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PROCEDURES FOR DETERMINING UNCERTAINTY OF MEASUREMENT RESULTS

1. Introduction

1. At its 35th Session of the Codex Committee on Methods of Analysis and Sampling, the Committee agreed to develop procedures for determining uncertainty of measurement results including sub-sampling, sample processing and analysis (REP14/MAS, paragraph 86).

2. According to ISO/IEC 17025 (1), testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement. This information document provides procedures to estimate the measurement uncertainty without being prescriptive. The presented procedures should be regarded as practical examples, which are applicable in many day-to-day situations. The development of the examples cannot be exhaustive and in special situations, other rationale procedures might be applied. Furthermore it does not apply situations, where legal specifications define special rules for the estimation of the measurement uncertainty (e.g. the empirical Horwitz equation). In order to consider as many analytical situations as possible, the procedures are developed for different classes of analytical methods. Multi-factor experimental designs, analysed by ANOVA, and Propagation of distributions using a Monte Carlo method are not included in this document but reference to literature is provided (2), (3).

3. In addition to the estimation of measurement uncertainty, this paper presents solutions for some practical problems depending on the measurement uncertainty:

- Checking the stability/validity of the estimated precision data
- Checking the equivalence of new/old methods or new/old standards of analyte for calibration taking into account the measurement uncertainty

2. Basic concepts

4. ISO/IEC 17025 allows a variety of approaches for estimating the uncertainty of measurement in testing:

- Laboratories shall have and shall apply procedures for estimating uncertainty of measurement.
- In the cases that the nature of the test method precludes rigorous, metrologically and statistically valid, calculation of measurement uncertainty, the laboratory shall at least attempt to identify all the components of uncertainty and make a reasonable estimation.
- Reasonable estimation of measurement uncertainty shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data.
- When estimating the uncertainty of measurement, all uncertainty components, which are of importance in the given situation shall be taken into account using appropriate methods (Sources contributing to the uncertainty include, but are not necessarily limited to, the reference standards and reference materials used, methods and equipment used, environmental conditions, properties and condition of the item being tested or calibrated, and the operator).
- For further information, see ISO 5725 and the Guide to the Expression of Uncertainty in Measurement (GUM).

5. In this paper, the approaches of the ISO/IEC 17025 are taken into account. The concepts of estimating the measurement uncertainty are based on international recommended guides (JCGM 100:2008: Evaluation of measurement data — Guide to the expression of uncertainty in measurement (GUM) (4), the EURACHEM / CITAC Guide CG 4: Quantifying Uncertainty in Analytical Measurement (5) and the JCGM 200:2008: International vocabulary of metrology — Basic and general concepts and associated terms (VIM)) (6), which are interrelated.

3. Evaluation of Measurement Uncertainty

6. Measurement uncertainty, which is a parameter of the test result, is based on precision data of the method, taking into account the steps of analysis that may include sub-sampling, sample processing and instrumental analysis. The uncertainty components are combined according to the error propagation rules. Basically, the source of the precision data determines the steps of analysis that are accounted for in the evaluation of measurement uncertainty, and N uncertainty standard $s_{1...N}$ (or relative standard deviations i.e. coefficients of variation $cv_{1...N}$) of the so called Type A evaluation (statistical analysis of series of experimental observations on one or more components of the analytical process) and of the so called Type B evaluation (usually based on a pool of comparatively reliable information) can be combined to the total standard uncertainty u (or relative total standard uncertainty u_{rel}) (GUM 5.1.2, 5.1.5, 5.1.6) :

$$u = \sqrt{(s_1^2 + s_2^2 + \dots + s_N^2)} \quad \text{or} \quad u_{rel} = \sqrt{(cv_1^2 + cv_2^2 + \dots + cv_N^2)} \quad *)$$

*) The formulas refer to measurands given by the sum and/or the difference of parameters (left) or given by the product and/or the quotient of parameters (right). Since in practice, most of the analytical measurands are given by formulas with products and/or quotients of parameters, in the following text the second formula will be used. For simplicity, the parameters are regarded as non-correlated.

7. This has the practical advantage that particular precision data from in-House method validation or from inter-laboratory method validation (after proving fitness for purpose of the particular test laboratory by verification of that precision data) can be used in combination. The following procedures are ordered according to the particular class of the analytical method.

