FAO-ESN/MON/AQA/81/8

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GEMS: Global Environmental Monitoring System

JOINT FAO / WHO
FOOD AND ANIMAL FEED CONTAMINATION
MONITORING PROGRAMME

ANALYTICAL QUALITY ASSURANCE OF MONITORING DATA

Prepared under the joint sponsorship of the

United Nations Environment Programme, the

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FAO-ESN/MON/AQA/81/8
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JOINT FAO/WHO FOOD AND ANIMAL FEED CONTAMINATION MONITORING PROGRAMME

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CONTENTS

	Page
Introduction	1
Analytical Quality Assurance - Aflatoxins B_1, B_2, G_1, G_2, and M_1 - M. Friesen and L. Garren International Agency for Research on Cancer Lyon, France	4
Analytical Quality Assurance - Cadmium and Lead M. E. Knowles and J. A. Burrell Ministry of Agriculture, Fisheries and Food Norwich, UK	28
Analytical Quality Assurance - Organochlorine Compounds S. A. Slorach and G. Ekström National Food Administration Uppsala, Sweden	65

ANALYTICAL QUALITY ASSURANCE OF MONITORING DATA

INTRODUCTION

The Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme was initiated in 1976 and is being carried out with support from the United Nations Environment Programme. The major objectives of this programme are:[1]

a) to collect selected data on levels of a limited number of chemical contaminants in individual foods and/or in total diet samples and to evaluate these data and produce and disseminate summaries and reviews of trends in certain types of food contamination enabling appropriate food control or resource management measures. The Programme is dependent on data generated in national monitoring programmes and the data to be collected will come from studies which have already been carried out in the various participating countries;

b) to obtain estimates of the intake via food of specific chemical contaminants, with a view to correlating these data with those on intake from other sources, thus enabling the total intake of the contaminant to be estimated. In appropriate cases, this intake will then be correlated with data on the levels of the contaminant in human tissues or body fluids, planned to be collected in other projects in the current WHO Health-Related Monitoring Programme;

c) to provide various Codex Alimentarius committees with information needed for incorporating maximum limits for specific contaminants in the development of international food standards;

d) to provide technical cooperation with the governments of countries wishing to initiate or strengthen food contamination monitoring programmes.

At present, 21 Collaborating Centres are participating in the Programme and data are being collected on certain contaminants in selected foods as follows:

1. Organo-chlorine compounds (DDT-complex, HCH-isomers, heptachlor, aldrin/dieldrin, HCB and PCBs) in:
   a. whole fluid cow's milk, whole dried milk, butter, edible fats and oils and fish,
   b. human milk,
   c. cereals, eggs, fresh fruits and vegetables, and
   d. total diet
2. Lead in:
   a. Canned, fruit, fruit juice and concentrates, mixed fruit juice for infants, vegetables and milk (all in cans with lead soldered seams),
   b. cereal flours, potatoes, vegetables of major importance, molluscs, crustaceans and kidney, and
   c. total diet.
3. Cadmium in:
   a. Molluscs, crustaceans, grains, cereal flours, potatoes and kidney, and
   b. total diet

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(1) Report of the Consultation on the Joint FAO/WHO Food and Animal Feed Contamination Monitoring - Phase II, Geneva, 14-18/6/77; WHO-FAD/FCM/77.9;FAO-ESN/MON/77.9.

4. Aflatoxins in:

   a. peanuts, tree nuts, maize and milk, and
   b. total diet.

A basic requirement for an international or national health-related environmental monitoring programme is that data generated by one activity be fully comparable to similar data produced elsewhere or at another time, and that the accuracy and precision of all data be known. This necessitates an active quality assurance programme[1].

At the end of 1979 development of data collection and processing had reached a stage which required an assessment of the comparability of the data; it was therefore decided to carry out analytical quality assurance (AQA) studies. A consultation was held in Geneva during February 1980 to plan this work[2]. Three institutes were selected to carry out the initial study of inter-laboratory comparability of analysis for the three groups of contaminants: aflatoxins, the heavy metals - lead and cadmium, and organochlorine compounds.

All Collaborating Centres were invited by WHO to participate in each of the three studies. For practical reasons a maximum of five laboratories in each country were invited to participate.

