Some legislation imposing maximum limits on contaminants is framed on the understanding that uncorrected results will be used for enforcement purposes.

RECOMMENDATIONS IN THE GUIDELINES

10. The following recommendations are made regarding the use of recovery information in these Guidelines: They have been endorsed by IUPAC, ISO and EUROCHEM. AOACI endorses the substance of the Guidelines but not the Recommendations.

1. In general results should be corrected for recovery, unless there are overriding reasons for not doing so. Such reasons would include the situation where a limit (statutory or contractual) has been established using uncorrected data, or where recoveries are close to unity. However, it is of over-riding importance that all data, when reported, should a) be clearly identified as to whether or not a recovery correction has been applied and b) if a recovery correction has been applied, the amount of the correction and the method by which it was derived should be included with the report. This will promote direct comparability of data sets. Thus, in all situations correction functions, should be established based on appropriate statistical considerations, documented, archived and available to the client.
2. Recovery values should always be established as part of method validation, whether or not recoveries are reported or results are corrected, so that measured values can be converted to corrected values and *vice versa*.
3. When the use of a recovery factor is justified, the method of calculation should be given in the method.
4. IQC control charts for recovery should be established during method validation and used in all routine analysis. Runs giving recovery values outside the control range should be considered for re-analysis in the context of acceptable variation, or the results reported as semi-quantitative.

CONCLUSIONS

11. Variable practice in handling information recovery is an important cause of the non-equivalence of data. To mitigate its effects the practice of reporting analytical data after the application of an appropriate correction factor should be normally encouraged. Where, however, an enforcement limit is based on data which has not had a correction factor applied, the present situation of reporting "raw" data will continue for the foreseeable future.

Detailed descriptions of recovery experiments and their results should be properly recorded. If it is known or suspected that a proportion of the native analyte in the test material is not extractable by the analytical procedure, the procedure must be qualified as determining only "available" analyte. Such qualification should be specified on analytical certificates. No valid compensation can be made, or should be attempted, for the "bound" analyte, which a recovery model does not represent.

It should be recognised that there is a dual role for recovery determinations in analytical measurement, that is, for (a) quality control purposes and (b) for deriving recovery values. In the latter application, more extensive and detailed data are required.

Recommendations to CCMAS

12. It is recommended that the Committee:

- Discusses the principle of requiring laboratories to correct analytical results for recovery for goods moving in international trade,

- Recommends that the Codex Alimentarius Commission endorses the use of the Guidelines for Codex purposes.

References

1. "Protocol for the Design, Conduct and Interpretation of Method Performance Studies", ed. W. Horwitz, *Pure Appl. Chem.*, 1988, **60,** 855-864, revised 1995 *Pure Appl. Chem.*, 1995, **67**, 331-343.

2. "The International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories", ed. M. Thompson and R. Wood, *Pure Appl. Chem.*, 1993, **65**, 2123-2144. (Also published in *J. AOAC International*, 1993, **76,** 926-940.

3. "Guidelines on Internal Quality Control in Analytical Chemistry Laboratories", ed. M. Thompson and R. Wood, *Pure Appl. Chem.*, 1995, **67,** 649-666.

4. Harmonised Guidelines For The Use Of Recovery Information In Analytical Measurement, Michael Thompson, Steven L R Ellison, Ales Fajgelj, Paul Willetts and Roger Wood, *Pure Appl. Chem.*, 1999, 71, 337 – 348

5. "Common Problems in Data Interpretations: Correction Factors", William Horwitz and Richard Albert, *Inside Laboratory Management*, 1997, July, 4 - 5.

ANNEX II

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ANALYTICAL, APPLIED, CLINICAL, INORGANIC AND PHYSICAL CHEMISTRY DIVISIONS INTERDIVISIONAL WORKING PARTY FOR HARMONIZATION OF QUALITY ASSURANCE SCHEMES FOR ANALYTICAL LABORATORIES*

HARMONISED GUIDELINES FOR THE USE OF RECOVERY INFORMATION IN ANALYTICAL MEASUREMENT

(Technical Report)

Resulting from the Symposium on Harmonisation of Quality Assurance Systems for Analytical Laboratories, Orlando, USA, 4-5 September 1996 held under the sponsorship of IUPAC, ISO and AOAC INTERNATIONAL

Prepared for publication by

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Chairman: M. Parkany,1995-97, (Switzerland); A. Fajgelj, 1997- , (IAEA, Austria); *Members*: T.B. Anglov (Denmark); K. Bergknut (Norway); K.G. Boroviczeny (Grmany); Carmen Camara (Spain); K. Camman (Germany); Jyette Molin Christensen (Denmark); S. Coates (AOAC Int., USA); W.P. Cofino (The Netherlands); P. De Bievre (Belgium); T.D. Geary (Australia); T. Gills (USA); A.J. Head (UK); J. Hlavay (Hungary); D.G. Holcombe (UK); P.T. Holland (New Zealand); W. Horwitz (USA); A. Kallner (Sweden); H. Klich (Germany); J. Kristiansen (Denmark); Helen Liddy (Australia); E.A. Maier (Belgium); H. Muntau (Italy); C. Nieto De Castro (Portugal); E. Olsen (Denmark); Nancy Palmer (USA); S.D. Rasberry (USA); M. Thompson (UK); M.J. Vernengo (Argentina); R. Wood (UK).