3.1 Standard Methods

8. For Standard Methods, the advantage consists of the appropriate validation including precision data. Generally, these data are based on extensive inter-laboratory method validation, mostly performed according to ISO 5725 1-6 (7). A basic assumption underlying ISO 5725-1 (8) is that, for a standard measurement method, repeatability will be, at least approximately, the same for all laboratories applying the standard procedure, so that it is permissible to establish one common average repeatability standard deviation s_r which will be applicable to any laboratory. However, any laboratory should, by carrying out a series of measurements under repeatability conditions, verify that the average repeatability standard deviation is applicable under given conditions (ISO 5725-6). The reproducibility standard deviation s_R of the standard method is obtained by combining s_r with the between-laboratory standard deviation s_L (ISO 5725-2) (9).

3.1.1 Empirical Standard Methods

9. Empirical methods achieve comparability between laboratories measuring the same material with no intent to obtain an absolute measure of the true amount of analyte present. Corrections for method bias or matrix effect are ignored by convention. For an empirical method, for which collaborative trial data are available, at least the in-house repeatability should be evaluated and proven to be comparable to that s_r predicted by the collaborative trial and documented in the method i.e. the repeatability standard deviation of the particular laboratory should be less or equal s_r (EURACHEM Example A6). A priori, no bias contribution must be considered and it is therefore appropriate to use the relative reproducibility standard deviation (i.e. the coefficient of variation) cv_R values from the collaborative trial or method publication as relative standard uncertainty u_{rel} in an appropriate range of analyte levels (EURACHEM 7.6.3).

10. In most cases, the collaborative trials providing homogenised material do not cover preparation steps (e.g. grinding, drying), and therefore, the uncertainty contributions of that analytical part must be additionally taken into consideration (EURACHEM 7.6.1), provided that the contribution is significant (i.e. $>1/3$ cv_R (EURACHEM 7.2.2)).

11. Uncertainty contribution of preparation: One subsample of the batch should be divided in as many parts as practical (at least 10 parts for sufficient statistical power). Each part should be prepared separately (e.g. grinding, drying), and the preparation should be analysed under identical analytical conditions (i.e. in a short time period using the same calibration) giving the relative standard deviation of preparation cv_P . The relative standard measurement uncertainty u_{rel} is given by the combination:

$$u_{rel} = \sqrt{(cv_R^2 + cv_P^2)}$$

12. Collaborative trials provide homogenised material and in the case of significant laboratory sample inhomogeneity, the uncertainty contribution of subsampling must be considered. The significance might be assessed by using a homogeneity check like ISO 13528 (10), Annex B by comparing the relative between-subsamples standard deviation cv_{Ss} with the relative standard deviation for proficiency assessment cv_{σ} (σ is used for the estimation of the z-scores) of the standard method. The sample may be considered to be adequately homogeneous if , $cv_s \leq 0.3 cv_{\sigma}$.

13. The between-subsamples standard deviation s_s might be estimated by the procedure given in ISO 13528, Annex B1 and using the formula given in ISO 13528, Annex B3:

14. Select a number g of the subsamples from the laboratory sample at random, where $g \geq 10$.

- Prepare two test portions from each subsample using techniques appropriate to the test material to minimize between-test-portion differences.
- Taking the $2g$ test portions in a random order, obtain a measurement result on each, completing the whole series of measurements under repeatability conditions.
- Calculate the general average \bar{x} , within-subsamples standard deviation s_w , and between-subsamples standard deviation s_s , giving the relative standard deviation of sample inhomogeneity $cv_{Ss} = s_s / \bar{x}$

15. The relative standard measurement uncertainty u_{rel} is given by the combination:

$$u_{rel} = \sqrt{(CV^2_R + CV^2_{Ss})}$$

16. In formulas for calculating the analytical result, the influence of subsampling differences due to inhomogeneity and preparation variability can be implemented as factors, which are dispersed around 1 (EURACHEM A4.3).