The analytical quality assurance study on aflatoxins was organized by the International Agency for Research on Cancer, Lyon, France. Samples of raw peanut meal, deoiled peanut meal, and yellow cornmeal with aflatoxins B_1, B_2, G_1, and G_2 and a sample of lyophilised cow's milk with aflatoxin M_1, were sent to the 30 laboratories in 11 countries which participated in the test. The results of the analyses of these samples were included with results from the laboratories already participating in the on-going Aflatoxin Check Sample Survey Programme being carried out by the International Agency for Research on Cancer with support from the US Food and Drug Administration.

The analytical quality assurance study on lead and cadmium was organized by the Food Laboratory, Ministry of Agriculture, Fisheries and Food, Norwich, UK. Samples of freeze dried vegetables and shellfish with lead and cadmium as contaminants were sent out and results were received from 37 laboratories in 13 countries which participated in this test.

The analytical quality assurance study for organochlorine compounds was organized by the National Food Administration, Uppsala, Sweden. Mixtures of contaminants selected from the group of organochlorine pesticides and PCBs on which data are currently being collected in the monitoring programme in iso-octane, soya bean oil or butter fat (butter oil) were sent to the 34 laboratories in 13 countries participating in the exercise.

Each of the above-mentioned coordinating institute has analyzed and evaluated the data submitted by the participating Collaborating Centres and the three reports as prepared follow.

Analytical quality assurance (AQA) should be an integral part of analytical work and not an exercise carried out only occasionally. In order to ensure the validity of the data being collected in the monitoring programme, it is important that the national authorities concerned are encouraged to include AQA within their monitoring programme. Results of the current AQA studies allow few conclusions to be drawn concerning the reliability of previously collected data. However, an important contribution of a continuing AQA programme should be to improve the overall reliability of data collected as a result of positive feed-back to those laboratories performing poorly in the AQA exercises.

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(1) WHO Health-Related Environmental Monitoring Programme - Working Document EHE/74.1.

(2) Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme. Analytical Quality Assurance - Report of a Consultation held in Geneva, 27-29 February 1980. HCS/FCM/80.1.

At the recently held Technical Advisory Committee meeting[1], the Committee recommended that AQA studies be included as a regular part of this Programme's future activities and these studies should be organized by selected coordinating institutes to ascertain improvements in particular laboratories identified as requiring training. The performance of each laboratory will be appraised, and comments on that performance given by the AQA coordinating institutes. This dialogue will be maintained and may in developing countries be supplemented by training visits to individual participating laboratories.

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(1) Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme Report of the second session of the Technical Advisory Committee Geneva, 27 April - 1 May 1981, WHO-EFP/81.15; FAO-ESN/MON/TAC-2/81/5

ANALYTICAL QUALITY ASSURANCE

- AFLATOXINS B_1, B_2, G_1, G_2, and M_1 -

by

M. Friesen and L. Garren
International Agency for Research on Cancer
Lyon, France

Contents | Page

		Page
1.	INTRODUCTION	5
2.	ORGANISATION OF THE SURVEY	5
3.	RESULTS	6
	3.1 Frequency distribution of results	6
	3.2 Frequency distribution of methods	6
	3.3 Statistical analysis of results by method	6
4.	DISCUSSION	7
	4.1 Raw Peanut Meal	7
	4.2 Deoiled Peanut Meal	8
	4.3 Yellow Corn Meal	8
	4.4 Lyophilized Milk	8
	4.5 General Discussion	8
5.	ACKNOWLEDGEMENTS	9
6.	PARTICIPATING LABORATORIES	10
Figures 1 - 4		12 - 18
Tables 1 - 6		19 - 24
REFERENCES		25

1. INTRODUCTION

At a meeting held in Geneva in February 1980, a group of experts from laboratories participating in the joint FAO/WHO Food and Animal Feed Monitoring Programme undertook to verify the quality of analytical results generated by the programme's collaborating centres around the world. As a part of its on-going Aflatoxin Check Sample Survey Programme(45), the International Agency for Research on Cancer (IARC) was asked to organize and carry out the segment of this effort involving the analysis of aflatoxins in various food stuffs.

The following report outlines the results of the overall check sample programme for 1980 and separates out, where feasible and useful, the results coming from the smaller subgroup of laboratories participating as a part of the joint FAO/WHO Programme.

Participation in the Aflatoxin Check Sample Survey Programme is open to any laboratory wishing to compare its analytical results with those of a large group of laboratories using the same or different methods. The service is offered free of charge to participating laboratories and individual results are not released to other participants. In addition to its usefulness to individual laboratories as concerns quality assurance, the statistical analysis of results can aid in comparing the results obtained through the use of certain widely employed methods or techniques.