Harmonised guidelines for the use of recovery information in analytical measurement (Technical Report)

Synopsis. ISO, IUPAC and AOAC INTERNATIONAL have co-operated to produce agreed protocols or guidelines on the "Design, Conduct and Interpretation of Method Performance Studies" [1] on the "Proficiency Testing of (Chemical) Analytical Laboratories" [2] and on "Internal Quality Control in Analytical Chemistry Laboratories" [3]. The Working Group that produced these protocols/guidelines was asked to prepare guidelines on the use of recovery information in analytical measurement. Such guidelines would have to outline minimum recommendations to laboratories producing analytical data on the internal quality control procedures to be employed.

A draft of the guidelines was discussed at the Seventh International Symposium on the Harmonisation of Quality Assurance Systems in Chemical Laboratory, sponsored by IUPAC/ISO/AOAC INTERNATIONAL, held in Orlando, USA, 4-5 September 1996 . Proceedings from that Symposium are available [4].

The purpose of these guidelines is to outline the conceptual framework needed for considering those types of analysis where loss of analyte during the analytical procedure is inevitable. Certain questions cannot be satisfactorily addressed, and hence remain irreducibly complex, unless such a conceptual framework is established. The questions at issue involve (a) the validity of methods for estimating the recovery of the analyte from the matrix of the test material, and (b) whether the recovery estimate should be used to correct the raw data to produce the test result. The types of chemical analysis most affected by these considerations are those where an organic analyte is present at very low concentrations in a complex matrix.

CONTENTS

FOREWORD

It is recognised that the use of recovery information to correct/adjust analytical results is a contentious one for analytical chemists. Different sectors of analytical chemistry have different practices. Formal legislative requirements with regard to the use of recovery factors also vary sector-to-sector. It is the aim of IUPAC, however, to prepare general Guidelines which may be seen to aid the preparation of the "best estimate of the true result" and to contribute to the comparability of the analytical results reported.

This document attempts to give Guidelines that are intended to be general in their scope and give recommendations that reflect common practice best able to achieve the above. However, specific sectors of analytical chemistry will need to develop these Guidelines for their own requirements and the recommendations are not, therefore, to be seen as binding for all areas of analytical chemistry.

1. INTRODUCTION

The estimation and use of recovery is an area where practice differs among analytical chemists. The variations in practice are most obvious in the determination of analytes such as veterinary drug residues and pesticide residues in complex matrices, such as foodstuffs and in environmental analysis. Typically, such methods of analysis rely on transferring the analyte from the complex matrix into a much simpler solution that is used to present the analyte for instrumental determination. However, the transfer procedure results in loss of analyte. Quite commonly in such procedures a substantial proportion of the analyte remains in the matrix after extraction, so that the transfer is incomplete, and the subsequent measurement gives a value lower than the true concentration in the original test material. If no compensation for these losses is made, significantly discrepant results may be obtained by different laboratories. Even greater discrepancies arise if some laboratories compensate for losses and others do not.

Recovery studies are clearly an essential component of the validation and use of all analytical methods. It is important that all concerned with the production and interpretation of analytical results are aware of the problems and the basis on which the result is being reported. At present, however, there is no single well-defined approach to estimating, expressing and applying recovery information. The most important inconsistency in analytical practice concerns the correction of a raw measurement, which can (in principle) eliminate the low bias due to loss of analyte. The difficulties involved in reliably estimating the correction factor deter practitioners in some sectors of analysis from applying such corrections.

In the absence of consistent strategies for the estimation and use of recovery information, it is difficult to make valid comparisons between results produced in different laboratories or to verify the suitability of those data for the intended purpose. This lack of transparency can have important consequences in the interpretation of data. For example in the context of enforcement analysis, the difference between applying or not applying a correction factor to analytical data can mean respectively that a legislative limit is exceeded or that a result is in compliance with the limit. Thus, where an estimate of the *true concentration* is required, there is a compelling case for compensation for losses in the calculation of reported analytical result.

These Guidelines provide a conceptual framework for consistent decisions on the estimation and use of recovery information in the various sectors of analytical science.

2. DEFINITIONS AND TERMINOLOGY USED IN THE GUIDELINES

General analytical terminology is assumed to be accepted when these Guidelines are read, but specific definitions of the terms most pertinent to the Guidelines are given below:

Recovery: Proportion of the amount of analyte, present in or added to the analytical portion of the test material, which is extracted and presented for measurement.

Surrogate: Pure compound or element added to the test material, the chemical and physical behaviour of which is taken to be representative of the native analyte.

***Surrogate Recovery**:* Recovery of a pure compound or element specifically added to the test portion or test material as a spike. (Sometimes called "marginal recovery".)