3.1.2 Non-empirical Standard Methods

17. For non-empirical (rational) standard methods, the trueness is an issue, which must be considered in the estimation of measurement uncertainty. The current procedure applies to the situation where no bias is to be taken into account. But this assumption must be proven by appropriate recovery experiments. Contrary to empirical methods, many rational standard methods are supplied by certified reference materials. As an alternative, samples can be spiked with a known level of the analyte, bearing in mind the different behaviour of the spiked substance and the native counterpart.

18. In a first step, the standard uncertainty u (by multiplying u_{rel} with the concentration of the analyte) should be estimated according to Procedure 3.1.1. The bias b from the recovery experiments is compared with that uncertainty and can be neglected if $b \ll u$. Otherwise, the bias is significant (EURACHEM 7.15).

19. In the case that the collaborative trial did not cover effects of different matrices and the matrix might have an impact on the analytical result, the corresponding uncertainty contribution must be additionally taken into consideration. In principle the uncertainty contribution of matrix might be estimated by the same procedure as for assessing the uncertainty contribution of inhomogeneity of the laboratory sample, corresponding to ISO 13528, Annex B3.

20. In this case, recovery experiments (e.g. spiking of samples) should be applied on samples from different matrices (with preference of matrices, which do not contain the analyte).

21. Select a number g (as many as practical) of matrices, where $g \geq 10$.

- Prepare two test portions from each of the g matrices using techniques appropriate to the test material to minimize between-test-portion differences.
- Obtain a measurement result on each, completing the whole series of measurements under repeatability conditions.
- Calculate the general average \bar{x} , within-matrices standard deviation s_w , and between-matrices standard deviation s_M , giving the relative standard deviation of sample inhomogeneity $cv_M = s_M / \bar{x}$

22. The relative standard measurement uncertainty u_{rel} is given by the combination:

$$u_{rel} = \sqrt{(CV^2_R + CV^2_M)}$$

23. In case that all the contributions of cv_P , cv_S and cv_M are significant (i.e. greater than one third of the maximal cv), the relative standard measurement uncertainty u_{rel} is the combination of the four contributions if applicable :

$$u_{rel} = \sqrt{(CV^2_R + CV^2_P + CV^2_S + CV^2_M)}$$

24. Where bias is significant compared to the combined uncertainty, the analytical result might be corrected for the bias, making due allowance for the uncertainty of the correction or the observed bias and its uncertainty might be reported in addition to the result. In case of correction, the relative uncertainty of the bias CV_B must be estimated by recovery experiments and combined with the other uncertainty contributions if applicable (EURACHEM Example A4):

$$U_{rel} = \sqrt{CV_R^2 + CV_P^2 + CV_S^2 + CV_M^2 + CV_B^2}$$

25. Notice: It should be avoided to take the effect of bias (this is not the uncertainty of bias) into account by enlarging the “uncertainty” assigned to the result instead of correcting for bias. Evaluating the uncertainty of a measurement result should not be confused with assigning a safety limit to some quantity (Guide to the expression of uncertainty in measurement (GUM), 6.3.1).

3.2 In-House Methods

26. Contrary to standard methods, for in-House methods no published standard precision data are available. Therefore, they are subjects of extensive validation procedures. Despite of ad-hoc situations, the validation provides precision data. Nevertheless, in case that the in-House method is a modification of a corresponding standard method, the estimation of precision should focus on the uncertainty contributions of that modification. The uncertainty contributions should be compared to the relative reproducibility standard deviation (i.e. coefficient of variation) CV_R values from the collaborative trial or standard method publication. If the uncertainty contribution of modifications is negligible, it is appropriate to use CV_R as relative standard uncertainty U_{rel} and to proceed according to Procedures 3.1.

27. There are two general approaches (Type A and B) to estimate the reproducibility precision CV_R :

- Type A: The combination of the repeatability precision of all single steps of analysis (e.g. weighing, drying, extracting, diluting and analytical measurement) with the involved calibrations and other uncertainty sources (e.g. purity of reference standards, experience of test personnel)
- Type B: Reproducibility precision estimated as far as possible over an extended time period allowing natural variation of all impact factors.

