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bcr information

REFERENCE MATERIALS

The certification of the contents (mass fractions) of Cd, Pb, Se, Cu, Zn, Fe and Mn in wholemeal flour and lyophilized brown bread reference materials

Wholemeal flour	CRM No 189
Brown bread	CRM No 191

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CORRIGENDUM

to the certification report on BCR-191

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1) On **page 3**, last paragraph, second line, and

on **page 40**, first paragraph, second line,

40 g should be replaced by **25 g.**

2) As a result of the revision of the certificates in April 2007, certified values and uncertainties should read as follows:

page 39 (Table 6):

	Certified value µg/g	**Uncertainty µg/g**
Cu	2.63	0.07

3) Additional Material Information

On the following pages “indicative values” should be read as “additional material information” (at least):

III, V, 1, 28, 32, 35 (4 x), 36, 37, 38, 39 and 62

NOTE:

This certification report also deals with **BCR-189.** As **BCR-189** is exhausted, certificate and report were not revised accordingly. All parts in the report referring to **BCR-189** are **obsolete**.

April 2007

ABSTRACT

The report describes the preparation and certification of a wholemeal flour (CRM 189) and a lyophilised brown bread (CRM 191) for their contents (mass fractions) of elements of toxicological and nutritional importance : Cd, Pb, Se, Cu, Zn, Fe and Mn. Indicative values are also given for As, Ca, Cl, Cr, Hg, Mg, Na, Ni, P and K.

Details are given of a preliminary intercomparison of methods for these elements in a wholemeal flour sample, homogeneity and stability studies on the two reference materials and the results and evaluation of the certification exercise which involved 21 European laboratories. Summaries of the certification methods are also presented. The report concludes with a discussion of the most common sources of error in determining the elements of interest and the steps to be taken to control them.

RESUME

Le rapport décrit la préparation et la certification d'une farine (complète) de blé (CRM 189) et d'un pain noir lyophilisé (CRM 191) pour leurs teneurs (fraction de masse) en éléments présentant une importance nutritionnelle et toxicologique: Cd, Pb, Se, Cu, Zn, Fe et Mn. A titre indicatif, les teneurs en As, Ca, Cl, Cr, Hg, Mg, Na, Ni, P et K sont aussi données.

Il décrit en détail une intercomparaison des méthodes utilisées pour l'analyse de ces éléments dans un échantillon de farine complète de blé, les études d'homogénéité et de stabilité des 2 matériaux de référence, ainsi que les résultats et l'évaluation de la campagne de certification à laquelle 21 laboratoires européens ont pris part. Les résumés des méthodes utilisées pour la certification sont aussi présentés. Le rapport se termine par une discussion des sources d'erreurs les plus communes rencontrées dans la détermination de ces éléments et les mesures à prendre pour les contrôler.

GLOSSARY OF TERMS

AAS	Atomic absorption spectrometry (flame)
APDC	Ammonium Pyrrolidine Dithiocarbamate
BPA	Baphothenanthroline (4,7-diphenyl-1,10,phenanthroline)
CVAAS	Cold vapour atomic absorption spectrometry
DCP	Direct current plasma emission spectrometry
DDDC	Diethylammonium diethyldithiocarbamate
DDTC	Diethyl dithiocarbamate
DEDPA	Diethyl dithiophosphoric acid (ammonium salt)
DPASV	Differential pulse anodic stripping voltammetry
ETAAS	Electrothermal atomic absorption spectrometry
FLUOR	Fluorimetric measurement
HAAS	Hydride formation atomic absorption spectrometry
ICP	Inductive coupled plasma emission spectrometry
IDMS	Isotope dilution mass spectrometry
INAA	Instrumental neutron activation analysis
MIBK	Methyl Iso-Butyl Ketone (4-methyl-2-pentanone)
RNAA	Neutron activation analysis with radiochemical separation
SPEC	Spectrometry (visible light and near UV)
ZAAS	Electrothermal atomic absorption spectrometry (Zeeman background correction)
2,3 DAN	2,3 diamino naphtalene

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1 Introduction

The two reference materials, RM 189 Wholemeal flour and RM 191 Brown bread, have been prepared as part of a broader programme to provide certified reference materials for the elemental analysis of the major dietary components. Already certified in this series are three milk powders (1) (CRM nos. 063, 150 and 151) and three animal tissues (2) (CRM nos. 184 Bovine muscle, CRM 185 Bovine liver and CRM 186 Pig kidney).

The importance of cereals in general and bread in particular for the human diet is clearly demonstrated by the annual European per capita consumption for wheat which for the period 1980/81 was 76 kg (3).

Thus, whilst cereal products are important sources of nutrients, the high consumption rate makes it essential to minimise and control the levels of potentially toxic species.

CRMs 189 and 191 have been certified for their Pb, Cd, Se, Cu, Zn, Fe and Mn contents and will find application in public health, industrial, agricultural and nutritional laboratories, where it is necessary to both ensure and demonstrate the accuracy of these determinations. Indicative values are provided for As, Ca, Cl, Cr, Mg, Na, Ni, P and K. In addition, several experienced laboratories provided data for Hg and demonstrated that the content of this element in both materials is less than 5 ng/g.

2 PARTICIPATING LABORATORIES

2.1 Preparation of Materials

Joint Research Centre, CEC, Ispra, I

2.2 Homogeneity Studies

Gesellschaft für Strahlen- und Umweltforschung, Neuherberg, D

2.3 Stability Studies

Gesellschaft für Strahlen- und Umweltforschung, Neuherberg, D

2.4 Certification Measurements

Bundesforschungsanstalt für Getreide- und Kartoffelverarbeitung, Detmold, D
Centro di Radiochimica, CRN, Pavia, I
Energie Centrum Nederland (ECN), Petten, NL
Gesellschaft für Strahlen- und Umweltforschung, Neuherberg, D
Greater Manchester Council, Public Analyst's Laboratory, Manchester, UK
Institut d'Hygiène et d'Epidémiologie, Brussels, B
Institut für Spektrochemie und Angewandte Spektroskopie, Dortmund, D
Kernforschungsanlage Jülich, Jülich, D
Laboratory of the Government Chemist, London, UK
Max-von-Pettenkofer-Institut des Bundesgesundheitsamtes (BGA), Berlin, D
Plasmon Dietetici Alimentari SpA, Milan, I
Rijksinstituut voor Volksgezondheid end Milieuhygiene, RIVM, Bilthoven, NL
Rijks-Kwaliteitsinstituut voor Land en Tuinbouwprodukten (RIKILT),
Wageningen, NL
Rijksuniversiteit Gent, Gent, B
Risø National Laboratory, Roskilde, DK
Service Central d'Analyse, CNRS, Vernaison, F
The Agricultural Institute, Wexford, IRL
The Animal and Grassland Research Institute, Maidenhead, UK
Universitaire Instelling Antwerpen, Antwerp, B
Universität Regensburg, Institut für Chemie, Regensburg, D
Vrije Universiteit Brussel, Brussels, B

2.5 Statistical Evaluation

Community Bureau of Reference, CEC, Brussels, B

3 PREPARATION OF THE MATERIALS

Commercial wholemeal flour of north Italian origin was seived through a 125 μm screen. The fraction retained was ground in a ZrO_2 ball-mill, re-seived and the two fractions having a particle size of less than 125 μm were combined.

Commercial sliced brown bread, obtained from the Oberfranken region of the FRG was freeze-dried, ground in a ZrO_2 planetary-mill and seived through a 125 μm screen. The fraction retained was reground, seived again and the two fractions having a particle size less than 125 μm were combined.

The batches of material were homogenised in a PVC drum equipped with internal mixing vanes during a period of two weeks.

XRF was employed to demonstrate that thorough mixing had been achieved and the materials were bottled by hand in units of 40 g. In order to minimise risk of de-mixing during the bottling procedure, the contents of the drum were re-mixed after each series of 40 bottles had been completed.

4 HOMOGENEITY

4.1 General

The homogeneity of the reference materials was investigated both with respect to the elements to be certified and several other elements which serve as general indicators of homogeneity. 20 randomly drawn bottles were sampled. Homogeneity was judged by comparison of the observed variance of the results with the estimated variation due to the method (see § 4.3 below).

4.2 Analytical Methods and their Repeatabilities

The analytical methods used to assess homogeneity were selected, having regard to the repeatability afforded at the expected concentrations, and for sample intakes in the range of 10 - 100 mg.

4.2.1 Differential Pulsed Anodic Stripping Voltammetry (DPASV) (Pb, Cd & NI)

DPASV was applied after pressure digestion with $HNO_3/HClO_4/H_2SO_4$ at 160 °C for 12 hours in a PTFE bomb equipped with a quartz insert (4). Careful control of blank values allowed good repeatabilities for these three elements.

4.2.2 Flameless Atomic Absorption Spectroscopy (HAAS)

The homogeneity of (Se) was studied by flameless AAS after pressure digestion employing the conditions described above (§4.2.1) and reduction to the hydride.

4.2.3 Inductively Coupled Plasma Spectroscopy (ICP) (Cu, Zn, Mn, Fe, Mg, P)

ICP was used to provide simultaneous homogeneity data for a number of elements, the digestion conditions being as described above (§4.2.1). Under carefully controlled conditions (5), the procedure yielded repeatabilities of the order (coefficients of variation) of 5 - 10 % for most of the elements studied.

4.2.4 Instrumental Neutron Activation Analysis (INAA)

A limited homogeneity study was made by INAA on the bottled samples at an early stage in the project as part of a survey of the homogeneity of a series of candidate cereal reference materials.

The study was restricted to between-bottle homogeneity using 50 mg samples.

Samples were exposed to either short-term or long-term irradiation and, after allowing suitable decay time, the induced activities were measured with a γ- spectrometer using a Ge(Li) detector having a relative efficiency of about 6 % and an average resolution of 2 keV over the range of energies considered.

a) Short-term irradiation (Mn & Na)
Samples were irradiated for 10 minutes, it being assumed that the neutron flux remained constant during the irradiation period. After allowing a decay period of 12 hours, the samples were counted for 1000 seconds.

b) Long-term irradiation (Se, Cu, Zn, Co and Cr)
Samples were irradiated for 40 hours in a rotating rig in order to average out any fluctuations or inhomogeneities in the neutron flux. Samples were allowed to decay for 20 days and were then counted for 4000 seconds.

Only elements which were considered to yield results of adequate precision as predicted by the counting statistics were included in the evaluation of homogeneity.

4.3 Results and Discussion

The results of the homogeneity studies for RMs 189 and 191 are presented in Tables 1a and 1b, where the observed variation of the results (coefficient of variation) is compared with the variation estimated for the method.

In the case of the wet chemical procedures, the method repeatability, is estimated from the known instrumental precision at the appropriate elemental concentration and takes account of the laboratory's previous and extensive experience of these measurements in similar matrices and homogeneity studies.

The overall variability of the INAA results is made up of factors, some of which can be estimated (weighing and counting errors) and others which cannot (e.g. random errors due to geometrical variations within the counting vials, variations in thickness and packaging density of the samples in vials).

Inhomogeneity of the materials may be evaluated by comparison of the observed, overall variation with the estimated variation derived from weighing and counting statistics. Since it was not possible to account for all sources of variability, this approach may lead to an over-estimate of inhomogeneity.

Taken overall the results show that both materials are acceptably homogeneous with respect to the elements examined with the possible exception of Cr which, when determined by INAA and a radio-chemical separation procedure gave a wider range of results than could be accounted for by method imprecision.

Taking account of the sample intake employed in the homogeneity studies and in the final certification measurements, a minimum sample size of 200 mg is recommended.

Table 1a : Results of Homogeneity Study for RM 189 - Wholemeal Flour

Element (approx. mass fraction)	Method	Samples (20 replicates in each case)	Predicted CV%	Observed CV%
Cd	DPASV	10 mg w - b	10	6.5
70 ng/g		50 mg b - b	10	8.5
		100 mg b - b	10	8.5
Pb	DPASV	100 mg w - b	10	10
370 ng/g		50 mg b - b	10	11
		100 mg b - b	10	11
Se	HAAS	100 mg w - b	10	8
130 ng/g		50 mg b - b	10	8
		100 mg b - b	10	8
Cu	ICP	10 mg b - b	3	3.7
6 µg/g		50 mg b - b	2.5	2.4
		100 mg b - b	2	1.3
Zn	ICP	10 mg b - b	3.5	3.7
60 µg/g		50 mg b - b	3	1.5
		100 mg b - b	2.5	1.5
	INAA	50 mg b - b	3.5	4.0
Fe	ICP	10 mg b - b	3.5	4
70 µg/g		50 mg b - b	3	1.6
		100 mg b - b	2.5	1.3
Mn	ICP	10 mg b - b	3.5	3.4
60 µg/g		50 mg b - b	3	2.6
		100 mg b - b	2.5	2.6
	INAA	50 mg b - b	3	2.8
Co*	INAA	50 mg b - b	12.5	14
Cr	INAA	50 mg b - b	7	11.2
65 ng/g				
Mg	ICP	10 mg b - b	2	1.9
2 mg/g		50 mg b - b	2	2.1
		100 mg b - b	2	1.4
Ni	ETAAS	10 mg b - b	10	10
400 ng/g		50 mg b - b	10	10
		100 mg b - b	10	12
P	ICP	10 mg b - b	2.5	2.3
5.3 mg/g		50 mg b - b	2	1.2
		100 mg b - b	2	1.1

N.B. w - b = within bottle
b - b = between bottle

* no value for Co content avalable

Table 1b : Results of Homogeneity Study for RM 191 - Brown Bread

Element (approx. mass fraction)	Method	Samples (20 replicates in each case)	Predicted CV%	Observed CV%
Cd 30 ng/g	DPASV	100 mg w - b	10	12.5
		50 mg b - b	10	12
		100 mg b - b	10	11
Pb 200 ng/g	DPASV	100 mg w - b	15	22
		50 mg b - b	15	13
		100 mg b - b	15	12
Se 30 ng/g	HAAS	100 mg w - b	10	8
		50 mg b - b	10	9
		100 mg b - b	10	8
Cu 2.5 µg/g	ICP	10 mg b - b	3.5	5.6
		50 mg b - b	3	1.5
		100 mg b - b	2	1
	INAA	50 mg b - b	3	2.8
Zn 20 µg/g	ICP	10 mg b - b	3	3
		50 mg b - b	2.5	3.3
		100 mg b - b	2	1.5
	INAA	50 mg b - b	4.5	4.1
Fe 40 µg/g	ICP	10 mg b - b	3	3.3
		50 mg b - b	2.5	1.8
		100 mg b - b	2	2.3
Mn 20 µg/g	ICP	10 mg b - b	2.5	3.0
		50 mg b - b	2	1.3
		100 mg b - b	2	1.5
	INAA	50 mg b - b	3.5	4.8
Cr 68-360 ng/g	INAA	50 mg b - b	7	13.2
Mg 500 µg/g	ICP	10 mg b - b	2	4.1
		50 mg b - b	2	2.8
		100 mg b - b	2	2.5
Na 10 mg/g	INAA	50 mg b - b	4.5	4.1
Ni 500 ng/g	ETAAS	10 mg b - b	10	10
		50 mg b - b	10	10
		100 mg b - b	10	8
P 2 mg/g	ICP	10 mg b - b	2.5	1.9
		50 mg b - b	2	1.0
		100 mg b - b	2	1.1

N.B. w - b = within bottle
b - b = between bottle

5 STABILITY

It is well established that under dry storage conditions, cereals and products such as lyophilised bread are resistant to spoilage by moulds or bacteria, provided the moisture content is low. The moisture content of CRMs 189 and 191 remained at around 6 % during the first three years of their life and is not expected to rise during storage.