Native Analyte: Analyte incorporated into the test material by natural processes and manufacturing procedures (sometimes called “incurred analyte”). Native analyte includes “incurred analyte” and “incurred residue” as recognised in some sectors of the Analytical Community. It is so defined to distinguish it from analyte added during the analytical procedure.

***Empirical Method of Analysis**:* A method that determines a value which can be arrived at only in terms of the method *per se* and serves by definition as the only method for establishing the measurand. (Sometimes called “defining method of analysis”.)

***Rational Method of Analysis**:* A method that determines an identifiable chemical(s) or analytes(s) for which there may be several equivalent methods of analysis available.

3. PROCEDURES FOR ASSESSING RECOVERY

3.1 Recovery Information from Matrix Reference Materials

In principle, recoveries could be estimated by the analysis of matrix reference materials. The recovery is the ratio of the concentration of analyte found to that stated to be present. Results obtained on test materials of the same matrix could, in principle, be corrected for recovery on the basis of the recovery found for the reference material. However, several problems potentially beset this use of the reference materials, namely: (a) the validity of any such recovery estimate depends on the premise that the analytical method is otherwise unbiased; (b) the range of appropriate matrix reference materials available is limited; and (c) there may be a matrix mismatch between the test material and the most appropriate reference material available.

In the last instance the recovery value obtained from the reference material would not be strictly applicable to the test material. The shortfall applies especially in sectors such as foodstuffs analysis where reference materials have to be finely powdered and dried to ensure homogeneity and stability. Such treatment is likely to affect the recovery in comparison with that pertaining to fresh foods of the same kind. However, matrix mismatch is a general problem in the application of recovery information, and is treated separately in Section 3.3.

3.2 Recovery Information from Surrogates

Where (certified) reference materials are unavailable, the recovery of analyte can be estimated by studying the recovery of an added compound or element that is regarded as a surrogate for the native analyte. The degree to which this surrogate is transferred into the measurement phase is estimated separately and this recovery can, if appropriate, be attributed also to the native analyte. This procedure in principle allows the loss of analyte to be corrected, and an unbiased estimate of the concentration of the native analyte in the original matrix to be made. Such a ‘correction-for-recovery’ methodology is implicit or explicit in several distinct methods of analysis and must be regarded as a valid procedure if it can be shown to be properly executed.

In order for this procedure to be valid the surrogate must behave quantitatively in the same way as analyte that is native in the matrix, especially in regard to its partition between the various phases. In practice that equivalence is often difficult to demonstrate and certain assumptions have to be made. The nature of these assumptions can be seen by considering the various types of surrogate that are used.

3.2.1 Isotope Dilution

The best type of surrogate is an isotopically-modified version of the analyte which is used in an isotope dilution approach. The chemical properties of the surrogate are identical with, or very close to, those of the native analyte and, so long as the added analyte and the native analyte come to effective equilibrium, its recovery will be the same as that of the analyte. In isotope dilution methods the recovery of the surrogate can be estimated separately by mass spectrometry, or by radiometric measurement if a radioisotope has been used, and validly applied to the native analyte. The achievement of effective equilibrium is not always easy, however.

In some chemical systems, for example in the determination of trace metals in organic matter, the native analyte and the surrogate can be readily converted into the same chemical form by the application of vigorous reagents that destroy the matrix. This treatment converts organically bound metal into simple ions that are in effective equilibrium with the surrogate. Such a simple procedure is usually effective in the determination of trace elements, but might not apply to a pesticide residue. In the latter instance the analyte may be in part chemically bound to the matrix. Vigorous chemical reagents could not be used to release the analyte without the danger of destroying it. The native analyte and surrogate cannot come into effective equilibrium. The recovery of the surrogate is therefore likely to be greater than that of the native analyte. Thus even for this best type of surrogate, a bias in an estimated recovery may arise. Moreover, the application of the isotope dilution approach is limited by the availability and cost of isotopically enriched analytes.

3.2.2 Spiking

A less costly expedient, and one very commonly applied, is to estimate in a separate experiment the recovery of the analyte added as a spike. If a matrix blank (a specimen of the matrix containing effectively none of the analyte) is available the analyte can be spiked into that and its recovery determined after application of the normal analytical procedure. If no matrix blank is available, the spike can be added to an ordinary test portion that is analysed alongside an unspiked test portion. The difference between these two results is the recovered part of the added analyte, which can be compared with the known amount added. This type of recovery estimate is called here the 'surrogate recovery' (the added analyte acts as a surrogate for the native analyte). It is analogous to the method of standard additions. It suffers from the same problem as that encountered with isotopically modified analyte, namely that added analyte may not come to effective equilibrium with the native analyte. If the added analyte is not so firmly bound to the matrix as the native analyte, the surrogate recovery will tend to be high in relation to that of the native analyte. That circumstance would lead to a negative bias in a corrected analytical result.