2. ORGANISATION OF THE SURVEY

In the current series, laboratories participated in the analysis of samples of raw peanut meal, deoiled peanut meal, and yellow corn meal for aflatoxins B_1, B_2, G_1 and G_2 and a sample of lyophilised cow's milk for aflatoxin M_1. The raw and deoiled peanut meal samples were naturally contaminated. The milk sample was obtained by feeding aflatoxin B_1 to a cow under laboratory conditions. The yellow corn meal sample was obtained by spiking a locally purchased sample.

Participants were provided with 120 g portions of raw peanut meal, deoiled peanut meal and yellow corn meal and 25 g portions of lyophilised milk from bulk samples which had been thoroughly mixed and controlled for homogeneity. Laboratories were requested to analyse each sample in duplicate using either the same method or two different methods. The latter option was provided to give an opportunity for laboratories experimenting with more than one method or those developing new methods to obtain comparative data.

Aflatoxin standards were provided for those laboratories requesting them. Participants were instructed to use these standards only after having verified their concentration. IARC furnished standards were used in about 75% of the analyses for overall programme. This figure increased to about 90% for FAO/WHO participants except in the case of lyophilised milk.

Samples contaminated with aflatoxins B_1, B_2, G_1 and G_2 were distributed to 152 laboratories; results were received from 123 laboratories (81%). The sample involving aflatoxin M_1 was distributed to 117 laboratories; results were received from 81 (69%). Overall participation involved laboratories in 31 countries. FAO/WHO involvement concerned 30 laboratories in 11 countries (see section 6).

A preliminary report of the results was distributed to participants soon after all the results were received at IARC.

3. RESULTS

3.1 Frequency distribution of results

Frequency distributions of the results reported for the concentration of aflatoxins B_1, B_2, G_1 and G_2 and M_1 in the four kinds of food tested by laboratories participating in the overall programme are given in section (a) of figures 1, 2, 3 and 4. Corresponding distributions for the subgroup of FAO/WHO participants are given in section (b) of the same figures. These graphs include all results received with the single bar set off to the right of each graph representing those results excluded from the statistical analysis as outliers (see section 3.3). Each such bar corresponds to a group of results covering a small concentration range which can differ from one graph to another. (For example, in Figure 1 (a) aflatoxin B_1, a bar of height 17 corresponding to a concentration of 225 ug/kg indicates that 17 results lie within the range 200.1 to 225.0 ug/kg).

3.2 Frequency of use of methods

Table 1 summarizes the frequency of use of methods according to sample matrix for participants in the overall programme and for the subgroup of FAO/WHO participants. For the analysis of aflatoxins B and G, only two methods were used by enough participants to justify their separate statistical analysis, the so-called CB[1] and BF[4] methods. Results obtained using the so-called BGA method [2,3] were included with results for the CB method as the former method involves only slight modifications of the latter. For the analysis of aflatoxin M_1, again only two methods were treated separately from the others: AOAC Method I[37] and AOAC Method II[38].

All four of the above mentioned methods use Thin Layer Chromatography (TLC) in the final quantification step. In order to permit some comparison with the second most frequently used quantitative analytical technique, all results involving the use of High Performance Liquid Chromatography (HPLC) in the final quantification step were grouped together. It is important to note that extraction and clean-up techniques vary widely for these methods.

The remaining results which all involve TLC, except for one laboratory using an enzyme-immunoassay technique[35], are grouped under the heading "Others".

Finally, it should be noted and emphasized that participants in the programme were allowed to and did often make modifications in established published methods. In addition, the quality of results grouped under a given method can vary widely as the laboratories involved range from those just beginning aflatoxin analysis to those who have carried out such analysis routinely for many years. For these reasons, statistical analyses made in this paper should be interpreted as referring to comparisons of laboratories using certain methods and not to comparisons of the methods themselves.

3.3 Statistical analysis of results by method

Results of the statistical evaluation for laboratories participating in the overall programme are given in section (a) of tables 2, 3, 4 and 5. Analogous results are given for the FAO/WHO subgroup of participants in section (b) of the same tables. For each of the method groups, as well as for a group including the ensemble of all results reported, the mean, standard deviation (SD), coefficient of variation (CV) in % and number of results (N) involved have been calculated.