28. In practice, a combination of these types is usually necessary and convenient.

3.2.1 Established In-House Methods

3.2.1.1 Type A estimation:

29. The uncertainty components associated with the potential sources of uncertainty are identified, quantified as standard deviations, and combined according to the appropriate rules, to give a combined standard uncertainty. The sources are for example:

- Standard substances (certified uncertainty/purity)
- Physical/chemical variability (extraction, derivatisation, stoichiometry)
- Application of measuring devices for preparation of the test samples (balances, pipettes, thermometers etc.)
- Application of analytical instruments (stability, calibration, contamination etc.)
- Different experience of test personnel

30. The procedure begins with the critical reflection of the formula of the measurand i.e. the relationship between the result and the input values. All parameters are to be checked for their uncertainty relevance. Therefore, for example, the uncertainty of the sample preparation is separated into the individual steps of weighing, homogenizing, drying, extracting, diluting etc., which are to be combined, e.g.:

$$CV_P = \sqrt{CV_{weigh}^2 + CV_{hom}^2 + CV_{dry}^2 + CV_{ext}^2 + CV_{dil}^2}$$

31. The uncertainty of weighing itself, for example, is estimated from the separate contributions of calibration and traceability (including certified uncertainty of the weights) and the uncertainty of the reading (analogue/digital-display):

$$S_{weigh} = \sqrt{S_{cal}^2 + S_{read}^2}$$

32. Obviously, the subject of Type A estimation is too complex to be sufficiently described in the current paper. Therefore, for further information, reference is made to the JCGM 100:2008: Evaluation of measurement data — Guide to the expression of uncertainty in measurement (GUM) and the EURACHEM / CITAC Guide CG 4: Quantifying Uncertainty in Analytical Measurement.

3.2.1.2 Type B estimation:

33. This type of estimation should be performed as far as possible under reproducibility conditions allowing natural variation of all impact factors. Basically this should include all conjectural components (subsampling, matrices, preparation and analysis) but for sufficient statistical power, that would require a very high number of experiments. Therefore, it is recommended to start at the situation, which is similar to the participation on collaborative trials (homogenised and dried material of a particular matrix) and to implement the additional components.

34. To this end, the in-House estimation of reproducibility precision cv_R should take into account all parts of the analysis, which basically would be involved in case of participation on a corresponding inter-laboratory validation of a standard method. That includes at least the extraction/derivatisation/digestion procedures (recovery variation) and the complete measurement process including calibration and traceability.

35. A typical test sample containing an appropriate amount of analyte (e.g. homogenised and dried for stability) might be analysed several times over a period of time, using different analysts and equipment where possible (e.g. the results of measurements on quality control samples) thus verifying reproducibility conditions (EURACHEM 7.7.2).

36. The relative reproducibility standard deviation estimated by use of the following procedures is comparable to that from a corresponding collaborative trial, which does not cover effects of sample preparation, different matrices and subsampling. In order to take into account these uncertainty components, they should be combined with cv_R as described in Procedures 3.1.

37. For the identification and uncertainty estimation of bias, the approaches described in the Procedure 3.1.2 have to be applied.

38. In case that the uncertainty might depend on analyte levels, the precision experiments should be carried out at different levels in any case, according to ISO/IEC 17025, including the level, which is relevant for compliance assessment. The significance of influence might be checked by the F-test or the Cochran test for homogeneity of the variances at different levels.

39. Finally, the uncertainty of the calibration standards or of the reference materials (traceability) should be considered, even though this uncertainty contribution is negligible in most cases.

3.2.1.2.1 ISO 5725-3 Approach

40. An appropriate norm-consistent approach might be the as-far-as-possible-application of the procedure given in ISO 5725-2 where the reproducibility standard deviation s_R of an inter-laboratory method validation is obtained by combining the mean repeatability standard deviation s_r of all laboratories with the between-laboratory standard deviation s_L . That inter-laboratory study approach is also applied in ISO 16140 (11) for validation of microbiological methods.

41. According to ISO 5725-3 (12), precision estimated in one laboratory is the so called intermediate precision measure, which is smaller than the reproducibility standard deviation based on inter-laboratory method validation and hence more practical for the individual laboratory.