3.2.3 Internal Standards

A third type of surrogate used for recovery estimation is the internal standard. When internal standardisation is used in recovery experiments the surrogate is an entity chemically distinct from the analytes, and therefore will not have identical chemical properties. However, it will normally be selected so as to be closely related chemically to the analytes, thus representing their chemical behaviour to the highest degree practicable. The internal standard would be used, for example, in recovery estimation where numerous analytes are to be determined in the same matrix and marginal recovery experiments would be impracticable for each of them individually. The question of practicability goes beyond the costs of handling numerous analytes: some analytes (for example, new veterinary residues, or metabolites) may not be available as pure substances. While it may be the most cost-effective expedient in some circumstances, the internal standard at best is technically less satisfactory than the spike as a surrogate, because its chemical properties are not identical with those of the analytes. Biases in both directions could result from the use of a recovery estimate based on an internal standard. Internal standards may also be used for other purposes.

3.3 Matrix Mismatch

Matrix mismatch occurs when a recovery value is estimated for one matrix and applied to another. The effect of matrix mismatch would be manifest as a bias in the recovery in addition to those considered above. The effect is likely to be most serious when the two matrices differ considerably in their chemical nature. However, even when the matrices are reasonably well matched (say two different species of vegetable) or nominally identical (for example, two different specimens of bovine liver), the analytical chemist may be forced to make the unsubstantiated assumption that the recovery is still appropriate. This would clearly increase the uncertainty in the recovery and in a recovery-corrected result. Matrix mismatch can be avoided in principle by a recovery experiment (for example, by spiking) for each separate test material analysed. However, such an approach will often be impracticable on a cost-benefit basis so a representative test material in each analytical run is used to determine the recovery.

3.4 Concentration of Analyte

The recovery of the surrogate or the native analyte has up to this point been treated as if it were independent of its concentration. This is unlikely to be strictly true at low concentrations. For instance a proportion of the analyte may be unrecoverable by virtue of irreversible adsorption on surfaces. However, once the adsorption sites are all occupied, which would occur at a particular concentration of analyte, no further loss is likely at higher concentrations. Hence the recovery would not be proportional to concentration. Circumstances like this should be investigated during the validation of an analytical method, but a complete study may be too time-consuming for *ad hoc* use.

4. SHOULD RECOVERY INFORMATION BE USED TO CORRECT MEASUREMENTS?

Seemingly a strong case can be made either for correcting results for recovery or for leaving them uncorrected. Regardless of these explicit arguments, however, analytical chemists are often obliged to comply with normal practice or documented procedure in their application area. The arguments listed here are not necessarily correct in every circumstance.

4.1 Arguments for Correction

- The purpose of analytical science is to obtain an estimate of the true concentration of the native analyte with an uncertainty that is fit for purpose.

- The true concentration can be estimated only if significantly low recoveries of analyte are corrected.

- An uncorrected bias due to low recovery means that results will not be universally comparable, not transportable and therefore unfit to support mutual recognition.

- Methods of correction advocated are isomorphic with perfectly acceptable analytical techniques such as internal standardisation and isotope dilution and therefore not suspect in principle.

- Although some uncertainty is inevitably associated with correction factors, that uncertainty can be estimated and incorporated into a combined uncertainty for the final result.

4.2 Arguments against Correction

- Estimated recoveries based on a surrogate may be higher than the corresponding value for the native analyte. The resultant corrected result would still have a negative bias.

- Estimated correction factors may be of doubtful applicability because they may vary among different matrices and for different concentrations of analyte.

- Estimated correction factors often have a high relative uncertainty, whereas uncorrected results usually have the smaller relative uncertainty associated with volumetric and instrumental measurement alone. (However, the uncertainty is small only if no contribution from the bias is included). Therefore corrected results will have a high relative uncertainty, sufficiently high if made explicit to create an unfavourable impression among those unfamiliar with the problems of analysis. This in turn might affect the credibility of science in the enforcement of legislation.

- Relatively small deviations from unity in correction factors could arise largely through random errors rather than a systematic loss of analyte. In that circumstance correction could make the absolute uncertainty of the result greater.

- Some legislation imposing maximum limits on contaminants is framed on the understanding that uncorrected results will be used for enforcement purposes.

4.3 Rational and Empirical Methods

Analytical measurements generally strive to estimate the measurand, that is, the true value of the concentration of the analyte, with an uncertainty that is fit for purpose. It is only on that basis that results can be completely

comparable. However, it must be recognised that this stance applies equally to 'rational' and 'empirical' methods of analysis [5]. In a rational method the measurand is the total concentration of the analyte in the test material. In an empirical method the measurand is the concentration of the "analyte" that can be measured in the test material by the specific procedure applied, and the result is traceable to the method. Therefore, if the method is regarded as empirical, the concentration measured is necessarily close* to the true value. In that case the measurand is the concentration of the 'extractable' analyte.