Instability with respect to the elemental contents could, in principle, occur by loss of elements that are volatile in the free state or which form volatile compounds by absorption/desorption at the surface of the container or, through changes in the mass of the matrix (other than changes in moisture content which are allowed for in the determination).

A 48 week stability study was performed in which samples of the two reference materials were stored in closed bottles at 4 °C, 20 °C and 30 °C. The contents of Cd, Pb, Hg and Se were periodically determined by ASV (Pb and Cd), CV-AAS (Hg) and Hydride-AAS (Se). The contents of each bottle were shaken before analysis on each occasion and the sample intake was 100 mg. The results are summarised in Table 2a and 2b.

It is evident that there is no detectable change in the Cd, Pb, Hg or Se contents of the materials under any of the conditions tested.

Table 2a : Stability of RM 189 (Wholemeal Flour) with respect to Pb, Cd, Hg, and Se contents at 3 temperatures[a,b]

Element	Storage temp. °C		TIME (weeks) 0	6	12	24	48	Overall mean $\bar{x}$	SD
Cd ng/g	4	$\bar{x}$	67.6	68.0	68.7	68.5	69.7	68.5	0.8
		SD	2.9	2.6	3.0	3.5	3.0		
	20	$\bar{x}$	67.6	68.3	68.6	69.7	69.0	68.6	0.8
		SD	2.5	3.2	2.1	2.1	2.6		
	30	$\bar{x}$	69.3	67.3	69.0	69.0	69.0	68.7	0.8
		SD	2.1	2.5	3.6	2.6	2.0		
Pb ng/g	4	$\bar{x}$	380	386	390	380	383	383	4.2
		SD	20	28	36	26	30		
	20	$\bar{x}$	380	386	387	370	367	382	7.3
		SD	20	30	35	36	15		
	30	$\bar{x}$	383	376	390	383	376	382	5.8
		SD	21	21	10	10	21		
Hg (arbitary units)	4	$\bar{x}$	48.6	50.0	48.3	48.3	49.3	49.9	0.7
		SD	2.1	3.0	2.1	3.5	2.5		
	20	$\bar{x}$	50.0	48.0	49.0	50.0	49.3	49.3	0.8
		SD	2.0	2.6	3.6	2.0	2.1		
	30	$\bar{x}$	47.7	47.6	48.7	48.3	47.6	48.0	0.5
		SD	2.1	2.9	2.1	3.2	3.8		
Se (arbitary units)	4	$\bar{x}$	120	126	130	123	123	124	4
		SD	10	11	10	12	15		
	20	$\bar{x}$	127	123	120	123	126	124	3
		SD	15	6	10	12	12		
	30	$\bar{x}$	120	123	120	127	120	122	3
		SD	10	15	10	6	10		

N.B. (a) Materials stored in closed bottles and mixed before sub-sampling
(b) Each result is the mean of 3 determinations (sample intake = 100 mg)

Table 2b : Stability of RM 191 (Brown Bread) with respect to Pb, Cd, Hg, and Se contents at 3 temperatures[a,b]

Element	Storage temp. °C		TIME (weeks) 0	6	12	24	48	Overall mean $\bar{x}$	SD
Cd ng/g	4	$\bar{x}$	27.6	27.6	27.3	28.0	27.7	27.6	0.3
		SD	2.1	3.2	3.5	3.0	1.5		
	20	$\bar{x}$	27.6	28.3	29.0	27.3	28.7	28.2	0.7
		SD	1.5	2.1	2.0	3.2	1.5		
	30	$\bar{x}$	27.0	25.0	28.7	28.0	28.7	27.5	1.5
		SD	2.0	3.6	1.5	2.0	2.5		
Pb ng/g	4	$\bar{x}$	173	176	167	186	190	178	9
		SD	15	15	15	12	10		
	20	$\bar{x}$	180	180	173	186	173	178	6
		SD	10	20	15	15	15		
	30	$\bar{x}$	173	190	176	180	187	181	7
		SD	15	10	21	20	15		
Hg (arbitary units)	4	$\bar{x}$	18	19	18	19	19	18.6	0.4
		SD	2	1	2	2	1		
	20	$\bar{x}$	19	20	18	19	18	19.0	0.9
		SD	2	2	3	2	2		
	30	$\bar{x}$	18	18	19	18	17	18.1	0.6
		SD	2	2	3	1	2		
Se (arbitary units)	4	$\bar{x}$	96	100	103	103	103	101	3.1
		SD	6	10	6	6	6		
	20	$\bar{x}$	103	100	97	103	103	101	2.7
		SD	6	10	6	6	1		
	30	$\bar{x}$	100	103	103	97	103	101	2.8
		SD	10	6	6	6	6		

N.B. (a) Materials stored in closed bottles and mixed before sub-sampling
(b) Each result is the mean of 3 determinations (sample intake = 100 mg)

6 PRELIMINARY INTERCOMPARISON OF METHODS

6.1 General

Before embarking on the certification procedure, a preliminary intercomparison was undertaken in which 24 laboratories determined trace elements by whichever method they preferred in a sample of wholemeal flour.

The objectives of the preliminary study were two-fold :

- to establish the level of agreement between the results by different methods and laboratories;
- to identify the most accurate methods for a final certification exercise.

The laboratories represented a wide range of interests, including those of public health, environment and the cereal industry. In addition, several laboratories which were particularly experienced with the application of INAA and IDMS took part.

6.2 Results

The results of this study are summarised in Tables 3 and 4. Table 3 presents the ranges of laboratory mean values observed in the preliminary intercomparison and compares them with the ranges of results submitted for certification, i.e. before results were rejected on technical grounds.

Table 3 : Comparison of the ranges of results observed in the preliminary intercomparison with those of results submitted for the final certification of RM 189 (p is the number of sets of results)

	Preliminary Intercomparison		Certification Exercise (results submitted for certification of RM 189)	
	Range of mean values	p	Range of mean values	p
Cd ng/g	38 - 208	18	61 - 78(125)[1]	14(15)[1]
Pb_2 ng/g	285 - 5073	18	243 - 460	17
Hg^2 ng/g	1 - 42	7	1 - 8	5
Se ng/g	46 - 140	9	108 - 150	10
Cu µg/g	3 - 8	18	(4.5)[1] 6.2 - 7.2	12(13)[1]
Zn µg/g	46 - 61	21	52 - 65	17
Mn_2 µg/g	50 - 78	15	59 - 73	14
Ca^2 µg/g	416 - 524	6	519 - 525	3
Ni^2 µg/g	359 - 490	5	335 - 440	3

[1] Range distorted by one extreme value
[2] Not certified

Table 4 compares the means (mean of means) and standard deviations of the means of the results of the preliminary intercomparison (after removal of extreme values), with those obtained from the results accepted for certification of RM 189 (Wholemeal Flour)

Table 4 : Comparison of the mean ($\bar{x}$) and standard deviations of the mean (S_M) of the preliminary intercomparison and final certification measurements for RM 189

		Preliminary Intercomparison[1]			Certification Exercise[2]		
		$\bar{x}$	S_M	p	$\bar{x}$	S_M	p
Cd	ng/g	69	20	16	71.3	5	12
Pb	ng/g	381	63	16	379	20	13
Se	ng/g	99	31	9	132	14	11
Cu	µg/g	6.1	0.9	16	6.4	0.2	10
Zn	µg/g	53.3	4	21	56.5	3	13
Fe	µg/g	64.7	8	15	68.3	3	13
Mn	µg/g	61.5	7	15	63.3	3	12

[1] Results obtained after removal of extreme values
[2] Results accepted for certification

6.3 Conclusions

It is evident from Table 3 that there are serious discrepancies in the results of determinations of Cd and Pb. Se and Hg also gave poor agreement, although it should be noted that the probable Hg content of the Wholemeal flour sample was around 2 ng/g, (see Table 8) a level which is below the capabilities of most laboratories where 'clean-bench' facilities are not available.

The agreement for the minor elements Cu, Zn and Mn was likewise considered to be unsatisfactory, especially considering that the contents were well above the limits of determination of most modern methods.

The preliminary study demonstrated the need for certified reference materials to allow laboratories to improve and control their performance. In addition, the study allowed many of the principal sources of errors to be identified and provided the basis for the selection of appropriate methods and laboratories for the final certification exercise.

7 DESIGN OF THE CERTIFICATION EXERCISE AND METHODS EMPLOYED

7.1 Design of the Certification Exercise

Participants were required to make at least five fully independent determinations of specified elements. The measurements for each element were made on different days and, samples for analysis were taken from at least two different bottles.

Participants were requested to take all necessary steps to ensure that the measurements were accurate. Particular care was to be taken to ensure that balances and volumetric apparatus were correctly calibrated and, that calibrants were of known and proven purity or stoichiometry. The contents of the bottles were required to be mixed before each analytical sub-sample was taken. The results were expressed on a dry-mass basis, the dry-mass correction factor being determined on a separate portion of sample, taken from the same bottle, immediately prior to the analysis of each analytical sub-sample. The dry mass correction factor was determined by oven-drying to constant mass at 103 °C. Full details of the procedure used are given in Chapter 11 (Instructions for Use).

7.2 The Methods Employed

Care was taken to ensure that the methods employed for each element to be certified varied both with respect to sample preparation and final determination.

Essential details of these methods are presented in Tables 5a to 5c, together with the sample intakes. The methods are given next to the corresponding laboratory codes and should be read in conjunction with the discussion of the results (Chapter 8)

Table 5a : Summary of Methods Employed for the Determination of Cd and Pb in RM 189 (Wholemeal flour) and RM 191 (Brown bread)

Lab.	Element	Sample size mg	Sample Pretreatment	Final determination
4	Cd	100	Pressure dign. at 160 °C for 12 hours with HNO_3/$HClO_4$/H_2SO_4; evaporate & redissolve in $HClO_4$	ASV
	Pb	100	" "	ASV
7	Cd	1000	Pressure dign. at 180 °C for 16 hours with HNO_3/HF; addition of HNO_3; evaporate; redissolve & elimination of interfering compounds with active charcoal.	ASV
	Pb	1000	" "	ASV
1	Cd	200-300	Open dign. at 200 °C for 4 hours with HNO_3/$HClO_4$	ASV
	Pb	200-300	" "	ASV
2	Cd	500-900	Open dign. at 200-250 °C for 5 hours HNO_3/$HClO_4$; evaporate to dryness & redisolve.	ASV
	Pb	500-900	" "	ASV
32	Cd	2000	Addn. of H_2SO_4/HNO_3; temp. programmed dry ashing to 500 °C for 8 hours, dissolution in HCl/NaOAc.	ASV
	Pb	2000	" "	ASV

43	Cd	1000	Addn. of $Mg(NO_3)_2/HNO_3$; Dry ashing at 450 °C for 8 - 9 hours; dissolution in HCl.	ASV
	Pb	1000	" "	ASV
17	Cd	5000	Open dign. with HNO_3/H_2SO_4 at 340°C for 40 mins.; chelation with DDDC & APDC & solvent (MIBK) extraction.	AAS
	Pb	5000	" "	AAS
16	Cd	2000	Open dign. with $HNO_3/HClO_4/H_2SO_4$ at about 250 °C for 6 hours; separation and preconcentration of the DEDPA complexes on charcoal.	AAS
	Pb	2000	" "	AAS
13	Cd	2000	Open dign. with $HNO_3/HClO_4$ for 2 days at 200°C.	ET-AAS
	Pb	2000	" "	ET-AAS
23	Pb	1000	Open dign. at 200 °C for 3 hours with $HClO_4/HNO_3$. Extraction with APDC in MIBK	ET-AAS
16a	Cd	300-400	Combustion in oxygen, trapping of combustion products on cold finger, dissolution by refluxing with HNO_3	Z-AAS
	Pb	300-400	" "	Z-AAS
16b	Pb	2000	Open dign. with $HNO_3/HClO_4/H_2SO_4$ at about 250°C for 6 hours; separation and pre-concentration of the DEDPA	ET-AAS

			complexes on charcoal.	
	Cd	2000	" "	ET-AAS
33	Cd	1000	Open dign; with HNO_3/H_2SO_4 for 3 days at about 350 °C; extraction with dithizone/toluene & back extraction into 0.5 mol.l^{-1} HNO_3	ET-AAS
	Pb	1000	" "	ET-AAS
4	Cd	100	Pressure dign. with HNO_3 for 12 hours at 160 °C	Z-AAS
	Pb	100	" "	Z-AAS
11	Cd	1000-2000	Dry-ashing at 550 °C for 4 hours; dissolution in HNO_3	Z-AAS
	Pb	1000-2000	" "	Z-AAS
7	Cd	1000	Addn. of ^{112}Cd; pressure dign. at 180 °C for 16 hours with HNO_3/HF; addn. of $HClO_4$; evaporation & redissolve in HCl; anion exchange & uptake in $HClO_4$	IDMS Thermal Ionisation
	Pb	1000	Addn. of ^{206}Pb; as above except residue redissolved in HBr prior to anion exchange	IDMS Thermal Ionisation
10	Cd	800-1500	Addn. of ^{116}Cd; open dign. at 120 °C for 2 hours with HNO_3/H_2O_2 (twice) followed by electrolytic deposition.	IDMS Thermal Ionisation