42. A typical test sample (homogenised and dried) is analysed over a period of time on n different days by different analysts (with a new extraction/digestion, recalibration). The different days with different analytical conditions simulate the situations in the different laboratories. Each of the days, a number of k replicates of the particular extract/digest are measured with the results $x_{j=1...k}$ under repeatability conditions (measurement within a short time, the same instrument and calibration used by the same operator) and the following parameters are calculated:

- Each day i : From the replicate results $x_{j=1...k}$ the mean value \bar{x}_i and the repeatability standard deviation (stddev) $s_{r i}$ are estimated.

$$\bar{x}_i = 1/k \sum x_{j=1...k}$$

$$s_{r i} = \text{stddev}(x_{j=1...k})$$

- From the repeatability standard deviations of the different days $s_{r i=1...n}$, the mean repeatability standard deviation $s_{r \text{ mean}}$ is calculated.

$$s_{r\ mean} = \sqrt{(1/n \sum s_{r\ i=1...n}^2)}$$

- The "between-laboratory" standard deviation s_L of the mean values $\bar{x}_{i=1...n}$ of the different days is calculated .

$$s_L = \text{stddev}(\bar{x}_{i=1...n})$$

Finally, according to ISO 5725-2, the reproducibility standard deviation is given by:

$$s_R = \sqrt{(s_{r\ mean}^2 + s_L^2)}$$

The relative reproducibility standard deviation (coefficient of variation) is given by:

$$CV_R = s_R/X ,$$

wherein X is the total mean value of the mean values of all the days $\bar{x}_{i=1...n}$

$$X = 1/n \sum \bar{x}_{i=1...n}$$

3.2.1.2.2. Duplicate Approach

43. As an alternative to the above-mentioned ISO 5725-3 approach, the overall run-to-run variation (reproducibility standard deviation) can be performed with a number n of duplicate tests (homogenised samples each divided into two test samples, each of the test samples subjected to complete extraction/digestion and determination procedure including recalibration)(EURACHEM 7.7.2 and A4.4).

- For each duplicate test i , the relative differences $\delta_{rel\ i}$ of the two particular results $x_{1\ i}$ and $x_{2\ i}$ (the difference δ_i divided by the mean \bar{x}_i) and the standard deviation (stddev) of the relative differences $s_{\delta_{rel}}$ are calculated:

$$\delta_i = x_{1\ i} - x_{2\ i}$$

$$\delta_{rel\ i} = \delta_i / \bar{x}_i \quad \text{where} \quad \bar{x}_i = (x_{1\ i} + x_{2\ i})/2$$

$$s_{\delta_{rel}} = \text{stddev}(\delta_{rel\ i=1...n})$$

- This standard deviation is divided by $\sqrt{2}$ to correct from a standard deviation for pairwise differences to the standard uncertainty for the single values giving the relative standard uncertainty:

$$CV_R = s_{\delta_{rel}} / \bar{X} / \sqrt{2}$$

3.2.2 Ad-hoc Methods

44. In most cases, ad-hoc methods are based on standard or well-established in-House methods. They are expanded substantially (e.g. to other analytes or matrices) and will not generally require complete revalidation, but the procedure, which was described in the first paragraph of Procedures 3.2 is highly recommended. In order to get an acceptable statistical power, as many replicates as practical of the test (including all relevant parts of method) should be performed. The comparison of the resulting relative standard deviation with the relative standard uncertainty of the basic method gives information about the precision equivalence of the ad-hoc method. Where appropriate, the uncertainty of the basic method should be reported.

45. Completely new developed ad-hoc methods are not covered by the basic guidelines used in the current paper.

4. Estimation of Expanded Measurement Uncertainty

46. The combined relative standard measurement uncertainty u_{rel} , which was obtained by applying one of the above described procedures, is the basis for the expanded measurement uncertainty U . It is obtained by multiplying the standard measurement uncertainty by a coverage factor k . The interval given by the result $X \pm U$ encompasses a large fraction of the distribution of values, which could reasonably be attributed to the measurand.

47. For the level of confidence required (normally 95%), for most purposes it is recommended to set $k=2$. In case that the combined uncertainty is based on only few observations (less than about seven i.e. less than six degrees of freedom ν), however, k should be set equal to the two-tailed value of Student's t-factor (note that the 95% one-sided confidence limit is equivalent to the 90% two-sided confidence limit) for the so called effective number of degrees of freedom ν_{eff} associated including that 'statistical low power'-contribution. The details are described in the Guide to the expression of uncertainty in measurement (GUM), Annex G.4.1.