However, regarding methods as empirical does not in itself cause results to comply with the requirement of equivalence. Empirical results will be "equivalent" throughout a particular analytical sector only where a single method protocol (rather than a family of similar protocols) is in use for a particular determination. In some sectors, where methods have stabilised or are specified in regulations, such a single empirical method protocol will be widely used. However, in many sectors the methodology is subject to continuous evolution and single protocols are not available. In such circumstances only recovery-corrected results would be equivalent.

5. ESTIMATION OF RECOVERY

There is no generally applicable procedure for estimating recovery that is free from shortcomings. However, it is possible to conduct a 'thought experiment' in which an ideal procedure is used. This provides a reference point for real procedures. In this ideal procedure a definitive analytical method is available: the analyte can be determined by a method that is completely unbiased with no recovery losses. The method is too resource-intensive for use in routine analysis, but there is an alternative routine method with imperfect recovery. The recovery obtained in the routine method is estimated by using both methods to analyse a large set of typical test materials, a set that covers the required range of matrices and analyte concentrations. This gives the recovery (and its uncertainty) for the routine method for any conceivable situation.

In practice there may be no such definitive method available for reference, so reference materials or surrogate studies have to be used for the estimation of recovery. However, reference materials are few, and lack of resources restricts the range of test materials that can be used to estimate recovery by using surrogates. Additionally, the use of surrogates in itself adds an uncertainty to a recovery estimate because it may not be possible to determine whether some proportion of the native analyte is covalently or otherwise strongly bound to the matrix and hence not recoverable.

A strategy commonly employed to handle this problem is to estimate recovery during the process of method validation. Recoveries are determined over as wide a range of pertinent matrices and analyte concentration as resources allow. These values are then held to apply during subsequent use of the analytical method. To justify that assumption, all routine runs of the method must contain a reference material (or spiked samples) to act as internal quality control. This helps to ensure that the analytical system does not change in any significant way that would invalidate the original estimates of the recovery.

The following points are therefore suggested as requiring consideration, even if lack of resources prevents their complete execution in practice.

5.1 Representative Recovery Studies

The entire range of matrix types for which the method will be applied should be available for the method validation. Moreover, several examples of each type should be used to estimate normal range of recoveries (the uncertainty) for that matrix type. If it is likely that the history of the material will affect the recovery of the analyte (for example, the technical processing or cooking of foodstuffs), then examples at different stages of the processing should be procured. If this range cannot be encompassed in the validation, there will be an extra uncertainty associated with the matrix mismatch in the use of the recovery. That uncertainty may have to be estimated from experience.

An appropriate range of analyte concentrations should be investigated where that is technically and financially possible, because the recovery of the analyte may be concentration-dependent. Consider adding an analyte to a matrix at several different levels. At very low levels the analyte may be largely chemisorbed at a limited

* Close, but not identical with the true value. Different laboratories may execute the protocol slightly differently, introducing systematic error, and there is also a repeatability (random) error contribution.

number of sites on the matrix, or irreversible adsorbed onto surfaces of the analytical vessels. Recovery at this concentration level might be close to zero. At a somewhat higher level, where the analyte is in excess of that so adsorbed, the recovery will be partial. At considerably higher concentrations, where the adsorbed analyte is only a small fraction of the total analyte, the recovery may be effectively complete. The analytical chemist may need to have information about recovery over all of these concentration ranges. In default of complete coverage, it may be suitable to estimate recovery at some critical level of analyte concentration, for example at a regulatory limit. Values at other levels would have to estimated by experience, again with an additional uncertainty.

When spiking is applied to a matrix blank then the whole range of concentration can be conveniently considered. When the concentration of the native analyte is appreciable the spike added should be at least as great, to avoid incurring a relatively large uncertainty in the surrogate recovery.

5.2 Internal Quality Control

The principles and application of internal quality control (IQC) are described elsewhere [3]. The purpose of IQC is to ensure that the performance of the analytical system remains effectively unchanged during its use. The concept of statistical control is crucial in IQC applied to routine analysis (as opposed to *ad hoc* analysis). When applied to recovery, IQC has some special features that have to be taken into account. This IQC of recovery can be addressed in two distinct ways, depending on the type of control material that is used.

(a) A matrix-matched reference material can be used as a control material. The recovery for this material and an initial estimate of its between-run variability are determined at the time of method validation. In subsequent routine runs the material is analysed exactly as if it were a normal test material, and its value plotted on a control chart (or the mathematical equivalent). If the result for a run is in control, then the validation-time estimate of the recovery is taken as valid for the run. If the result is out of control, further investigation is required, which may entail the rejection of the results of the run or possibly a re-investigation of the recovery. It may be necessary to use several control materials, depending on the length of the run, the analyte concentration range *etc*.

(b) Spiked materials can also be used for quality control. As usual, initial estimates of the average recovery and its between-run variability are made during method validation, and are used to set up a control chart. Either of two variant approaches can be used in routine analysis, depending on the stability of the material: (a) a single long-term control material (or several such materials) can be prepared for use in each routine run, or (b) all, or a random selection, of the test materials for the run can be spiked. In either instance the surrogate recovery is plotted on a control chart. While the recovery remains in control it can be deemed to apply to the test materials generally. Of the two alternative methods, the latter (involving the actual test materials) is probably the more representative, but also the more demanding.