Prior to statistical analysis, outlying results ($P = 0.05$) have been excluded according to the test of Thompson (44). Briefly, a result is considered to be an outlier when:

$$\frac{\text{(value in question) - (mean of all non-outlying results)}}{\text{(SD of all non-outlying results)}}$$

is greater than 3.6. The number of outliers for a given sample or method can be found in Figures 2 through 5.

For laboratories submitting results involving duplicate analyses using a single method, only the first reported result was included in the statistical analysis. Results from duplicate analyses involving two different methods were analysed statistically as individual results.

All values were handled as reported except as follows:

Trace was assigned a value of 1 ug/kg

Values listed as (<) were taken as one-half the value

Values indicating an interference were rejected from the analysis

Values listed as ($\leq$) were taken as equal to the values

One listed as 10-15 ug/kg was taken as 12.5 ug/kg

Values listed as ($\simeq$) were taken as equal to the value.

Brackets connecting method groups indicate a significant statistical difference (t test; $P = 0.05$) between mean values for laboratories using the two methods in question. Such comparisons were not carried out with results in the category "all" methods.

4 DISCUSSION

4.1 Raw Peanut Meal

This sample was naturally contaminated at relatively high levels of aflatoxins B_1 and B_2 and at relatively low or near zero levels of aflatoxins G_1 (79% of results $\leq$ 1 ug/kg) and G_2 (94% of results $\leq$1 ug/kg). The large number of zero results make calculation of statistics for G_1 and G_2 meaningless. Although the absolute spread of results is very wide for aflatoxins B_1 and B_2, means obtained by the four method groups are statistically comparable and coefficients of variation of 50-60% are relatively good. The mean of the results from laboratories using the CB method are in general the highest with the group of laboratories using the EEC method having the best coefficient of variation.

Significant statistical differences in means for the FAO/WHO laboratories using the HPLC as compared to CB or BF methods or other as compared to CB methods may come about in part due to the small number of results involved. Results for this subgroup involving aflatoxins B_1 and B_2 are, however, generally higher than those found for the overall programme.

4.2 Deoiled Peanut Meal

This sample was also naturally contaminated at a relatively high level of aflatoxin B_1 with much lower levels of B_2, G_1 and G_2. Significantly lower means were found for results from laboratories using the BF as compared to the CB, EEC or other methods, in the case of aflatoxin B_1 and B_2 for laboratories using other methods as compared to EEC or CB methods for aflatoxin G_1 and for laboratories using HPLC and other methods as compared to the CB method for aflatoxin G_2. Although only 10 results were from laboratories using the EEC method, coefficients of variation were consistently lower for this group. This may in part be due to the low number of zeros and outliers reported by laboratories employing this method (see table 6).

The relatively high percentage of zero values reported by laboratories using "other" methods should also be underlined. It is important to note that for aflatoxin G_1, 37% of the results (43 zeros + 9 outliers out of 142 results) were from laboratories either unable to detect the compound or detecting an excessively large amount. For the case of aflatoxin G_2, the analogous figure is 42% (44 zeros + 15 outliers out of 142 results). For aflatoxin B_2, which appears to be present at a level only slightly higher than G_1, the figure drops to 11% (11 zeros + 5 outliers out of 142) and for aflatoxin B_1, present at a relatively high concentration to 5% (2 zeros + 5 outliers out of 142 results). The variation in the concentrations of the 4 aflatoxins present in this sample illustrate well the increased difficulties encountered by laboratories as the concentration of contaminant decreases.

Again the results obtained by the FAO/WHO subgroup were in general higher than for the overall group, however, the small number of results make comparisons difficult.

4.3 Yellow Corn Meal

This sample was artifically contaminated in the laboratory at the following levels: B_1, 14 ug/kg ; B_2, 9 ug/kg ; G_1, 16 ug/kg and G_2, 8 ug/kg. There were no statistically significant differences in means for the 4 method groups concerned for any of the four aflatoxins, nor were the means significantly different from the actual spiked levels.

Results were again similar in the FAO/WHO subgroup.

4.4 Lyophilized Milk

This sample was naturally contaminated with aflatoxin M_1 under laboratory conditions by feeding a diet contaminated with aflatoxin B_1. The resulting contaminated milk was lyophilized and the aflatoxin concentration reduced to a reasonable value by mixing with non-contaminated milk powder.

Significant differences in means are noted between the AOAC Method I and the HPLC and other methods groups. There is close agreement in means for results from laboratories using either AOAC Method I or II. The coefficient of variation is lowest for the group of 12 laboratories using HPLC methods.