Pb	800-1500	Addn. of ^{206}Pb; then as above.	IDMS Thermal Ionisation

Table 5b : Summary of Methods Employed for the Determination of Se in RM 189 (Wholemeal flour)and RM 191 (Brown bread)

Lab.	Sample size mg	Sample Pretreatment	Final deter-mination
32	300	Pressure digestion with HNO_3 followed by $HClO_4$ treatment; evap.; reduction with HCl; complexation with 3,4-DAN and extraction into cyclohexane	Fluorimetry (ext 376 nm em 520 nm)
15	500	Open digestion with $HNO_3/HClO_4$ (80 °C - 30 min, 120 °C - 15 min and then 200 °C until clear solution); complexation with 2,3-DAN and extraction into cyclohexane	Fluorimetry (ext 376 nm em 520 nm)
1	200	Open digestion with $HNO_3/H_2SO_4/HClO_3/HCl$ at 20 - 310 °C during 2 hours; reduction with HCl followed by $NaBH_4$	H-AAS
16a	100	Open digestion with $HNO_3/HClO_4$ at 25-210 °C during 3 hours; pre-reduction with 5M HCl at 95 °C; reduction with $NaBH_4$	H-AAS
19	1000	Open digestion with HNO_3 (25 °C - 8 hours; 70 °C - 7 hours; 120 °C - 7 hours); $HClO_4$ (210 °C) then HCl reduction 100 °C - 15 minutes. Final reduction with $NaBH_4$	H-AAS

33 (189 only)	500	Open dign. with $HNO_3/HClO_4/HCl$ for 2 days at 230 °C. Pre-reduction with 5M HCl (110 °C for 30 min) followed by addn. of $HONH_2Cl$	H-AAS
4	100	Pressure digestion with $HNO_3/HClO_4/H_2SO_4$ at 160 °C for 12 hours Pre-reduction with HCl at 80 °C; reduction to hydride.	H-AAS
16b	200	Combustion in oxygen, trapping of combustion products on cold finger, dissolution by refluxing with HNO_3	H-AAS
3	250	Neutron irradiation for 28 hours (flux: $2x10^{12}$ $cm^{-2}.s^{-1}$)	γ-counting of ^{75}Se
4	100	Neutron irradiation for 36 hours (flux: $10^{13} cm^{-2}.s^{-1}$)	γ-counting of ^{75}Se
5	100	Neutron irradiation for 50 hours (flux: 1.2×10^{12} $cm^{-2}.s^{-1}$)	γ-counting of ^{75}Se at 136 and 264 keV
7	100	Neutron irradiation for 20 s (flux: 5.10^{13} $cm^{-2}.s^{-1}$), wait 5 s., count during 20 s.	γ-counting of ^{77m}Se at 162 keV
9	500	Neutron irradiation for 20 min (flux: 10^{13} $cm^{-2}.s^{-1}$); digestion in H_2SO_4/HNO_3; precipitation as Se; dissolution in HNO_3; extraction into MIBK	γ-counting of ^{81m}Se at 103 keV

Table 5c : Summary of Methods Employed for the Determination of Cu, Zn, Fe & Mn in RM 189 (Wholemeal flour) and RM 191 (Brown bread)

Lab.	Element	Sample size mg	Sample Pretreatment	Final determination
32a	Cu	200	Bomb digestion at 150 °C for 3 hours.	AAS
	Zn	"	" "	AAS
	Mn	"	" "	AAS
32b	Cu	2000	Addition of K_2SO_4/HNO_3; temp. programmed dry ashing up to 500 °C during 8 hours; dissolve ash in HCl & buffer to pH 2-3 with 2mol.l^{-1} NaOAc.	ASV
32c	Fe	100-150	Open digestion with HNO_3/ K_2SO_4/H_2SO_4 followed by H_2O_2; complexation with BPA and extraction into 3-methyl-1-butanol	SPEC
17	Cu	5000	Open digestion at 340 °C for 40 min with HNO_3/H_2SO_4	AAS
	Zn	"	" "	"
	Fe	"	" "	"
	Mn	"	" "	"
22	Cu	700-1200	Open digestion with H_2SO_4/ HNO_3; boiling for 1 hour with HNO_3 then under reflux half-hour with H_2SO_4	AAS
	Zn	"	" "	"
	Fe	"	" "	"
	Mn	"	" "	"

23	Cu	500	Open digestion at 200 °C 3 hours with $HClO_3/HNO_3$	ET-AAS
	Zn	"	"	AAS
	Fe	"	"	AAS
	Mn	"	"	AAS
39	Cu	3000	Dry-ashing at 440 °C for 6 hours after addition of 1 ml conc. HNO_3. Dissolve ash in 10 % HCl, evaporate and re-dissolve.	AAS
	Zn	"	"	"
	Fe	"	"	"
	Mn		"	"
43	Cu	1000	Addition of HNO_3; dry ashing to 450 °C during 8-9 hours	AAS
	Zn	"	"	"
	Fe	"	"	"
	Mn	"	"	"
14	Cu	200 "	Dry ashing at 520 °C for 16 hours; ash dissolved in dilute HNO_3	DCP
	Zn	"	"	"
	Fe	"	"	"
	Mn	"	"	"

2	Cu	500-900	Open digestion with $HNO_3/HClO_4$	ICP
	Zn	"	"	"
	Fe	"	"	"
	Mn	"	"	"
4a	Cu	100	Pressure digestion at 160 °C for 12 hours with HNO_3	ICP
	Zn	"	"	"
	Fe	"	"	"
	Mn	"	"	"
4b	Zn	100	Neutron irradiation for 36 hours (flux: 10^{13} $cm^{-2}.s^{-1}$	γ-counting of ^{65}Zn
	Fe	100	"	γ-counting of ^{59}Fe
3	Zn	250	Neutron irradiation for 28 hours (flux : $2x10^{12}$ $cm^{-2}.s^{-1}$)	γ-counting of ^{65}Zn
	Fe	"	"	γ-counting of ^{59}Fe
5	Cu	200	Neutron irradiation for 10 hours (flux:1 x 10^{11} $cm^{-2}.s^{-1}$); pressure digestion with HNO_3/H_2SO_4; ion exchange with CuS	γ-counting of ^{64}Cu
	Zn	"	Neutron irradiation for 50 hours (flux: 1.2 x 10^{12} $cm^{-2}.s^{-1}$)	γ-counting of ^{65}Zn
	Fe	"	"	γ-counting of ^{59}Fe
	Mn	"	Neutron irradiation for 60 s. (flux: 4.5 x 10^{12} $cm^{-2}.s^{-1}$)	γ-counting of ^{56}Mn

7	Zn	70	Neutron activation for 6 hours (flux: 5 x 10^{12} $cm^{-2}.s^{-1}$)	γ-counting of ^{65}Zn
	Mn	600	Neutron activation for 10 min (flux: 3 x 10^{11} $cm^{-2}.s^{-1}$)	γ-counting of ^{65}Mn
9	Zn	500	Neutron activation for 12 hours (flux: 4.5 x 10^{13} $cm^{-2}.s^{-1}$)	γ-counting of ^{65}Zn
	Fe	"	" "	γ-counting of ^{59}Fe
	Mn	"	Neutron activation for 1 min (flux: 2.5x10^{13} $cm^{-2}.s^{-1}$	γ-counting of ^{56}Mn
10	Cu	800-1500	Open digestion at 120 °C for 2 hours, with HNO_3/H_2O_2 (twice); addition of ^{65}Cu spike & cathodic deposition.	IDMS Thermal ionisation
	Zn	200-400	As above, but addition of ^{68}Zn as spike.	" "

8 RESULTS OF THE CERTIFICATION EXERCISE AND DISCUSSION

All results submitted by the participants are given in Tables I a and b to VII a and b, in Annex A. Each set of results is identified by the laboratory code and, the abbreviation of the analytical method.

8.1 Technical Evaluation

The results were subjected to detailed technical evaluation at a meeting of the participants, where inaccurate results and the reasons for them were identified as far as possible, in order to enable selection of the most reliable certification data.

Particular attention was paid to possible systematic errors arising either because of inaccuracies in a method or because of weaknesses in the application of an otherwise satisfactory procedure. For detection of systematic errors, the results of each laboratory for a given element and method were also compared in the two reference materials, as far as differences in elemental content allowed.

The results of Labs. 11 and 13 for Pb and Cd were generally discordant in both reference materials. This suggested an underlying weakness in the methods employed and their results for these elements were discarded.

The sets of results of Lab. 17 (AAS - solvent extraction) for Cd and Pb were generally more scattered than expected. After detailed technical and statistical scrutiny, it was concluded that the laboratory had worked near the limits of determination and all its results for Pb and Cd were rejected.

The conclusions of the technical evaluation of each element and material are as follows.

N.B. Results submitted for certification are given in the Tables indicated (Tables I a & b to VII a & b, in Annex A).

a) Cadmium (Methods : Table 5a)

Cd in RM 189 Wholemeal Flour (70 ng/g) (Results - Table Ia)

All results other than those of Labs. 11, 13 and 17 were accepted.

Cd in RM 191 Brown Bread (30 ng/g) (Results - Table Ib)

Results of Labs. 11, 13 and 17 were rejected.

b) Lead (Methods : Table 5a)

Lab. 33 reported a recurring problem with lead contamination and withdrew its results.

Pb in RM 189 Wholemeal Flour (380 ng/g) (Results - Table IIa)

All results, other than those of Labs. 11, 13, 17 and 33, were accepted on technical grounds.

Pb in RM 191 Brown Bread (185 ng/g) (Results - Table IIb)

The results of Labs. 11, 13, 17 and 33 were rejected. Labs. 1 and 4 had initially recorded low results by ASV after wet digestion. On investigation, both laboratories found that their original digestion methods (open digestion for 12 hours with $HNO_3/HClO_4$ and, bomb digestion for 12 hours at 160 °C with $HNO_3/HClO_4/H_2SO_4$ respectively) had been insufficient to destroy all organic matter, residues of which had interfered in the ASV determination.

On modification of the digestion conditions (see Method Descriptions, Table 5a), higher results were obtained which agreed with those of other techniques. The results of Lab. 16 (16A-ETAAS after oxygen combustion) were inconsistent with the other results from this laboratory and were withdrawn on grounds of insufficient sensitivity.

c) Selenium (Methods : Table 5b)

Se in RM 189 Wholemeal Flour (130 ng/g) (Results - Table IIIa)

All results submitted were accepted on technical grounds.

Se in RM 191 Brown Bread (25 ng/g) (Results - Table IIIb)

In spite of the relatively small range of the results for this Se content (12 to 32 ng/g), there was insufficient overlap of the sets of results to allow calculation of a mean value for certification purposes. This problem was exacerbated by the very high precision achieved for the four lower sets of results. The Se content of RM 191 is given as an indicative value (25 ng/g).

d) Copper (Methods : Table 5c)

Lab. 39 withdrew its widely spread results for both reference materials on grounds of insufficient sensitivity of its AAS method. (The widely spread results of lab 14 (DCP) were eventually rejected on statistical grounds in both materials, see section 8.2.2).

Cu in RM 189 Wholemeal Flour (6 µg/g) (Results - Table IVa)

All results other than those of Lab. 39 were accepted on technical grounds.

Cu in RM 191 Brown Bread (2.5 µg/g) (Results - Table IVb)

The results of Lab. 2 by ICP were low and inconsistent with its ASV results. It was noted that all the ICP results of Lab. 2 were low for Cu, Zn, Fe and Mn in Brown Bread and were rejected on grounds of suspected incomplete digestion of this material. The results of Lab. 23 were considered to be unrealistically low and, together with those of Lab. 39, were rejected.

e) Zinc (Methods : Table 5c)

Both Labs. 7 (INAA) and 17 (AAS) had found their methods to give consistently high results when judged against other certified reference materials, and withdrew their results.

Zn in RM 189 Wholemeal Flour (56 µg/g) (Results - Table Va)

The results of Lab. 7 and 17 were withdrawn. Both sets of Lab. 4 (INAA and ICP) were inexplicably high and, being inconsistent with previous results obtained by the laboratory on this material, were withdrawn on grounds of a suspected calibration error.

Zn in RM 191 Brown Bread (19 µg/g) (Results - Table Vb)

In addition to the results of Labs. 7 and 17, the AAS results of Lab. 39 were withdrawn because of an unacceptably high spread at this Zn content. The low results of Lab. 2 were rejected because of suspected incomplete digestion of the material (see also results for Cu, Fe and Mn in RM 191).

f) Iron (Methods : Table 5c)

Fe in RM 189 Wholemeal Flour (68 µg/g) (Results - Table VIa)

All results submitted were accepted on technical grounds.

Fe in RM 191 Brown Bread (40 µg/g) (Results - Table VIb)

The low results of Lab. 2 by ICP were rejected because of suspected incomplete digestion of RM 191.

f) Manganese (Methods : Table 5c)

The results of Lab.14 by DCP were inexplicably high in both materials and were eliminated.

Mn in RM 189 Wholemeal Flour (63 µg/g) (Results - Table VIIa)

Inspite of the higher than expected between-laboratory dispersion for the AAS methods, there was no evidence of method dependent effects and all results (other than those of Lab. 14) were accepted.

Mn in RM 191 Brown Bread (20 µg/g) (Results - Table VIIb)

The results of Lab. 14 were rejected as were the low ICP results of Lab. 2 (cf. Lab. 2 results for Cu, Zn and Fe).