There is a tendency for the role of IQC to be confused with the simple estimation of recovery (where deemed appropriate). It is better to regard IQC results solely as a means of checking that the analytical process remains in control. The recovery estimated at method validation time are usually more accurate for application to subsequent in-control runs, because more time can be spent on studying their typical levels and variability. If real-time spiking is used to correct for recovery, this is more like a species of calibration by standard additions.

6. UNCERTAINTY IN REPORTING RECOVERY

Uncertainty is a key concept in formulating an approach to the estimation and use of recovery information. Although there are substantive practical points in the estimation of uncertainty that (at the time of writing) remain to be settled, the principle of uncertainty is an invaluable tool in conceptualising recovery issues. A definition of uncertainty, key references and an extended discussion are given in the Appendix.

When loss of analyte occurs in an analytical procedure, two uncertainties need to be separately considered. First, there is the uncertainty u_x associated only with the determination, namely that due to gravimetric, volumetric, instrumental, and calibration errors. That relative uncertainty u_x/x will be low unless the concentration of the analyte is close to the detection limit. Second, there is the uncertainty u_R on the estimated

recovery R. Here the relative uncertainty u_R/R is likely to be somewhat greater. If the raw result is corrected for recovery, we have $x_{corr} = x / R$ (*i.e.*, the correction factor is 1/R). The relative uncertainty on x_{corr} is given by

$$\frac{u_{corr}}{x_{corr}} = \sqrt{\left(\left(\frac{u_x}{x}\right)^2 + \left(\frac{u_R}{R}\right)^2\right)},$$

which is necessarily greater than u_x/x and may be considerably greater. Hence correction for recovery seems at first sight to degrade, perhaps substantially, the reliability of the measurement.

Such a perception is incorrect. Only if the method is regarded as empirical (and this has drawbacks in relation to comparability as seen above) is u_x the appropriate uncertainty. If the method were taken as rational, and the bias due to loss of analyte were not corrected, a realistic estimate of uncertainty u_x' would have to include a term describing the bias. Hence u_x'/x would be at least comparable with, and may be even greater than, u_{corr}/x_{corr} .

This topic is developed in more detail in the Appendix.

6.1 Estimating Uncertainty in a Recovery

The approaches to the estimation of the uncertainty of a recovery provided here are necessarily tentative, and may be expected to be rapidly superseded as detailed studies become available. The important principles are as follows.

(a) The recovery and its standard uncertainty may both depend on the concentration of the analyte. This may entail studies at several concentration levels. Subsequent comments in this section apply to a single level of concentration.

(b) The main recovery study should involve the whole range of matrices that are included in the category for which the method is being validated. If the category is strict (*e.g.*, bovine liver) a number of different specimens of that type should be studied so as to represent variations likely to be encountered in practice (*e.g.*, sex, age, breed, duration of storage etc.). Probably a minimum of ten diverse matrices are required for recovery estimation. The standard deviation of the recovery over these matrices is taken as the main part of the standard uncertainty of the recovery.

(c) If there are grounds to suspect that a proportion of the native analyte is not extracted, then a recovery estimated by a surrogate will be biased. That bias should be estimated together with its contribution to the uncertainty budget.

(d) If a method is used outside the matrix scope of its validation, there is a matrix mismatch between the recovery experiments at validation time and the test material at analysis time. This could result in extra uncertainty in the recovery value. There may be problems in estimating this extra uncertainty. It would probably be preferable to estimate the recovery in the new matrix, and its uncertainty, in a separate experiment.

7. CONCLUSIONS

Variable practice in handling information recovery is an important cause of the non-equivalence of data. To mitigate its effects the practice of reporting analytical data after the application of an appropriate correction factor normally should be encouraged. Where, however, an enforcement limit is based on data that has not had a correction factor applied, the present situation of reporting “raw” data will continue for the foreseeable future.

Detailed descriptions of recovery experiments and their results should be properly recorded. If it is known or suspected that a proportion of the native analyte in the test material is not extractable by the analytical procedure, the procedure must be qualified as determining only "available" analyte. Such qualification should be specified on analytical certificates. No valid compensation can be made, or should be attempted, for the "bound" analyte, which a recovery model does not represent.

It should be recognised that there is a dual role for recovery determinations in analytical measurement, that is, for (a) quality control purposes and (b) for deriving recovery values. In the latter application, more extensive and detailed data are required.

8. RECOMMENDATIONS[1]

The following recommendations are made regarding the use of recovery information in these Guidelines:

1. Quantitative analytical results should be corrected for recovery unless there are specific reasons for not doing so. Reasons for not estimating or using correction factors include the situations where (a) the analytical method is regarded as empirical, (b) a contractual or statutory limit has been established using uncorrected data, or (c) recoveries are known to be close to unity. However, it is of over-riding importance that all data, when reported, should (a) be clearly identified as to whether or not a recovery correction has been applied and (b) if a recovery correction has been applied, the amount of the correction and the method by which it was derived should be included with the report. This will promote direct comparability of data sets. Correction functions should be established on the basis of appropriate statistical considerations, documented, archived and available to the client.