Results for the FAO/WHO group parallel those obtained for the overall study.

4.5 General Discussion

It is interesting to note that four cases were encountered in the four samples considered where aflatoxin concentration ranged between 5 and 10 ug/kg; aflatoxins B_2

and G_2 in the case of yellow corn meal, aflatoxin G_1 in the case of deoiled peanut meal and aflatoxin M_1 in the case of lyophilized milk. The number of zero results reported varies widely for these four cases, respectively (7/142 ; 5%), (14/142 ; 10%), (43/142 ; 30%) and (1/95 ; 1%). It is thus evident that at a given level of contamination, the aflatoxin in question and sample matrix also play a role in determining the difficulty of analysis.

Even though there are sometimes statistically significant differences between means for laboratories using certain methods, it is difficult to make statements about which method is better than another. We can however note that such differences often belong to laboratories in the group "other" methods. Hopefully those laboratories employing methods included in this group will profit from the results of this report to improve or abandon methods which do not give the expected results.

Finally, we can remark that in general results of the FAO/WHO subgroup parallel those of the overall group; however, the small size of some of the method groupings would have made impossible significant statistical comparisons if only these data were to have been considered.

5. ACKNOWLEDGEMENTS

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We also thank L. Stoloff of the U.S.F.D.A. in Washington D.C., USA for preparation of the standard samples of aflatoxins B and G and for helpful discussions on the analysis of the data.

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The results of this study will be submitted in the near future for publication in J. Assoc. Off. Anal. Chem.

6. PARTICIPATING LABORATORIES (FAO/WHO subgroup)

AUSTRIA

Contact Point:
J. Ettl, Ministry of Health and Environmental Protection, Vienna

Participating laboratory:
R. Pfleger, Institut fur Milchhygiene & Milchtechnologie, Veterinärmedizinische Universitat, Vienna

BRAZIL

Contact point:
W.H. Lara, Instituto Adolfo Lutz, Sao Paulo

Participating laboratories:
O. Marmo, CICOBRA, Sao Paulo
M. Sabino, Instituto Adolfo Lutz, Sao Paulo

GERMANY, FEDERAL REPUBLIC OF

Contact point:
F.K. Käferstein, Max von Pettenkofer-Institut des Bundesgesundheitsamtes, Berlin (West)

Participating laboratories:
S. El-Dessouki, Chemische Landesuntersuchungsanstalt, Stuttgart
W. Krönert, Max von Pettenkofer-Institut des Bundesgesundheitsamtes, Berlin (West)
A.H. Mack, Bundesforschungsanstalt für Getreide- und Kartoffel-verarbeitung,Detmolo
R. Woller, Chemisches Untersuchungsamt Trier, Trier

GUATEMALA

Contact Point:
M. de Campos, Unified Food Control Laboratory INCAP, Guatemala City

Participating laboratory:
M. de Campos, Unified Food Control Laboratory INCAP, Guatemala City

HUNGARY

Contact Point:
V. Cieleszky, Department of Toxicological Chemistry, Institute of Nutrition, Budapest

Participating laboratories:
G.Nemeth, National Feeding and Animal Breeding Inspectoring, Budapest
Hygienic and Epidemiological Station in county Békées
Hygienic and Epidemiological Station in county Hadju-Bihar
S. Koudela, Institute of Nutrition, Gyali ut 3/a

IRELAND

Contact point:
T. O'Toole, Department of Agriculture, Dublin

Participating laboratory:
M. Walsh, State Laboratory, Dublin

JAPAN

Contact point:
M. Uchiyama, National Institute of Hygienic Sciences, Tokyo

Participating laboratories:
T. Kashimoto, Osaka Prefectural Institute of Public Health, Osaka
N. Nose, 639-Kamiookubo-Higashi, Urawa Saitama
K. Saito, Tokyo Metropolitan Research Laboratory of Public Health, Tokyo
Y. Saito, E. Isohata & Y. Takeda, National Institute of Hygienic Sciences, Tokyo
Y. Sakabe, Nagoya City Health Research Institute, Nagoya

KENYA

Contact point:
J.N. Kaviti, National Public Health Laboratory, Nairobi

Participating laboratory:
J.N. Kaviti, National Public Health Laboratory, Nairobi

SWITZERLAND

Contact point:
R.P. Gerber, Federal Office of Public Health, Bern

Participating laboratories:
Laboratorio Cantonale d'Igiene, Lugano
F.Y. Tripet, Laboratoire Cantonal de Chimie, Genève
H. Vogt, Gemeinschaftslaboratorium, Belp