5. Methods for day-to-day-checking the acceptability of test results with regard to the measurement uncertainty

48. The checking methods described in this clause are based on a probability level of 95% and should be applied only to the case where s_R and s_r are known. Vice versa, they are an appropriate mean to check the stability/validity of the estimated precision data. As far as practical, the approaches should be combined (ISO 5725-6).

- Testing in duplicate under repeatability condition: The absolute difference between the two test results should be equal to or less than the repeatability limit $r = 2,8 s_r$.
- Testing in duplicate under reproducibility condition: The absolute difference between the two test results should be equal to or less than the reproducibility limit $R = 2,8 s_R$.
- Using Quality Control samples (typical test samples containing an appropriate amount of analyte, homogenised and dried for stability or CRM-samples): The result should be in agreement with the mean value $\pm 2s_R$. In order to realize trends, the use of control charts is highly recommended.

49. Taking into account the probability level of 95% (statistically one of twenty experiments might not meet the expectation), non-conforming test results should be considered as suspect, and therefore, the cause of the aberrant result should be investigated.

6. Method for checking the equivalence of new/old methods or new/old standards of analyte for calibration taking into account the measurement uncertainty (13)

50. A statistical test called the two one-side t-test (TOST) begins with the opposite null hypothesis; that the two mean values for the methods are not equivalent. A positive test for significance then results in demonstrating, at a specified confidence level, that the two data sets are equivalent (the nominal concentrations of the new/old standard solutions or results of the new/old methods on the same standard solution are not significantly different). The TOST requires the specification of a parameter called the acceptance criterion, θ (e.g. 10%), which represent the smallest difference in mean values for the two methods that is deemed as practically important.

51. The confidence interval (CI) for the difference in means at a specific level of confidence (usually 95%) is calculated by

$$CI = X_{new} - X_{old} \pm t_{90,(n_1+n_2-2)} \sqrt{(s_p^2(1/n_1 + 1/n_2))}$$

52. where X_{new} and X_{old} are the mean values of the nominal concentrations of the new/old standard solutions or results of the new/old methods on the same test material respectively, $t_{90,(n_1+n_2-2)}$ is the t-value at 90% confidence (note that the 95% one-sided confidence limit is equivalent to the 90% two-sided confidence limit) with $n_1 + n_2 - 2$ degrees of freedom, s_p is the estimate for the standard deviation under repeatability (comparison of solutions using the same calibration) or reproducibility (comparison of methods with all analytical steps) conditions and n_1 and n_2 are the numbers of new/old experiments.

53. If CI is completely contained within the range defined by $\pm \theta$ the nominal concentrations of standard solutions or the new and old methods are deemed equivalent.

7. Literature

- (1) ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories
- (2) M H Ramsey and S L R Ellison (eds.) Eurachem/EUROLAB/ CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approaches Eurachem (2007)

- (3) Evaluation of measurement data — Supplement 1 to the “Guide to the expression of uncertainty in measurement” — Propagation of distributions using a Monte Carlo method, JCGM 101:2008
- (4) Evaluation of measurement data — Guide to the expression of uncertainty in measurement (GUM), JCGM 100:2008
- (5) S L R Ellison and A Williams (Eds). Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (2012)
- (6) International vocabulary of metrology — Basic and general concepts and associated terms (VIM), JCGM 200:2008
- (7) ISO 5725-6:1994 Accuracy (trueness and precision) of measurement methods and results -- Part 6: Use in practice of accuracy values
- (8) ISO 5725-1:1994 Accuracy (trueness and precision) of measurement methods and results -- Part 1: General principles and definitions
- (9) ISO 5725-2:1994 Accuracy (trueness and precision) of measurement methods and results -- Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method
- (10) ISO STANDARD 13528:2005 Statistical methods for use in proficiency testing by interlaboratory comparisons
- (11) ISO 16140:2003 Microbiology of food and animal feeding stuffs -- Protocol for the validation of alternative methods
- (12) ISO 5725-3:1994 Accuracy (trueness and precision) of measurement methods and results - Part 3: Intermediate measures of the precision of a standard measurement method
- (13) Working Document of the Hungarian CCMAS committee