8.2 Statistical Evaluation

8.2.1 General

The sets of results found acceptable on technical and statistical grounds are represented in the form of "bar-charts" in Figs. I-VII of Annex A. The length l_i of a bar corresponds to the 0.95 confidence interval of the mean, computed as:

$$l_i = 2 \cdot t^{\nu_i}_{1-\alpha/2} \cdot s_i / \sqrt{n_i}$$

where $t^{\nu_i}_{1-\alpha/2}$ is the value of the student distribution for $\nu_i = n_i - 1$ degrees of freedom and a significance level of $\alpha = 0.05$.

For each accepted set of results (i), the mean value was calculated as the arithmetic mean of the individual measurements, i.e. as

$$\bar{x}_i = \frac{1}{n_i} \sum_{j=1}^{n_i} x_{ij}$$

n_i being the number of measurements carried out in the ith' laboratory (n_i=1...j). The corresponding standard deviation was estimated as :

$$s_i = \sqrt{\left\{ \frac{\sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)^2}{n_i - 1} \right\}}$$

The sets of results found acceptable on technical grounds have been further submitted to the following statistical tests :

- Kolmogorov-Smirnov-Lilliefors tests to assess the conformity of the distributions of individual results and of laboratory means to normal distributions;
- Nalimov test to detect 'outlying' values in the population of individual results and in the population of laboratory means;
- Cochran test to detect 'outlying' values in the laboratory variances (s_i);

- Bartlett test to assess the overall consistency of the variance values obtained in the participating laboratories;
- one way analysis of variance (F-test) to compare and estimate the between- and the within-laboratory components of the overall variance of all individual results.

The estimates of the within-laboratory standard deviation S_W and of the between-laboratory standard deviation S_B, derived from one way analysis of variance, show that for most elements certified in the two reference materials, the between-laboratory variation was the major source of variability between results. The laboratory mean values (and not the individual results) were used for the calculation of the overall mean of the results.

Pertinent information derived from the normality test and the Nalimov test applied to laboratory means populations are reported in Figs. I-VII, Annex A, as well as the outcome of Cochran and Bartlett's tests on the laboratory variance.

For Cochran and Nalimov tests, a value is called an 'outlier' when the hypothesis that it belongs to the populations of results considered, can be rejected with a 0.01 risk of error. For a 'straggler', the risk lies between 0.01 and 0.05.

When an 'outlying' value was detected, it was not rejected automatically. In particular, an 'outlier' of variance was only eliminated if the standard error of the mean ($s_i / \sqrt{n_i}$) of the set exceeded the standard deviation of the distribution of all laboratory means. (s)

Each certified value was calculated as the arithmetic means of the corresponding $\bar{x}_i$'s, i.e. as

$$\bar{x} = \frac{1}{p} \sum_{i=1}^{p} \bar{x}_i$$

where p is the number of sets accepted for certification after both statistical and technical scrutiny. $\bar{x}$ is featured as a vertical dotted line on the bar graphs (Fig. I to VII, Annex A).

The uncertainty of this estimate is the 0.95 confidence interval of the mean $\bar{x}$ with the following limits :

$$\bar{x} - t^{\nu}_{1-\alpha/2} \cdot s/\sqrt{p} \qquad \text{and} \qquad \bar{x} + t^{\nu}_{1-\alpha/2} \cdot s/\sqrt{p}$$

$t^{\nu}_{1-\alpha/2}$ is the value of the Student's t distribution for $\nu = p-1$ degrees of freedom and a significance level $\alpha = 0.05$. s is the standard deviation of the distribution of laboratory mean values. It is estimated as :

$$s = \sqrt{\left\{ \frac{\sum_{i=1}^{p} (\bar{x}_i - \bar{x})^2}{p - 1} \right\}}$$

8.2.2 Detailed Statistical Analysis

In view of the use of different methods of analysis for the certification of the various elemental contents, it is to be expected that the laboratory variances would vary considerably. Where the variances of the accepted results were not homogeneous, the within-laboratory standard deviations S_W (given in Figs. I-VII, Annex A) must be taken as indicative only.

Several sets of results were identified as variance outliers. The decision as to whether they should be rejected was based on both technical and statistical grounds (see § 8.2.1).

No outliers of mean were detected in the accepted results.

Cadmium (Figs. Ia and b)

RM 189 : The variances were not homogeneous. Although the set of Lab 33 was an outlier of variance, it was retained because the standard error did not exceed s.

RM 191 : The variances were not homogeneous. The straggling variance of Lab 33 satisfied the condition that the standard error did not exceed s.

Lead (Figs. IIa and b)

RM 189 : The variances were homogeneous and the within laboratory standard deviation (s_w) was greater than the between laboratory value (s_b). Although 5 sets of results did not satisfy the condition that their standard error did not exceed s, it was not considered justifiable to reject them under these circumstances, especially as their set-means were in good agreement with the overall mean. There were no outliers of variance.

RM 191 : The variances were homogeneous. There were no outliers of variance.

Selenium (Fig. IIIa)

RM 189 : The variances were not homogeneous. No outliers of variance were detected.

RM 191 : Not certified for Se

Copper (Figs. IV and b)

RM 189 : The variances were not homogeneous. The set of Lab 14 was rejected because its standard error exceeded s.

RM 191 : The variances were not homogeneous. The set of Lab 14 was rejected because its standard error exceeded s.

Zinc (Figs. Va and b)

RM 189 : The variances were not homogeneous. There were no outliers of variance.

RM 191 : The variances were not homogeneous. There were no outliers of variance.

Iron (Figs. VI and b)

RM 189 : The variances were not homogeneous. There were no outliers of variance.

RM 191 : The variances were not homogeneous. The set of Lab 5 (INAA) was an outlier of variance but was retained since its standard error did not exceed s.

Manganese (Figs. VIIa and b)

RM 189 : The variances were not homogeneous. The set of Lab 33 was an outlier of variance but was retained since its standard error did not exceed s.

RM 191 : The variances were not homogeneous. There were no outliers of variance.

9 THE CERTIFIED VALUES

The certified values and the corresponding uncertainties are presented in table 6.

The uncertainties are expressed as the 95% confidence intervals.

Table 6. Certified Elemental Contents and Uncertainties in RMs 189 and 191

Element	CRM 189 Wholemeal Flour Certified Value	Uncertainty	P	CRM 191 Brown Bread Certified Value	Uncertainty	P
Cd	71.3 ng/g	+/- 3.0 ng/g	12	28.4 ng/g	+/- 1.4 ng/g	12
Pb	379 ng/g	+/- 12 ng/g	13	187 ng/g	+/- 14 ng/g	12
Se	132 ng/g	+/- 10 ng/g	11	--		
Cu	6.4 µg/g	+/- 0.2 µg/g	10	2.6 µg/g	+/- 0.1 µg/g	8
Zn	56.5 µg/g	+/- 1.7 µg/g	13	19.5 µg/g	+/- 0.5 µg/g	13
Fe	68.3 µg/g	+/- 1.9 µg/g	13	40.7 µg/g	+/- 2.3 µg/g	12
Mn	63.3 µg/g	+/- 1.6 µg/g	12	20.3 µg/g	+/- 0.7 µg/g	11

10 INDICATIVE VALUES

Several participants determined elements additional to those required for certification.

Insufficient data are available for certification, however, these indicative values will be of assistance to laboratories concerned either with the assay of these elements in cereals and similar materials or, with the potential interference of the elements in the determination of the certified properties.

Apart from As and Cr, where, in each case, only one laboratory provided data, the indicative values are based on 2 - 4 sets of results, generally involving different methods.

As and Cr were determined by laboratories which are particularly experienced with these assays. It should be noted that Lab. 3, which determined Cr by a RNAA procedure, found evidence of inhomogeneity for this element for sample intakes of 200 mg and the results are consequently expressed in terms of the range. The results are presented in Tables 7a and 7b.

In view of the interest of the determination of Hg at very low levels, the results for this element are presented in more detail in Table 8, together with a brief description of the methods used. Technical evaluation of the results with the participants led to the conclusion that the results of Lab. 5 (RNAA) were too high and that the Hg content of RMs 189 and 191 are around 1 and 2 ng/g respectively.

Table 9 summarises the elemental contents of the two reference materials. The indicative values given are simply the arithmetic means of the individual set means. No attempt has been made to ascribe uncertainties to these values. It is suggested that the user employs the indicative values in conjunction with the more detailed data presented in Tables 7a and 7b.

Table 7a : Indicative Values (Mass fractions) for RM 189 Wholemeal Flour (expressed on a dry-mass basis)

Element	Lab.	Method	Results Mean	% CV
As (ng/g)	1	H-AAS	18	0.9
Ca (µg/g)	17	ICP	519	2
	22	AAS	525	1
	33	AAS	512	0.5
Cl (mg/g)	14	Volhardt Titration	0.69	25
	17	SPEC (via HgCNS)	0.61	3
Cr (ng/g)	3	RNAA	57-73	(n = 6)
Mg (mg/g)	17	ICP	1.9	5
	22	AAS	1.9	1
	33	AAS	1.8	4
Na (µg/g)	14	DCP	64	3
	17	AAS	30	30
	22	AES	28	30
	33	AAS	30	28
Ni (ng/g)	1	ASV	335	4
	4	ASV	440	11
	33	ET-AAS	348	14
P (mg/g)	17	SPEC (Mo Blue)	5.4	0.3
	33	SPEC (Mo Blue)	5.2	6
K (mg/g)	17	AAS	6.4	2
	22	AES	6.4	2
	33	AAS	6.0	11

Table 7b : Indicative Values (Mass fractions) for RM 191 Brown Bread (expressed on a dry-mass basis)

Element	Lab.	Method	Results Mean	% CV
As (ng/g)	1	H-AAS	23	5
Ca (µg/g)	17	ICP	408	3
	22	AAS	407	1
	33	AAS	415	4
Cl (mg/g)	14	Volhardt Titration	15.2	2
	17	SPEC (via HgCNS)	17.7	9
Cr (ng/g)	3	RNAA	68-360	(n = 6)
Mg (µg/g)	17	ICP	487	3
	22	AAS	542	2
	33	AAS	515	3
Na (mg/g)	14	DCP	9.1	2
	17	AAS	10.1	8
	22	AES	10.2	2
	33	AAS	10.8	6
Ni (ng/g)	1	ASV	406	3
	4	ASV	510	10
	33	ET-AAS	410	17
P (mg/g)	17	SPEC (Mo Blue)	2.1	3
	33	SPEC (Mo Blue)	2.0	6
K (mg/g)	17	AAS	3.1	7
	22	AES	3.2	1
	33	AAS	3.0	3
	33	ISE	3.1	0.1
Se (ng/g)	15	FLUOR	32	25
	32	FLUOR	19	8
	1	HAAS	15	3
	16A	HAAS	15	3
	19	HAAS	32	17
	4	HAAS	34	6
	16B	HAAS	12	14
	4	INAA	32	11
	9	RNAA	29	44

Table 8 : Summary of individual results and methods used for determination of Hg in RMs 189 and 191

Hg in RM 189 Wholemeal Flour (ng/g)

Lab.	Method	Results ng/g					$\bar{x}$	SD
1	CV-AAS (Bomb dign, HNO_3 140 °C for 2 hours)	0.91	0.92	1.1	1.09	1.03	1.01	.09
4	CV-AAS (Bomb dign., HNO_3 160 °C for 12 hours)	2					2	
16a	CV-AAS (Bomb dign., HNO_3 160 °C for 2 hours; concn. on Au)	0.91	1.21	0.91	0.67	0.66	0.87	0.22
16b	CV-AAS (Oxygen combustion, concn. on Au)	1.99	1.77	1.83	1.88	1.94	1.87	0.11
5[1]	RNAA	8.58	7.81	8.52	8.35	7.93	8.24	0.3

Hg in RM 191 Brown Bread ng/g

1	As for RM 189	1.57	1.68	1.6	1.56	1.63	1.6	0.05
4	" "	2					2	
16a	" "	1.18	1.18	1.34	1.08	1.14	1.18	0.10
16b	" "	2.92	3.54	3.26	3.02	3.47	3.24	0.27
5[1]	" "	11.65	10.31	9.97	11.41	11.73	10.97	0.90

[1] not included in calculation of indicative value

Table 9 : Summary of elemental contents (mass fractions) expressed on a dry-mass basis

Element	Wholemeal flour (RM 189)			Brown bread (RM 191)		
	mean	uncertainty	n	mean	uncertainty	n
CERTIFIED						
Cd ng/g	71.3	3.0	12	28.4	1.4	12
Pb ng/g	379	12	13	187	14	12
Se ng/g	132	10	11	-	-	-
Cu µg/g	6.4	0.2	10	2.6	0.1	8
Zn µg/g	56.5	1.7	13	19.5	0.5	13
Fe µg/g	68.3	1.9	13	40.7	2.3	12
Mn µg/g	63.3	1.6	12	20.3	0.7	11
INDICATIVE[1]						
As ng/g	18	-	1	23	-	1
Ca mg/g	0.52	-	3	0.41	-	3
Cl mg/g	0.7	-	2	16.5	-	2
Cr[2] ng/g	57 - 73	-	1	68 - 360	-	1
Hg ng/g	1	-	4	2	-	4
Mg mg/g	1.9	-	3	0.5	-	3
Na mg/g	0.04	-	4	10	-	4
Ni µg/g	0.38	-	3	0.44	-	3
P ng/g	5.3	-	2	2.1	-	2
K mg/g	6.3	-	3	3.1	-	
Se ng/g	certified (see above)		-	25[3]	-	8

[1] **N.B.** The indicative values are the arithmetic means of the means of accepted results submitted by the laboratories and accepted. No attempt has been made to ascribe uncertainties to them.

[2] Suspected inhomogeneity; range of results is given.

[3] See also Fig III Annex A (Bar chart of Results)

11 INSTRUCTIONS FOR USE

11.1 Storage, Handling and Minimum Sample Intake

CRMs 189 and 191 are supplied in dark glass, screw-cap bottles in units of circa 40 g. Samples should be stored in the cool and dark. Care must be exercised to avoid contamination of the materials during storage and handling. Particular care must be taken if it is desired to use them to determine the laboratory background for Hg.

The contents of the bottles should be thoroughly mixed before each test portion is taken.

The minimum sample intake is 200 mg.

11.2 Correction for Moisture Content

The certified values are expressed on a dry-mass basis.