2. Recovery values should always be established as part of method validation, whether or not recoveries are reported or results are corrected, so that measured values can be converted to corrected values and *vice versa*.

3. When the use of a recovery factor is justified, the method of its estimation should be specified in the method protocol.

4. IQC control charts for recovery should be established during method validation and used in all routine analysis. Runs giving recovery values outside the control range should be considered for re-analysis in the context of acceptable variation, or the results reported as semi-quantitative.

9. REFERENCES

1. *"Protocol for the Design, Conduct and Interpretation of Method Performance Studies"*, W Horwitz, *Pure Appl. Chem.*, 1988, **60**, 855- 864, revised 1995, **67**, 331-343.

2. *"The International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories"*, M Thompson and R Wood, *Pure Appl. Chem.*, 1993, **65**, 2123-2144. (Also published in *J. AOAC International*, 1993, **76**, 926-940.

3. *"Harmonised Guidelines For Internal Quality Control in Analytical Chemistry Laboratories"*, Michael Thompson and Roger Wood, J. Pure & Applied Chemistry, 1995, **67**, 49-56.

4. *"Quality Assurance for Analytical Laboratories"*, edited M. Parkany, Royal Society of Chemistry, London, UK, 1993.

5. *"Sense and Traceability"*, M. Thompson, *Analyst*, 1996, **121**, 285-288.

[1] IUPAC, ISO and EURACHEM embrace the scientific principles and recommendations of these Guidelines. AOAC INTERNATIONAL embraces the scientific principles but does not agree that analytical results should be corrected for recovery as a general policy.

APPENDIX: UNCERTAINTY IN REPORTING RECOVERY

The principle of uncertainty is a helpful tool in conceptualising recovery issues. The main intent of this Appendix is to indicate those principles. The estimation of uncertainty in recovery has yet to be studied in detail.

Definition of Uncertainty

Measurement Uncertainty is defined by ISO [1,2] as

> "A parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand",

with the note that "The parameter may be, for example, a standard deviation (or a given multiple of it), or the half width of an interval having a stated level of confidence". The ISO Guide recommends that this parameter should be reported as either a standard uncertainty, denoted *u*, defined as the

> "uncertainty of the result of a measurement expressed as standard deviation" or as an expanded uncertainty, denoted *U*, defined as
>
> "a quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could be attributed to the measurand". The expanded uncertainty is obtained by multiplying the standard uncertainty by a coverage factor, which in practice is typically in the range 2 to 3.

Definition of the Measurand

Clear definition of the measurand is crucial to uncertainty estimation and to the relevance or otherwise of recovery values. The most important issue here is whether the measurand is the amount of material actually present in the sample matrix (a rational method), or the response to a reproducible but otherwise essentially arbitrary procedure established for comparative purposes (an empirical method).

Recovery and Uncertainty

The recovery $R = c_{obs}/c_{ref}$ is the ratio of the observed concentration (or amount) c_{obs} obtained by the application of an analytical procedure to a material containing analyte at a reference level c_{ref}. c_{ref} will be (a) a reference material certified value, (b) measured by an alternative definitive method, or (c) defined by a spike addition. In a perfect separation R would be exactly unity. In reality, circumstances such as imperfect extraction often give observations that differ from the ideal. It is therefore good practice in validating an analytical method to estimate a recovery R for the analytical system. In such experiments, the recovery can be tested for significant departure from unity. Such a test considers the question "is $|R-1|$ greater than u_R, the uncertainty in the determination of R?", at some level of confidence. Table 2 gives some sources of the uncertainty in measured recovery. The experimenter then performs a significance test of the form

$|R-1|/u_R > t$: R differs significantly from 1

$|R-1|/u_R \leq t$: R does not differ significantly from 1

where t is a critical value based either on a 'coverage factor' allowing for practical significance or, where the test is entirely statistical, $t_{(\alpha/2,\, n-1)}$, being the relevant value of Student's t for a level of confidence 1-α.

Following such an experiment, four cases can be distinguished, chiefly differentiated by the use made of the recovery R.

(a) R is not significantly different from 1. No correction is applied.

(b) R is significantly different from 1 and a correction for R is applied.

(c) R is significantly different from 1 but, for operational reasons, no correction for R is applied

(d) An empirical method is in use. R is arbitrarily regarded as unity and u_R as zero. (Although there is obviously some variation in recovery in repeated or reproduced results, that variation is subsumed in the directly estimated precision of the method.)

The uncertainty may be handled in each of the cases above as follows.