UNITED KINGOM

Contact point:
M.E. Knowles, Ministry of Agriculture, Fisheries and Food, Norwich

Participating laboratories:
R.D. Coker, Tropical Products Institute, London
D.W. Pimbley, Ministry of Agriculture Fisheries and Food, Shardlow
M.J. Shepherd, Ministry of Agriculture Fisheries and Food, Norwich

UNITED STATES OF AMERICA

Contact point:

C. F. Jelinek, Food and Drug Administration, Washington, D.C.

Participating laboratories:
B.L. Joyner, U.S.D.A., F.S.Q.S., Dothan
L.J. Lipinski, U.S. Food and Drug Administration, New Orleans
M.E. Orlando, U.S. Food and Drug Administration, Los Angeles
J. Schade, U.S.D.A., W.R.R.C., Albany
R.D. Stubblefield and O.L. Shotwell, NRRC, Peoria

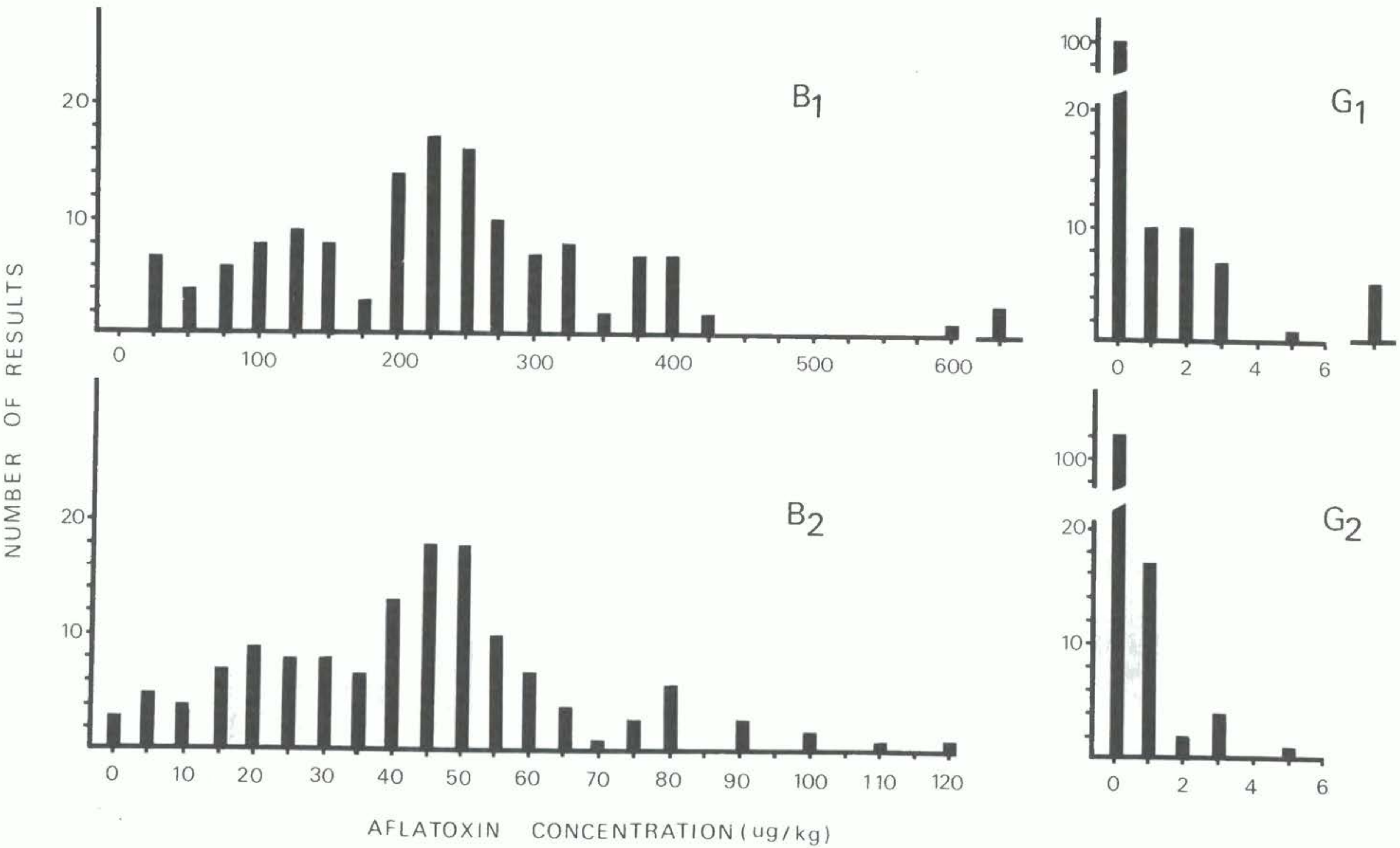

Fig. 1 (a). Distribution of results for the analysis of aflatoxins B_1, B_2, G_1, and G_2 in Raw Peanut Meal.

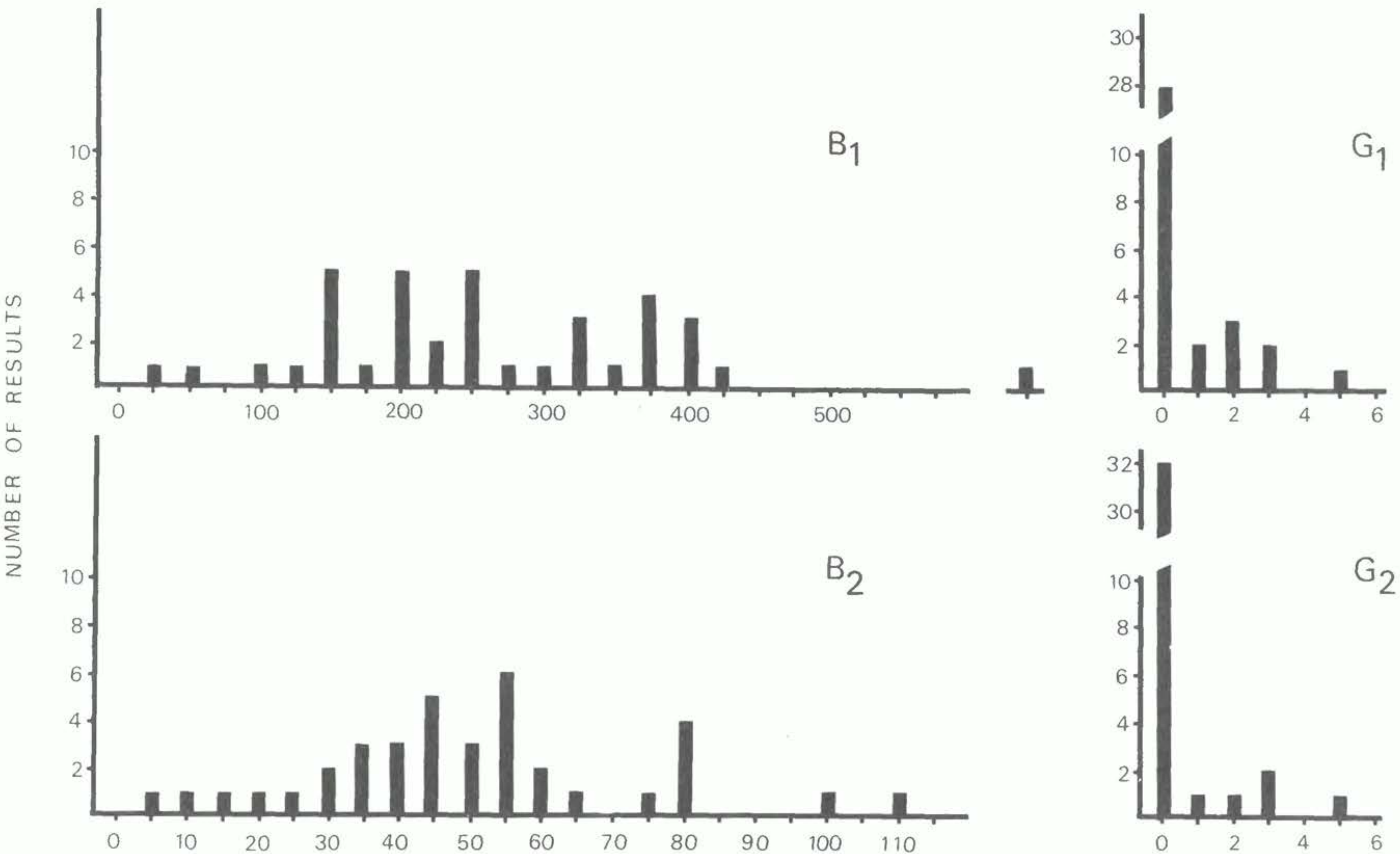

Fig. 1 (b). Distribution of results for the analysis of aflatoxins B_1, B_2, G_1, and G_2 in Raw Peanut Meal for the FAO/WHO subgroup.