The dry-mass correction is to be made on a separate sample of the contents of the bottle immediately before the analytical test portion is taken. The dry-mass correction is determined by the following procedure :

1) Principle

The residual mass of a test portion is determined after drying at atmospheric pressure in an oven at 103°C +/- 2°C to constant mass. The correction factor is given by the mass before drying divided by the mass after drying to constant mass.

2) Apparatus

i) Analytical balance

ii) Metal dishes - preferably of nickel or stainless steel.
The dishes must have lids which fit very well but which can readily be removed. Suitable dimensions are : diameter 60 to 30 mm and depth about 25 mm.

iii) Atmospheric pressure drying oven, well ventilated, thermostatically controlled, with temperature regulated at 103 +/- 2°C. The temperature should be uniform throughout the oven.

iv) Desiccator, containing an efficient, self-indicating drying agent.

3) Procedure

i) Uncover the dish and place it and its lid in the oven for about two hours.

ii) Place the lid on the dish and transfer the covered dish to the desiccator. Allow it to cool to room temperature and weigh it to the nearest mg.

iii) Put approximately 2 g of the sample in the dish, spread evenly over the bottom, cover the dish with the lid and weigh quickly to the nearest mg.

iv) Uncover the dish and put it and its lid in the oven for 2 hours.

v) Replace the lid, transfer the covered dish to the desiccator, allow it to cool to room temperature and weigh quickly to the nearest mg.

vi) Uncover the dish and heat it and its lid for further half-hour in the oven.

vii) Repeat process 3. v

viii) Repeat process 3. vi and 3. v until the decrease in mass between two successive weighings does not exceed 5 mg. If an increase in mass occurs, (e.g. because of oxidation), use the lowest mass obtained in the calculation of the dry mass correction.

4. Calculation of Results

The dry mass correction factor F is given by

$$F = \frac{\text{Mass of sample before drying}}{\text{Mass of sample after drying to constant mass}}$$

F should be expressed to three places of decimals.

11.3 Use of the Certified Values

Reference materials 189 and 191 may be employed in either of two ways :

a) For calibration purposes, in which case the values to be used are the certified mean values with the corresponding uncertainty

b) To check the performance of a method, provided that the user can demonstrate that the repeatability of the method is compatible with the repeatabilities of the certifying laboratories.

For this latter purpose, it should be verified that the standard error of the mean of the user's results ($s_i / \sqrt{n_i}$) is less than the standard deviation s as given in the Figs. I-XII, Annex A, for a particular element and material. Then, as an assessment of the performance of their technique, the users can check whether the mean of their results (y) lies within the limits :

(certified value $-2s$) $< y <$ (certified value $+2s$)

which is the interval containing about 95% of the population of the laboratory means.

References

1. The Certification of Trace Elements in three Samples of Skim Milk Powder. B. Griepink, E. Colinet, H. Marchandise, H. Gonska, H. Muntau : Milchw. 3g (11) 1984, 662

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ANNEX A

Bar charts and statistical summaries of results accepted for certification
(Figs. I-VII)

Summaries of results submitted for certification
(Tables 1 a, b to VII a, b)

CD IN WHOLEMEAL FLOUR NG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
4 ASV	5	78.00000	69.00000	73.00000	66.00000	75.00000	72.20000	4.76655
7 ASV	6	74.00000 72.00000	75.00000	73.00000	73.00000	73.00000	73.33334	1.03280
1 ASV	5	78.60000	77.80000	77.40000	79.30000	74.10000	77.43999	2.00574
2 ASV	5	71.30000	67.40000	70.70000	73.20000	72.10000	70.94000	2.18929
32 ASV	5	74.00000	71.60000	71.50000	71.40000	70.40000	71.78000	1.33116
43 ASV	5	79.00000	69.00000	67.00000	72.00000	70.00000	71.40000	4.61519
16 AAS	5	68.40000	70.00000	66.20000	65.60000	66.20000	67.27999	1.85796
17 AAS	5	58.00000	69.00000	69.00000	79.00000	62.00000	67.40000	8.01873
13 ETAAS	10	118.70000 135.10000	111.10000 140.10000	98.60000 141.20000	126.50000 142.20000	103.90000 141.10000	125.65000	16.72857
16 ETAAS	5	58.00000	60.80000	60.20000	66.50000	66.10000	61.52000	2.74718
33 ETAAS	20	79.00000 83.00000 81.00000 77.00000	87.00000 68.00000 75.00000 79.00000	97.00000 71.00000 81.00000 75.00000	84.00000 63.00000 72.00000 66.00000	87.00000 83.00000 83.00000 73.00000	78.20000	8.11496
4 ZAAS	5	64.00000	63.00000	70.00000	63.00000	68.00000	65.60000	3.20936
11 ZAAS	5	80.00000	77.00000	73.00000	79.00000	74.00000	76.60000	3.04959
7 IDMS	6	74.00000 73.00000	76.00000	78.00000	73.00000	72.00000	74.33334	2.25093
10 IDMS	5	70.70000	72.30000	70.50000	70.40000	72.20000	71.21999	.94710

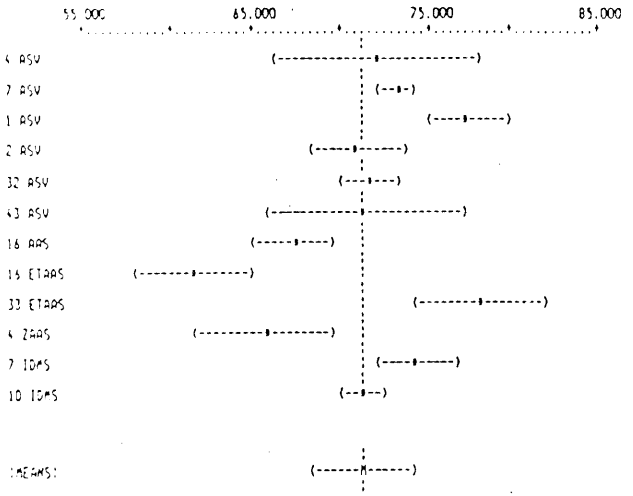
Table Ia : RM 189

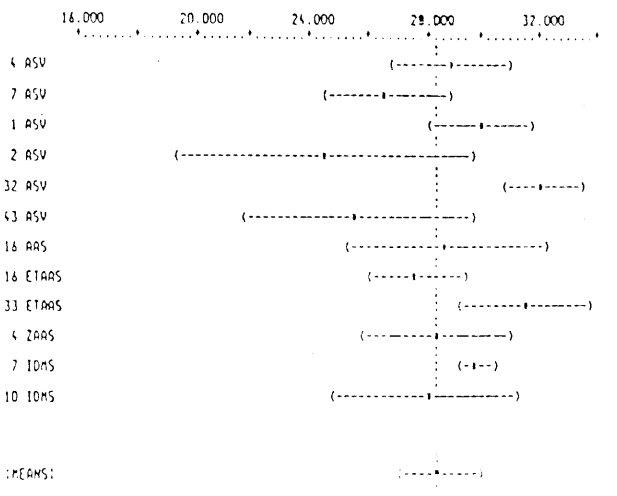
CD IN BROWN BREAD NG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
4 ASV	5	26.00000	29.00000	30.00000	29.00000	30.00000	28.80000	1.64317
7 ASV	6	24.00000 26.00000	26.00000	28.00000	30.00000	26.00000	26.66667	2.06559
1 ASV	5	30.70000	28.70000	28.10000	30.60000	31.30000	29.88000	1.39126
2 ASV	5	21.40000	29.10000	19.00000	25.60000	26.70000	24.40000	4.10192
32 ASV	5	30.10000	33.20000	31.90000	32.50000	32.40000	32.02000	1.06719
43 ASV	5	23.00000	27.00000	30.00000	22.00000	26.00000	25.60000	3.00998
16 AAS	5	28.30000	33.50000	26.70000	27.60000	27.20000	28.66000	2.79821
17 AAS	5	7.90000	33.00000	31.00000	28.00000	23.00000	24.58000	10.05644
13 ETAAS	10	30.60000 32.80000	29.10000 37.10000	30.00000 39.10000	34.90000 33.90000	30.40000 30.90000	32.88000	3.03457
16 ETAAS	5	28.30000	29.30000	28.10000	25.70000	27.10000	27.70000	1.36382
33 ETAAS	20	27.00000 32.00000 46.00000 30.00000	30.00000 33.00000 29.00000 34.00000	32.00000 32.00000 29.00000 26.00000	24.00000 36.00000 31.30000 27.00000	26.00000 33.00000 39.00000 32.00000	31.42000	4.96831
4 ZAAS	5	30.00000	28.00000	27.00000	31.00000	26.00000	28.40000	2.07364
11 ZAAS	5	18.00000	21.00000	18.00000	16.00000	21.00000	18.80000	2.16795
7 IDMS	6	30.60000 30.00000	28.90000	29.30000	29.20000	30.20000	29.70000	.66333
10 IDMS	5	24.70000	27.70000	29.30000	26.80000	31.50000	28.00000	2.56125

Table Ib : RM 191

Tables Ia and Ib : Results Submitted for Certification of Cd in RMs 189 and 191.





CD IN WHOLEMEAL FLOUR MG/G RM 189

Number of accepted sets of results	P	:	12
Number of accepted replicates	N	:	77
Outlying variance (Cochran test)		:	8.11496 is an outlier
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	71.27055
Std.dev.of distribution of means	S	:	4.71092
Within-labs std.deviation	Sw	:	4.94431
Between-labs std.deviation	Sb	:	4.65747
0.95 confidence interval of the mean		:	71.27055 +/- 2.99318

CD IN BROWN BREAD MG/G RM 191

Number of accepted sets of results	P		12
Number of accepted replicates	N		77
Outlying variance (Cochran test)		:	4.98841 is a straggler
Homogeneity of variances (Bartlett test)			Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)			Normal
Outlying mean values (Nalimov test)		:	None
Mean of means			28.43555
Std.dev.of distribution of means	S		2.20931
Within-labs std.deviation	Sw		3.30669
Between-labs std.deviation	Sb		[illegible]
0.95 confidence interval of the mean			28.43555 +/- 1.40373

Fig. I : Bar Charts and Statistical Summaries for Accepted Results for Cd

PB IN WHOLEMEAL FLOUR MG/G

DATA SET	NUM	REPLICATES.....					MEAN	ST.DEV
4 ASV	5	350.00000	390.00000	410.00000	430.00000	340.00000	384.00000	38.47077
7 ASV	6	429.00000 399.00000	437.00000	370.00000	380.00000	360.00000	395.83330	31.66438
1 ASV	5	378.00000	399.00000	372.00000	372.00000	414.00000	387.00000	18.73499
2 ASV	5	405.00000	432.00000	354.00000	390.00000	409.00000	398.00000	28.83574
32 ASV	5	353.00000	353.00000	354.00000	360.00000	379.00000	359.80000	11.12205
43 ASV	5	420.00000	370.00000	440.00000	400.00000	380.00000	402.00000	28.63564
16 AAS	5	399.00000	372.00000	361.00000	410.00000	421.00000	392.60000	25.36336
17 AAS	5	311.00000	343.00000	346.00000	403.00000	275.00000	335.60000	47.38987
13 ETAAS	10	248.20000 221.70000	282.00000 213.40000	262.20000 249.90000	284.20000 213.50000	250.60000 209.60000	243.53000	27.95302
16A ETAA	5	384.00000	379.00000	406.00000	400.00000	385.00000	390.80000	11.56287
16B ETAA	5	383.00000	345.00000	345.00000	405.00000	423.00000	380.20000	35.11695
23 ETAAS	6	308.00000 344.00000	349.00000	346.00000	346.00000	369.00000	343.66670	19.76529
33 ETAAS	20	425.00000 362.00000 430.00000 408.00000	388.00000 379.00000 402.00000 442.00000	359.00000 354.00000 326.00000 427.00000	327.00000 310.00000 467.00000 371.00000	353.00000 422.00000 425.00000 426.00000	390.15000	43.64965
4 ZAAS	5	360.00000	360.00000	390.00000	400.00000	330.00000	368.00000	27.74887
11 ZAAS	5	462.00000	485.00000	493.00000	435.00000	427.00000	460.40000	29.28822
7 IDMS	6	369.00000 377.00000	434.00000	390.00000	390.00000	347.00000	384.50000	29.02929
10 IDMS	5	343.40000	341.40000	330.30000	349.20000	357.10000	344.28000	9.91298

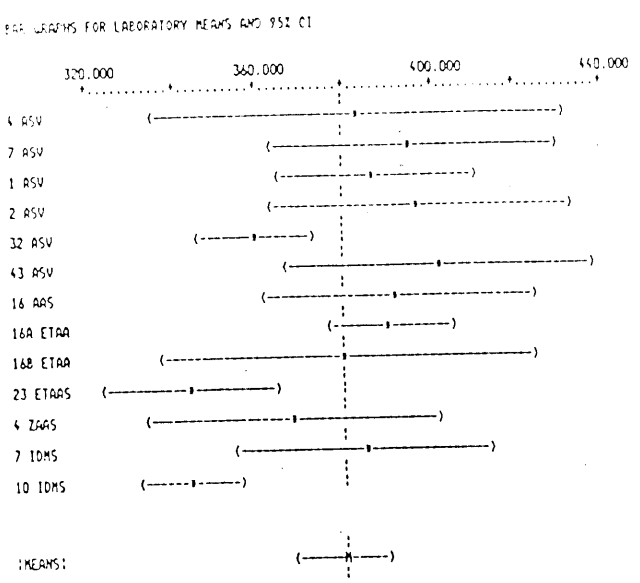
Table IIa : RM 189

PB IN BROWN BREAD MG/G

DATA SET	NUM	REPLICATES.....					MEAN	[illegible]
4 ASV	3	185.00000	190.00000	191.00000			188.66670	[illegible]
7 ASV	6	170.00000 185.00000	215.00000	171.00000	209.00000	200.00000	191.66670	[illegible]
1 ASV	5	187.00000	218.00000	185.00000	202.00000	232.00000	204.80000	[illegible]
2 ASV	5	146.00000	220.00000	119.00000	189.00000	165.00000	167.80000	[illegible]
32 ASV	5	161.00000	139.00000	163.00000	184.00000	159.00000	161.20000	[illegible]
43 ASV	5	150.00000	170.00000	160.00000	180.00000	130.00000	158.00000	[illegible]
16 AAS	5	220.00000	168.00000	141.00000	166.00000	183.00000	175.60000	[illegible]
17 AAS	5	130.00000	140.00000	150.00000	160.00000	170.00000	150.00000	[illegible]
13 ETAAS	10	181.10000 186.60000	132.00000 158.30000	148.70000 96.80000	132.50000 123.00000	177.90000 96.90000	143.38000	[illegible]
16A ETAA	5	252.00000	256.00000	259.00000	275.00000	210.00000	250.40000	[illegible]
16B ETAA	5	192.00000	183.00000	227.00000	227.00000	138.00000	193.40000	[illegible]
23 ETAAS	6	263.00000 223.00000	204.00000	230.00000	182.00000	235.00000	222.83330	[illegible]
33 ETAAS	20	243.00000 146.00000 376.00000 338.00000	219.00000 153.00000 183.00000 373.00000	223.00000 167.00000 146.00000 230.00000	208.00000 243.00000 231.00000 241.00000	253.00000 153.00000 201.00000 244.00000	228.55000	[illegible]
4 ZAAS	5	170.00000	180.00000	150.00000	220.00000	140.00000	172.00000	[illegible]
11 ZAAS	5	136.00000	132.00000	120.00000	126.00000	124.00000	127.60000	[illegible]
7 IDMS	6	179.00000 171.00000	233.00000	195.00000	176.00000	207.00000	193.50000	[illegible]
10 IDMS	5	233.00000	228.00000	199.00000	189.00000	254.00000	220.40000	[illegible]