(a) *R not significantly different from 1.* The experiment has detected no reason to adjust subsequent results for recovery. It might be thought that the uncertainty in the recovery is unimportant. However, the experiment could not have distinguished a range of recoveries between $1-ku_R$ and $1+ku_R$. It follows that there is still uncertainty about the recovery that should be taken into account in calculating the overall uncertainty. (An alternative view is that a correction factor of $R=1$ is implicitly applied, but the experimenter is uncertain that the value is exactly unity). u_R is therefore to be included in the uncertainty budget. However, it must not be included twice: uncertainty of recovery will often be included automatically in estimates of precision.

(b) *R differs from 1 and a correction is applied.* Since R is explicitly included in the calculation of the corrected result (*i.e.*, $c_{corr} = c / R$, where c is the raw result with an uncertainty u_c) it is clear that u_R must be included in the uncertainty budget. This leads to a combined uncertainty u_{corr} on the corrected result given by

$$\frac{u_{corr}}{c_{corr}} = \sqrt{\left(\left(\frac{u_c}{c}\right)^2 + \left(\frac{u_R}{R}\right)^2\right)} \quad .$$

u_{corr} would be multiplied by k (usually 2) to obtain the expanded uncertainty U.

(c) *R differs from 1 and no correction is applied.* Failure to apply a correction for a known systematic effect in a rational method is inconsistent with obtaining the best possible estimate of the measurand. It is less straightforward in this case to take recovery into account in calculating the overall uncertainty. If R is substantially different from unity, the dispersion of values that includes the measurand is not properly represented unless the uncertainty u_R is substantially increased. A simple and pragmatic approach that is sometimes adopted, when a correction b for a known systematic effect has not been applied, is to increase the expanded uncertainty on the final result to (U_c+b) where U_c is calculated assuming b is zero. For recovery, therefore, $U = U_c + (c/R - c)$. This procedure gives a pessimistic overall uncertainty, and departs from the ISO-recommended principle of treating all uncertainties as standard deviations.

Alternatively, if the correction for recovery is not applied because the analyst's judgement is that the difference is not meaningful in normal use, case (c) may be treated in the same way as case (a) after increasing u_R because the significance test should have used a value larger than u_R. This amounts to estimating u_R as $|1-R|/t$ where t is the critical value used in the significance test. This amplified uncertainty on the recovery should be included as in case (b). This will normally only be significant where u_R is comparable with or greater than $|1-R|$.

While either method will provide an estimate of uncertainty, both methods have similar drawbacks arising from the failure to correct the result to give a best estimate of the measurand. Both lead to overstatement of the uncertainty, and the range quoted around the result will include the measurand only near one extreme (usually the upper end), with the remainder of the range unlikely to contain the value with significant probability.

For recoveries of the order of 70%, the additional uncertainty contribution (before applying a coverage factor) will be close to 20% of the result. This is clearly not unreasonable given the size of recovery correction being ignored, but it does point strongly to the consequences for reported uncertainty of neglecting a substantial recovery correction.

There is therefore a clear choice if the customer is not to be misled by a result from a putative rational method. Either the recovery must be corrected or a substantially greater uncertainty must be quoted.

Finally, it should be noted that the foregoing discussion relates to the situation where a result and its uncertainty are obtained on a real scale and reported as such. The instance where an analyst provides an *interpretation* of a result (for example by stating that the value is “not less than...”) has not been considered. In this kind of interpretation, the analyst’s professional knowledge of the recovery and overall experimental uncertainty will be taken into account in the interpretation, and accordingly neither the recovery nor an uncertainty need necessarily be reported.

References

1. Guide to the Expression of Uncertainty in Measurement’, ISO, Geneva, 1993, (ISBN 92-67-10188-9)

2. *International Vocabulary of basic and general standard terms in Metrology*. ISO, Geneva, Switzerland 1993 (ISBN 92-67-10175-1)

Table 1. Sources of uncertainty in analytical chemistry

1.	Incomplete definition of the measurand (for example, failing to specify the exact form of the analyte being determined).
2.	Sampling - the sample measured may not represent the defined measurand.
3.	Incomplete extraction and/or pre-concentration of the measurand, contamination of the measurement sample, interferences and matrix effects.
4.	Inadequate knowledge of the effects of environmental conditions on the measurement procedure or imperfect measurement of environmental conditions.
5.	Cross contamination or contamination of reagents or blanks.
6.	Personal bias in reading analogue instruments.
7.	Uncertainty of weights and volumetric equipment.
8.	Instrument resolution or discrimination threshold.
9.	Values assigned to measurement standards and reference materials.
10.	Values of constants and other parameters obtained from external sources and used in the data reduction algorithm.
11.	Approximations and assumptions incorporated in the measurement method and procedure.
12.	Variations in repeated observations of the measurand under apparently identical conditions.

Table 2. Sources of uncertainty in recovery estimation

1	Repeatability of the recovery experiment
2	Uncertainties in reference material values
3	Uncertainties in added spike quantity
4	Poor representation of native analyte by the added spike
5	Poor or restricted match between experimental matrix and the full range of sample matrices encountered
6	Effect of analyte/spike level on recovery and imperfect match of spike or reference material analyte level and analyte level in samples.