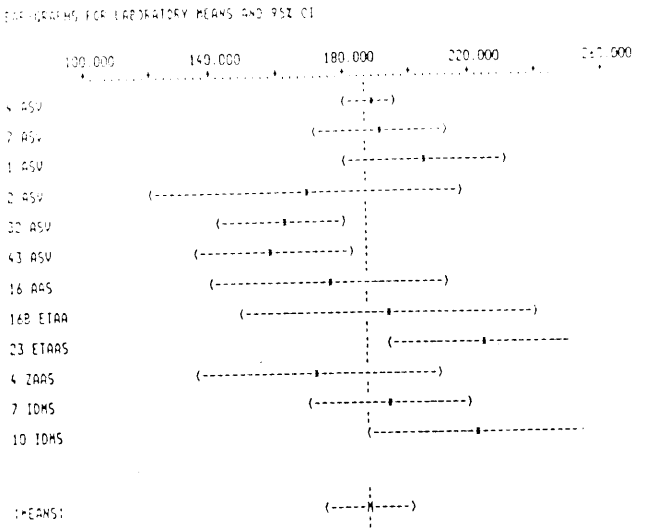
Table IIb : RM 191

Tables IIa and IIb : Results Submitted for the Certification of Pb in RMs 189 and 191



PB IN WHOLEMEAL FLOUR MG/G RM 189

Number of accepted sets of results	P	:	13
Number of accepted replicates	N	:	68
Outlying variance (Cochran test)		:	No outliers , no stragglers
Homogeneity of variances (Bartlett test)		:	Homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	379.28310
Std.dev.of distribution of means	S	:	19.49524
Within-labs std.deviation	Sw	:	25.97633
Between-labs std.deviation	Sb	:	16.10151
0.95 confidence interval of the mean		:	379.28310 +/- 11.78088



FE IN BROWN BREAD MG/G RM 191

Number of accepted sets of results	P	:	12
Number of accepted replicates	N	:	61
Outlying variance (Cochran test)		:	No outliers , no stragglers
Homogeneity of variances (Bartlett test)		:	Homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	187.48890
Std.dev.of distribution of means	S	:	21.42973
Within-labs std.deviation	Sw	:	26.40823
Between-labs std.deviation	Sb	:	18.36343
0.95 confidence interval of the mean		:	187.48890 +/- 13 [illegible]

Fig II : Bar Charts and Statistical Summaries of Accepted Results for Pb

SE IN WHOLEMEAL FLOUR MG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
15 FLUOR	10	130.00000 140.00000	110.00000 120.00000	140.00000 120.00000	130.00000 120.00000	140.00000 130.00000	128.00000	10.32796
32 FLUOR	5	130.00000	135.00000	136.00000	141.00000	142.00000	136.80000	4.86826
1 HAAS	5	132.00000	130.00000	132.00000	127.00000	123.00000	128.80000	3.83406
16A HAAS	5	98.00000	103.00000	113.00000	110.00000	118.00000	108.40000	7.95613
19 HAAS	10	133.00000 138.00000	197.00000 132.00000	156.00000 163.00000	147.00000 156.00000	148.00000 137.00000	150.50000	19.34052
4 HAAS	4	128.00000	140.00000	161.00000	164.00000		148.25000	17.21191
33 HAAS	5	122.50000	140.00000	135.40000	138.30000	121.40000	131.52000	8.89816
16B HAAS	5	113.00000	111.00000	111.00000	111.00000	105.00000	110.20000	3.03315
3 IHAA	5	159.00000	157.00000	146.00000	145.00000	147.00000	150.80000	6.64831
7 IHAA	6	135.00000 137.00000	138.00000	149.00000	115.00000	107.00000	130.16670	15.82930
9 RHAA	5	139.00000	125.00000	127.00000	120.00000	124.00000	127.00000	7.17635

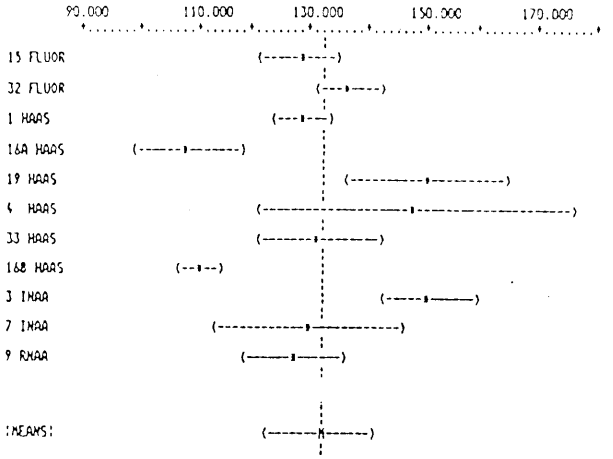
Table IIIa : RM 189

SE IN BROWN BREAD MG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
15 FLUOR	10	40.00000 30.00000	30.00000 40.00000	40.00000 20.00000	30.00000 40.00000	20.00000 30.00000	32.00000	7.88811
32 FLUOR	5	20.00000	21.00000	17.00000	18.00000	18.00000	18.80000	1.64317
1 HAAS	5	16.00000	15.00000	15.00000	15.00000	16.00000	15.40000	.54772
16A HAAS	5	15.20000	15.50000	15.00000	14.90000	16.20000	15.36000	.52249
19 HAAS	10	33.00000 38.00000	34.00000 26.00000	38.00000 25.00000	39.00000 35.00000	26.00000 26.00000	32.00000	5.67890
4 HAAS	5	33.00000	31.00000	33.00000	35.00000	34.00000	33.60000	1.94936
16B HAAS	5	11.00000	10.50000	14.50000	13.30000	12.90000	12.44000	1.66033
6 IHAA	5	35.00000	28.00000	29.00000	36.00000	31.00000	31.80000	3.56231
9 RHAA	5	37.00000	11.00000	22.00000	44.00000	32.00000	29.20000	12.94993

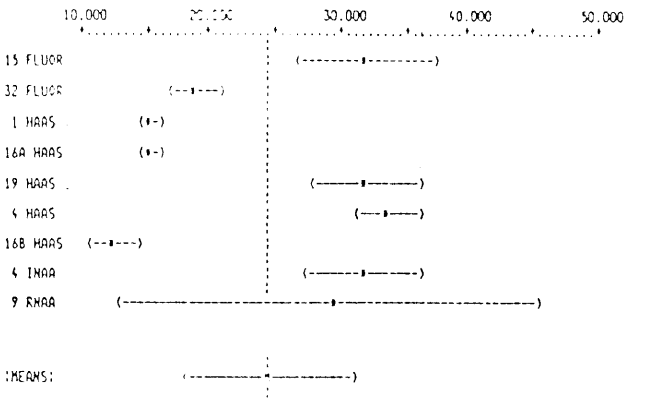
Table IIIb : RM 191

Tables IIIa and IIIb : Results Submitted for the Certification of Se in RMs 189 and 191



SE IN WHOLEMEAL FLOUR NG/G RM 189

Number of accepted sets of results	P	:	11
Number of accepted replicates	N	:	65
Outlying variance (Cochran test)		:	No outliers , no stragglers
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	131.85790
Std.dev.of distribution of means	S	:	14.38611
Within-labs std.deviation	Sw	:	11.87215
Between-labs std.deviation	Sb	:	13.28994
0.95 confidence interval of the mean		:	131.85790 +/- 9.66477



N.B. : Se in Brown Bread (RM 191) not certified
(See § 8.1)

Se in Brown Bread (ng/g) RM 191

Fig III : Bar Chart and Statistical Summary for Se in CRM 189.

N.B. : Bar Chart for Se in RM 191 given for information only.

CU IN WHOLEMEAL FLOUR µG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
32 AAS	5	6.46000	6.28000	6.37000	6.17000	6.11000	6.27800	.14272
17 AAS	5	6.50000	6.30000	6.30000	6.40000	6.50000	6.40000	.10000
22 AAS	10	6.64000 6.63000	6.63000 6.51000	6.51000 6.71000	6.67000 6.72000	6.75000 6.78000	6.65500	.09168
39 AAS	5	6.21000	5.14000	7.61000	6.69000	6.31000	5.99200	1.13882
23 ETAAS	6	6.60000 7.18000	6.48000	7.31000	6.29000	6.50000	6.72667	.41587
1 ASV	5	5.86000	6.31000	6.02000	5.81000	5.96000	5.99200	.19588
2 ASV	8	5.93000 5.95000	6.22000 6.61000	6.54000 6.00000	6.56000	6.14000	6.24375	.28720
14 DCP	4	6.39000	6.25000	7.06000	7.16000		6.71500	.46148
2 ICP	5	5.81000	6.40000	6.15000	5.87000	6.29000	6.10200	.25587
4 ICP	5	6.40000	6.60000	6.30000	6.50000	6.40000	6.44000	.11402
9 RNAA	5	6.42000	5.95000	6.17000	6.33000	6.26000	6.22600	.17953
10 IDMS	5	6.40000	6.40000	6.40000	6.47000	6.48000	6.43000	.04123

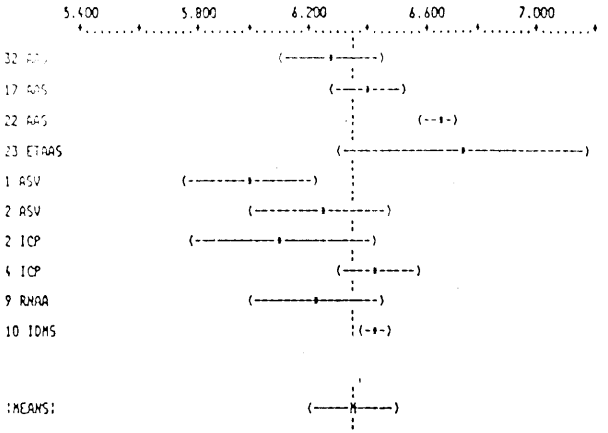
Table IVa : RM 189

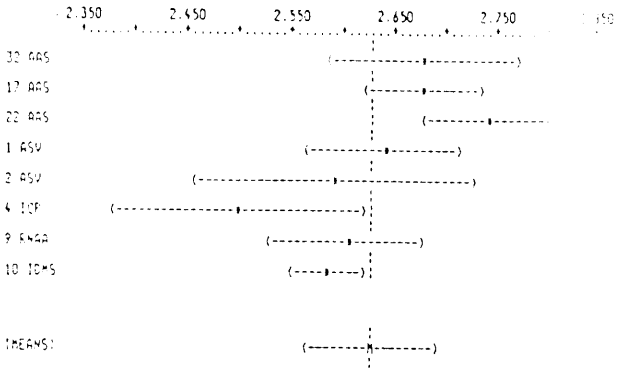
CU IN BROWN BREAD µG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
32 AAS	5	2.68000	2.81000	2.63000	2.65000	2.63000	2.68000	.07550
17 AAS	5	2.70000	2.70000	2.70000	2.60000	2.70000	2.68000	.04472
22 AAS	10	2.81000 2.90000	2.81000 2.72000	2.66000 2.81000	2.68000 2.77000	2.62000 2.66000	2.74400	.08959
39 AAS	5	2.68000	2.17000	2.10000	2.40000	3.57000	2.58400	.59601
23 ETAAS	7	1.99000 2.26000	2.04000 2.34000	2.25000	2.40000	2.47000	2.25000	.17933
1 ASV	5	2.74000	2.65000	2.61000	2.58000	2.63000	2.64200	.06058
2 ASV	10	2.57000 2.76000	2.59000 2.59000	2.94000 2.51000	2.73000 2.25000	2.57000 2.40000	2.59100	.19145
14 DCP	4	2.62000	2.99000	3.01000	2.65000		2.81750	.21125
2 ICP	5	2.32000	2.17000	2.48000	2.36000	2.24000	2.31600	.11845
4 ICP	5	2.60000	2.50000	2.60000	2.60000	2.40000	2.50000	.10000
9 RNAA	5	2.62000	2.54000	2.68000	2.64000	2.55000	2.60600	.05983
10 IDMS	5	2.62000	2.62000	2.59000	2.55000	2.56000	2.58400	.02881

Table IVb : RM 191

Tables IVa and IVb : Results Submitted for the Certification of Cu in RMs 189 and 191





CU IN WHOLEMEAL FLOUR µG/G RM 189

Number of accepted sets of results	P	:	10
Number of accepted replicates	N	:	59
Outlying variance (Cochran test)		:	.41587 is a straggler
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	6.34934
Std.dev.of distribution of means	S	:	.22935
Within-labs std.deviation	Sw	:	.21392
Between-labs std.deviation	Sb	:	.21937
0.95 confidence interval of the mean		:	6.34934 +/- .16407

CU IN BROWN BREAD µG/G RM 191

Number of accepted sets of results	P	:	8
Number of accepted replicates	N	:	50
Outlying variance (Cochran test)		:	.19145 is an outlier
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	2.62838
Std.dev.of distribution of means	S	:	.07496
Within-labs std.deviation	Sw	:	.10948
Between-labs std.deviation	Sb	:	.06483
0.95 confidence interval of the mean		:	2.62838 +/-

Fig IV : Bar Charts and Statistical Summaries of Accepted Results for Cu.

ZN IN WHOLEMEAL FLOUR µG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
32 AAS	5	55.20000	53.90000	54.50000	54.50000	54.00000	54.42000	.51672
17 AAS	5	58.00000	62.00000	60.00000	62.00000	61.00000	60.60000	1.67332
22 AAS	10	59.20000 58.50000	58.80000 58.60000	58.70000 56.80000	57.70000 56.20000	58.30000 57.80000	58.06000	.94560
23 AAS	7	59.90000 57.70000	57.80000 56.30000	56.70000	58.10000	59.10000	57.65714	1.72806
33 AAS	20	54.90000 56.20000 57.30000 60.80000	56.10000 56.00000 58.70000 59.90000	58.20000 56.40000 57.70000 59.10000	55.90000 56.00000 55.50000 59.60000	57.50000 53.20000 58.10000 58.20000	57.06499	2.07574
39 AAS	5	53.90000	56.50000	55.10000	52.70000	52.10000	54.06000	1.78550
43 AAS	5	56.70000	58.10000	57.60000	58.10000	58.10000	57.68000	.62610
1 ASV	5	54.90000	55.00000	52.20000	51.60000	52.60000	53.26000	1.58367
14 DCP	4	51.70000	53.10000	52.20000	51.00000		52.00000	.89318
2 ICP	5	57.30000	58.80000	59.90000	55.60000	56.70000	57.26000	2.15940
4 ICP	5	64.00000	63.00000	62.00000	65.00000	65.00000	63.80000	1.30384
3 INAA	5	63.70000	61.60000	60.20000	60.40000	60.90000	61.36000	1.41527
4 INAA	5	67.00000	63.00000	65.00000	66.00000	64.00000	65.00000	1.58114
5 INAA	5	54.40000	52.80000	53.10000	54.30000	53.30000	53.58000	.72595
7 INAA	6	68.20000 67.80000	68.00000	63.40000	70.00000	65.70000	67.18333	2.30340
9 INAA	5	58.10000	56.70000	58.40000	58.90000	58.10000	58.04000	.81731
10 IDMS	5	60.90000	58.90000	59.50000	59.40000	59.60000	59.66000	.74364

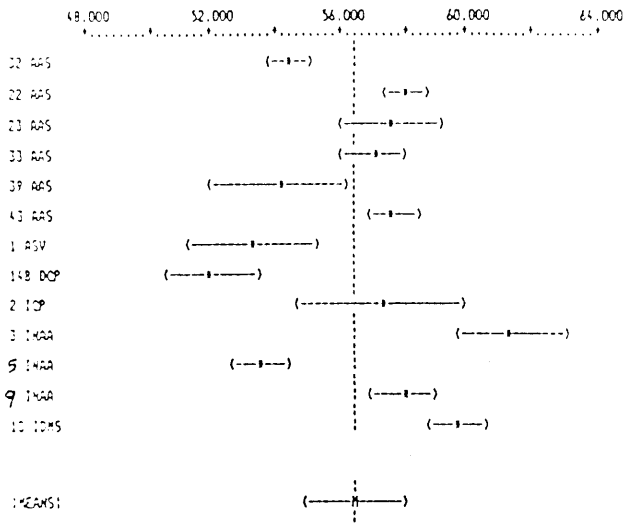
Table Va : RM 189

ZN IN BROWN BREAD µG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
32 AAS	5	19.40000	18.70000	19.70000	19.30000	19.50000	19.32000	.37683
17 AAS	5	21.00000	21.00000	21.50000	21.00000	21.00000	21.10000	.22361
22 AAS	10	20.00000 19.30000	19.80000 20.60000	20.40000 19.40000	20.30000 20.70000	19.70000 20.80000	20.10000	.53955
23 AAS	7	18.90000 19.00000	19.10000 19.40000	18.90000	19.00000	19.40000	19.10000	.21602
33 AAS	20	19.10000 18.80000 17.30000 19.20000	18.60000 18.60000 17.50000 18.20000	18.80000 21.20000 17.10000 18.90000	19.60000 17.60000 17.60000 18.10000	16.50000 18.00000 18.10000 19.10000	18.39500	1.03947
39 AAS	5	21.10000	17.70000	15.80000	20.50000	16.70000	18.36000	2.33624
43 AAS	5	18.40000	19.00000	18.40000	19.20000	18.60000	18.72000	.36332
1 ASV	5	20.50000	18.80000	18.90000	21.10000	19.40000	19.72000	1.03779
14 DCP	4	18.60000	18.40000	18.90000	18.60000		18.60000	.16330
2 ICP	5	17.70000	17.90000	16.50000	18.70000	19.00000	17.96000	.97877
4 ICP	5	20.00000	19.00000	18.50000	19.50000	21.00000	19.60000	.96177
3 INAA	5	19.40000	19.20000	19.90000	20.10000	19.40000	19.60000	.38079
4 INAA	5	19.50000	19.70000	18.80000	19.30000	19.50000	19.36000	.34351
5 INAA	5	20.40000	20.70000	18.80000	19.90000	21.40000	20.24000	.97108
7 INAA	6	21.60000 22.40000	20.90000	22.30000	20.90000	22.50000	21.76667	.74207
9 INAA	6	21.30000 21.00000	20.70000	20.70000	21.40000	21.20000	21.05000	.30166
10 IDMS	5	20.20000	20.50000	20.10000	20.10000	20.00000	20.18000	.19235

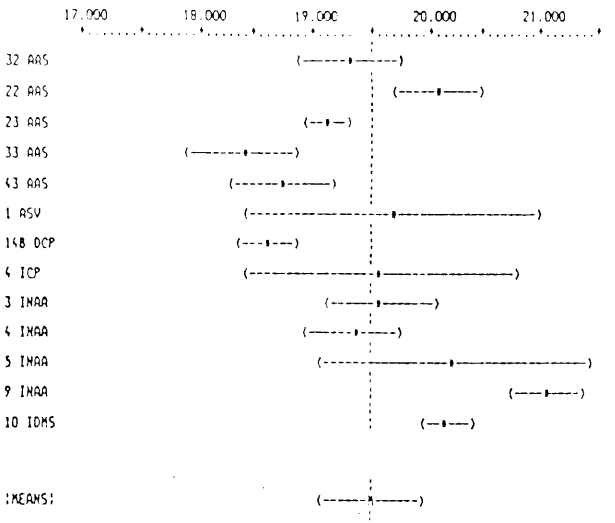
Table Vb : RM 191

Tables Va and Vb : Results Submitted for Certification of Zn in RMs 189 and 191



ZN IN WHOLEMEAL FLOUR µG/G RM 189

Number of accepted sets of results	P	:	13
Number of accepted replicates	N	:	86
Outlying variance (Cochran test)		:	No outliers , no stragglers
Homogeneity of variances (Bartlett test)		:	About homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	56.46940
Std.dev.of distribution of means	S	:	2.76255
Within-labs std.deviation	Sw	:	1.52263
Between-labs std.deviation	Sb	:	2.31203
0.95 confidence interval of the mean		:	56.46940 +/- 1.66940



ZN IN BROWN BREAD µG/G RM 191

Number of accepted sets of results	P	:	13
Number of accepted replicates	N	:	87
Outlying variance (Cochran test)		:	No outliers , no stragglers
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	19.53731
Std.dev.of distribution of means	S	:	.74578
Within-labs std.deviation	Sw	:	.71698
Between-labs std.deviation	Sb	:	.77539
0.95 confidence interval of the mean		:	19.53731 +/- .45067

Fig V : Bar Chart and Statistical Summaries for Accepted for Zn

FE IN WHOLEMEAL FLOUR µG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
17 AAS	5	71.00000	73.00000	71.00000	73.00000	72.00000	72.00000	1.00000
22 AAS	10	65.60000 66.70000	67.30000 66.40000	66.10000 66.10000	67.30000 67.70000	66.60000 68.70000	66.85000	.91439
23 AAS	7	74.80000 76.20000	70.90000 74.40000	67.00000	66.50000	73.60000	71.91428	3.87747
39 AAS	5	62.50000	63.30000	67.20000	74.30000	64.10000	66.28000	4.82411
43 AAS	5	72.80000	71.90000	74.80000	71.90000	73.70000	73.02000	1.24378
14 DCP	4	63.40000	61.50000	64.10000	63.70000		63.17500	1.15289
32 SPEC	5	67.30000	67.70000	68.90000	69.30000	69.00000	68.44000	.88204
2 ICP	5	71.00000	67.90000	60.00000	65.30000	69.10000	66.66000	4.25829
4 ICP	5	68.00000	70.00000	67.00000	68.00000	68.00000	68.20000	1.09545
3 INAA	5	74.60000	67.60000	69.00000	73.40000	73.00000	71.52000	3.03842
4 INAA	5	70.00000	73.00000	65.00000	66.00000	69.00000	68.60000	3.20936
5 INAA	5	63.90000	63.80000	68.60000	67.90000	68.40000	66.52000	2.45092
9 INAA	5	63.70000	61.90000	65.90000	65.20000	64.50000	64.24001	1.54208

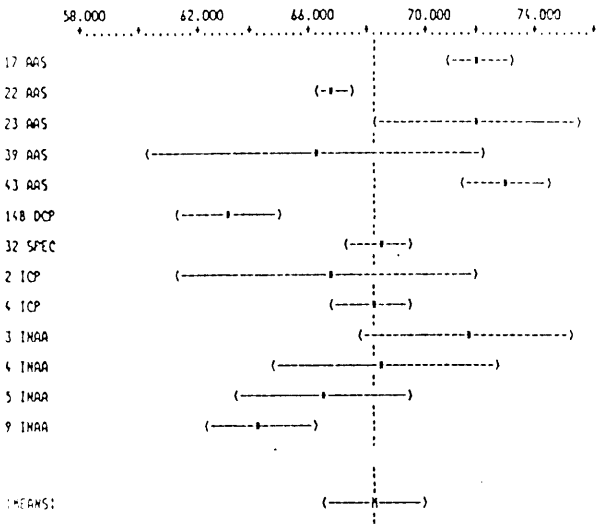
Table VIa : RM 189

FE IN BROWN BREAD µG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
17 AAS	5	42.00000	42.00000	41.00000	40.00000	41.00000	41.20000	.83666
22 AAS	10	40.60000 40.70000	38.50000 41.50000	40.90000 39.80000	42.30000 40.00000	41.60000 40.40000	40.63000	1.06672
23 AAS	7	46.10000 44.30000	48.40000 45.40000	43.40000	46.30000	50.70000	46.37143	2.48309
39 AAS	4	42.30000	36.70000	42.60000	43.90000		41.37500	3.19309
43 AAS	5	38.30000	37.80000	38.30000	39.20000	39.80000	38.68000	.80436
14 DCP	4	41.90000	45.40000	43.30000	44.40000		43.75000	1.50222
2 ICP	5	28.90000	32.30000	31.10000	30.10000	35.70000	31.62000	2.60231
4 ICP	5	36.00000	36.00000	35.00000	34.00000	36.00000	35.40000	.89443
32 SPEC	5	38.00000	39.40000	38.00000	39.00000	39.50000	38.78000	.73621
3 INAA	5	42.40000	40.80000	40.60000	40.20000	43.70000	41.54000	1.46901
4 INAA	5	33.00000	37.00000	36.00000	31.00000	38.00000	35.00000	2.91548
5 INAA	5	52.40000	48.30000	43.60000	48.40000	39.00000	46.30000	5.17977
9 INAA	5	36.20000	37.90000	39.50000	42.90000	41.90000	39.68000	2.76623

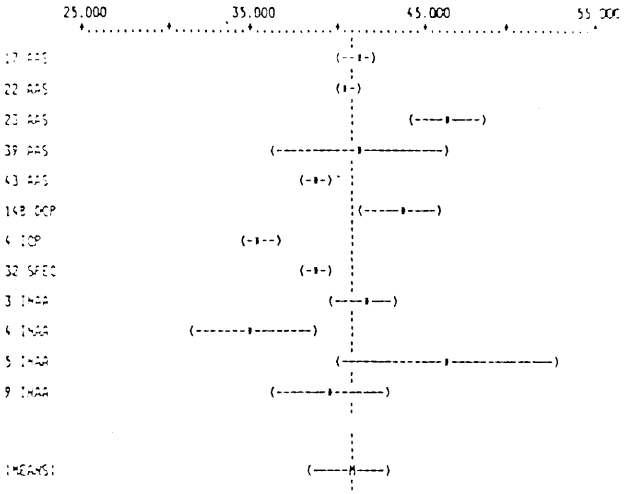
Table VIb : RM 191

Tables VIa and VIb : Results Submitted for the Certification of RMs 189 and 191



FE IN WHOLEMEAL FLOUR µG/G RM 189

Number of accepted sets of results	P	:	13
Number of accepted replicates	N	:	71
Outlying variance (Cochran test)		:	No outliers , no stragglers
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Malinov test)		:	None
Mean of means		:	68.26302
Std.dev.of distribution of means	S	:	3.08615
Within-labs std.deviation	Sw	:	2.61662
Between-labs std.deviation	Sb	:	2.76114
0.95 confidence interval of the mean		:	68.26302 +/- 1.86494



FE IN BROWN BREAD µG/G RM 191

Number of accepted sets of results	P	:	12
Number of accepted replicates	N	:	65
Outlying variance (Cochran test)		:	5.17977 is an outlier
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Malinov test)		:	None
Mean of means		:	40.72554
Std.dev.of distribution of means	S	:	3.61340
Within-labs std.deviation	Sw	:	2.28163
Between-labs std.deviation	Sb	:	3.46239
0.95 confidence interval of the mean		:	40.72554 +/- 2.29585

Fig VI : Bar Charts and Statistical Summaries for Accepted Results for Fe

MN IN WHOLEMEAL FLOUR µG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
32 AAS	5	65.80000	66.00000	66.40000	66.50000	65.80000	66.10000	.33166
17 AAS	5	69.00000	68.00000	65.00000	67.00000	67.00000	67.20000	1.48324
22 AAS	10	56.60000 58.00000	58.60000 58.80000	59.40000 57.30000	59.30000 59.10000	58.90000 59.90000	58.59000	1.01374
23 AAS	6	59.60000 58.20000	58.90000	57.80000	58.10000	60.20000	58.80000	.94446
33 AAS	14	73.40000 66.50000 53.90000	64.50000 66.50000 58.00000	62.20000 67.00000 62.40000	65.80000 65.70000 61.60000	59.50000 66.60000	63.68572	4.68580
39 AAS	5	61.30000	58.10000	66.30000	64.40000	64.80000	62.98000	3.27826
43 AAS	5	64.30000	64.30000	63.60000	65.60000	63.60000	64.28001	.81670
14 DCP	4	71.00000	68.60000	70.30000	73.40000		70.82500	1.99060
2 ICP	5	61.80000	62.80000	63.90000	60.60000	61.30000	62.08000	1.29699
4 ICP	5	65.00000	67.00000	64.00000	65.00000	63.00000	64.80000	1.48324
5 INAA	5	62.80000	64.00000	61.50000	63.50000	61.50000	62.66000	1.14149
7 INAA	6	62.50000 63.80000	63.10000	64.40000	63.40000	64.10000	63.55000	.69498
9 INAA	5	64.60000	64.90000	64.00000	65.70000	64.80000	64.80000	.61237

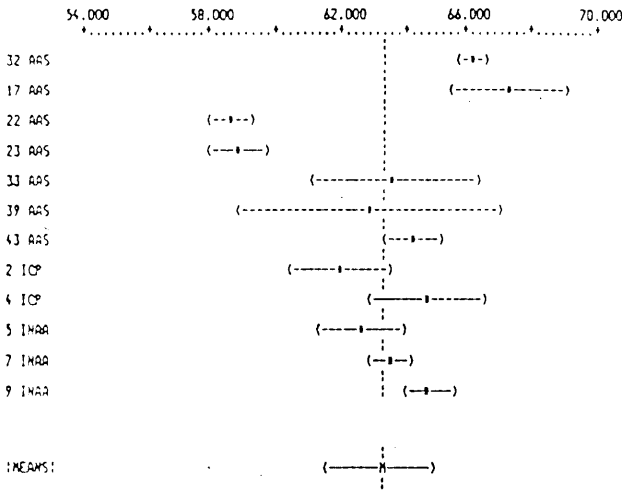
Table VIIa : RM 189

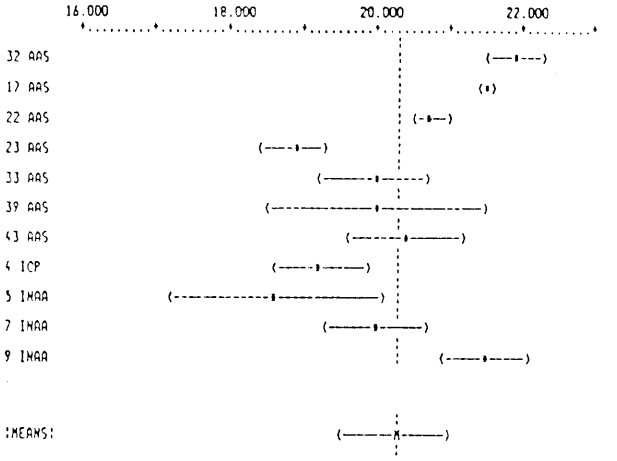
MN IN BROWN BREAD µG/G

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
32 AAS	5	22.00000	21.60000	21.60000	21.90000	22.40000	21.90000	.33166
17 AAS	5	21.40000	21.40000	21.50000	21.50000	21.60000	21.48000	.08367
22 AAS	10	20.20000 20.50000	20.80000 20.40000	21.00000 21.00000	20.90000 21.00000	21.00000 20.60000	20.74000	.29515
23 AAS	7	19.00000 19.50000	18.20000 19.10000	19.10000	18.30000	18.90000	18.87143	.44445
33 AAS	15	21.10000 20.20000 21.00000	21.30000 18.10000 20.20000	20.40000 20.50000 21.20000	19.10000 16.90000 22.10000	19.30000 18.80000 19.60000	19.98667	1.37210
39 AAS	5	19.40000	20.30000	20.90000	21.20000	18.20000	20.00000	1.21861
43 AAS	5	19.60000	20.80000	21.10000	20.70000	19.90000	20.42000	.63797
14 DCP	4	27.10000	23.80000	25.80000	27.00000		25.92500	1.53487
2 ICP	5	19.10000	16.50000	17.20000	18.50000	15.20000	17.30000	1.56045
4 ICP	5	19.60000	19.30000	19.50000	19.20000	19.60000	19.24000	.55045
5 INAA	5	17.90000	18.40000	19.60000	20.00000	17.30000	18.64000	1.13710
7 INAA	6	19.60000 20.10000	19.30000	21.00000	19.50000	20.50000	20.00000	.65727
9 INAA	5	21.00000	21.40000	22.30000	21.30000	21.50000	21.50000	.48477

Table VIIb : RM 191

Tables VIIa and VIIb : Results Submitted for the Certification of RMs 189 and 191





MN IN WHOLEMEAL FLOUR µG/G RM 189

Number of accepted sets of results	P	:	12
Number of accepted replicates	N	:	76
Outlying variance (Cochran test)		:	4.68580 is an outlier
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	63.29381
Std.dev.of distribution of means	S	:	2.57886
Within-labs std.deviation	Sw	:	2.43245
Between-labs std.deviation	Sb	:	2.47061
0.95 confidence interval of the mean		:	63.29381 +/- 1.63853

MN IN BROWN BREAD µG/G RM 191

Number of accepted sets of results	P	:	11
Number of accepted replicates	N	:	73
Outlying variance (Cochran test)		:	No outliers , no stragglers
Homogeneity of variances (Bartlett test)		:	Not homogeneous
Normality of distribution of mean values (Kolmogorov-Smirnov-Lilliefors test)		:	Normal
Outlying mean values (Nalimov test)		:	None
Mean of means		:	20.25255
Std.dev.of distribution of means	S	:	1.08507
Within-labs std.deviation	Sw	:	.86094
Between-labs std.deviation	Sb	:	.92748
0.95 confidence interval of the mean		:	20.25255 +/- .72897

Fig VII : Bar Charts and Statistical Summaries for Results Accepted for Mn.

Annex B

Common Sources of Error in the Determination of the Elemental Contents RM 189 and 191

Sources of error in elemental analysis have been extensively reviewed in the literature (6. Tölg 1985, 7. Tschöpel 1982, 8. Stoeppler 1979). However, it is useful to summarize some of the more common sources of error and to note particular problems that arose during this and related BCR exercises.

General

The results of the intercomparisons described in this and similar BCR reports lead to two broad conclusions:

Accuracy

In spite of the considerable care that participating laboratories take to ensure the accuracy of their measurements, the intercomparisons have frequently revealed substantial errors.

This is a simple demonstration of the fact that, unlike repeatibility, accuracy is both difficult to acheive and to demonstrate in the absence of some form of external comparison of measurements through for example, participation in intercomparisons or the use of certified reference materials.

Choice of Method

Whenever possible, certification has been based on the agreement of results of different methods (e.g. differing in sample digestion, work-up or end-method).

In view of the very high level of agreement that has been achieved between the various procedures, it can be concluded that most errors arise through failure of the application of a procedure and only very rarely from fundamental weakness in the underlying principles.

This does not imply the need for even more detail in the written descriptions of the methods but rather that the analyst has failed to apply some of the basic precautions required to achieve accurate results.

It is again stressed that accurate results can only be achieved through careful control of every step in the procedure, e.g. choice of calibrant (known purity, stoichiometry, etc.), calibration, representative sampling, sample digestion and intermediate steps (losses, incomplete recovery or conversions) and final determination (specificity and sensitivity).

In addition, the risk of contamination, especially by elements such as Fe and Hg which are ubiquitous in the laboratory, must be strictly controlled at every stage in the procedure. For trace analysis, 'clean' laboratories and laminar flow hoods are indispensable.

Calibration

Generally, calibrants provided by analytical supply houses are of good quality. However, errors may arise because of:

- variations in batch quality; new batches should be compared with previous ones before use.
- changes in stoichiometry during storage, e.g. of oxidation state or in the water of crystalisation may need to be corrected; pure metals may suffer oxidation and require surface etching before use, etc.

In the first intercomparison, several participants experienced serious errors when using multi-cassette soft-ware calibration systems for DCP and ICP.

It is essential for the achievement of accurate results from multi-element techniques, that they are individually calibrated for each element and for the expected concentration range.

In addition, it is important that the calibrant solution matches the final analyte solution with regard to major components (e.g. soluble solids, sugars, alcohol), viscosity, ionic strength and potentially interfering elements.

Sample Digestion

A wide variety of sample digestion procedures were employed in the preliminary and certification exerices.

In general, dry ashing gave rise to more errors than wet digestion, essentially because of the losses that can occur when volatile compounds such as metal halides are formed.

Losses of this nature also led in some cases (notably for Pd, Cd, Cu and Mn) to cross-contamination when high level samples had been ashed in the same oven before or at the same time as low-level ones.

It was, however, demonstrated that good results could be obtained by dry ashing provided certain precautions were taken, e.g.
- use of temperature programmed dry-ashing with a relatively long initial charring period, e.g. 5 hours at around 120°C followed by several periods at progressively increasing temperature;
- the use of ashing aids (e.g. HNO_3/H_2SO_4) especially when the halide content is high, to minimise risk of formation of volatile halides;
- avoidance of temperatures exceeding 450°C.

Care must be taken to ensure complete dissolution of the residue remaining after ashing, especially when there is a risk of formation of phosphate glasses (when the P content is high) or, of refractory oxides.

Wet digestion, especially in closed systems, avoids errors of the above kind but is subject to other difficulties.

ASV procedures require the absence of complexing anions; even acetate can cause interference. Therefore the demands on the digestion method are not only complete digestion of the sample but also destruction of potential complexing organic species.

This problem is illustrated by the observation that ASV results for Pb in CRM 191 (Brown Bread, certified Pb content 187 ng/g) were generally systematically lower that those by IDMS and ETAAS. Two laboratories subsequently repeated their measurements using a high pressure bomb which allowed higher digestion temperatures. In both cases, the new results were around 50 ng/g higher than before and in line with other results.

Intermediate Steps

Several procedures involved additional steps between sample digestion and the final determination, generally with the objective of improving sensitivity through concentration (e.g. chelation/solvent extraction of Pb, Cd and Cu; separation of DEDPA complexes of Pb and Cd on active charcoal).

Where laboratories do not have access to equipment of appropriate sensitivity, but have to rely on intermediate concentration stages, e.g. for low-level determination of Pb by AAS instead of ET-AAS, it must be remembered that every additional manipulation will introduce errors.

In general, the methods with the smallest number of manipulations are to be preferred.

Contamination

Contamination is an ever-present problem in trace elemental analysis. It is not possible to give an exhaustive list of sources of contamination, however, some of the more common ones are as follows:

- contact of sample or solutions with the laboratory atmosphere. This is particularly important for elements such as Fe and Hg, which are commonly present in the laboratory (especially so for Hg determinations in biological matrices, where Hg contents are usually very low and therefore sensitive to contamination);
- contact of solutions with glass (Ca, Mn, etc.);
- contact of solutions with plastic containers (Cd, Sn, etc.);
- cross-contamination and memory effects (surface contamination) during poorly controlled dry-ashing; diffusion of digest into cracks in the Teflon lining of a destruction bomb which has been used too often;
- contaminated reagents (acids, ashing aids, etc.);
- poor laboratory hygiene, e.g. smoking in the laboratory (Cd, etc.), dusts from cosmetic preparations (Zn, etc.).

It is essential that the analyst is highly critical of all potential sources of contamination if this important source of error is to be controlled.

Certified reference materials having very low elemental contents of particular elements are particularly well suited to the investigation of contamination in the laboratory, e.g.:

BCR Milk Powder	CRM 63	Hg 1.0 ug/g, Cd 2.9 ng/g
BCR Bovine Muscle	CRM 184	Hg 2.6 ng/g, Cd 13 ng/g
BCR Brown Bread	CRM 191	Se* 25 ng/g, Hg* 2 ng/g
BCR Single Cell Protein	CRM 274	Cd 30 ng/g, Pb 44 ng/g, Co 40 ng/g.

* Indicative valves.

It is stressed however, that the above materials must be stored and handled under non-contaminating "clean room" conditions, since otherwise their certification may be invalidated.

Finally, it is strongly recommended that laboratories employ appropriate quality assurance schemes to ensure that, once having achieved the required level of accuracy for a particular measurement, it is maintained in spite of changes in, for example, personnel and equipment. Certified reference materials provide a highly cost effective and convenient means of achieving this end.

European Communities — Commission

EUR 10997 — The certification of the contents (mass fractions) of Cd, Pb, Se, Cu, Zn, Fe and Mn in wholemeal flour and lyophilized brown bread reference materials
Wholemeal flour CRM No 189
Brown bread CRM No 191

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The report describes the preparation and certification of a wholemeal flour (CRM 189) and a lyophilized brown bread (CRM 191) for their contents (mass fractions) of elements of toxicological and nutritional importance: Cd, Pb, Se, Cu, Zn, Fe and Mn. Indicative values are also given for As, Ca, Cl, Cr, Hg, Mg, Na, Ni, P and K.

Details are given of a preliminary intercomparison of methods for these elements in a wholemeal flour sample, homogeneity and stability studies on the two reference materials and the results and evaluation of the certification exercise which involved 21 European laboratories. Summaries of the certification methods are also presented. The report concludes with a discussion of the most common sources of error in determining the elements of interest and the steps to be taken to control them.