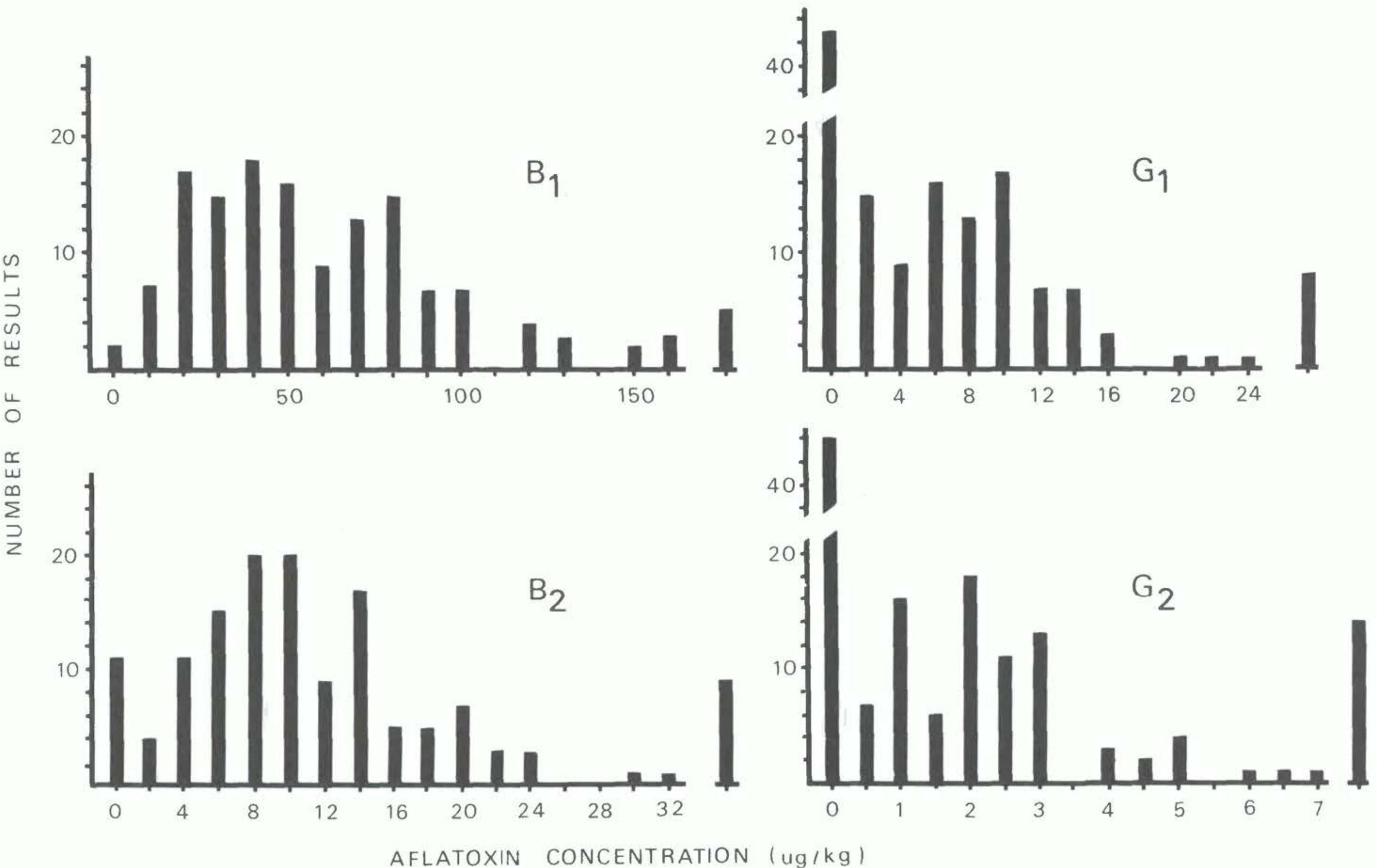

Fig. 2 (a). Distribution of results for the analysis of aflatoxins B_1, B_2, G_1 and G_2 in Deoiled Peanut Meal.

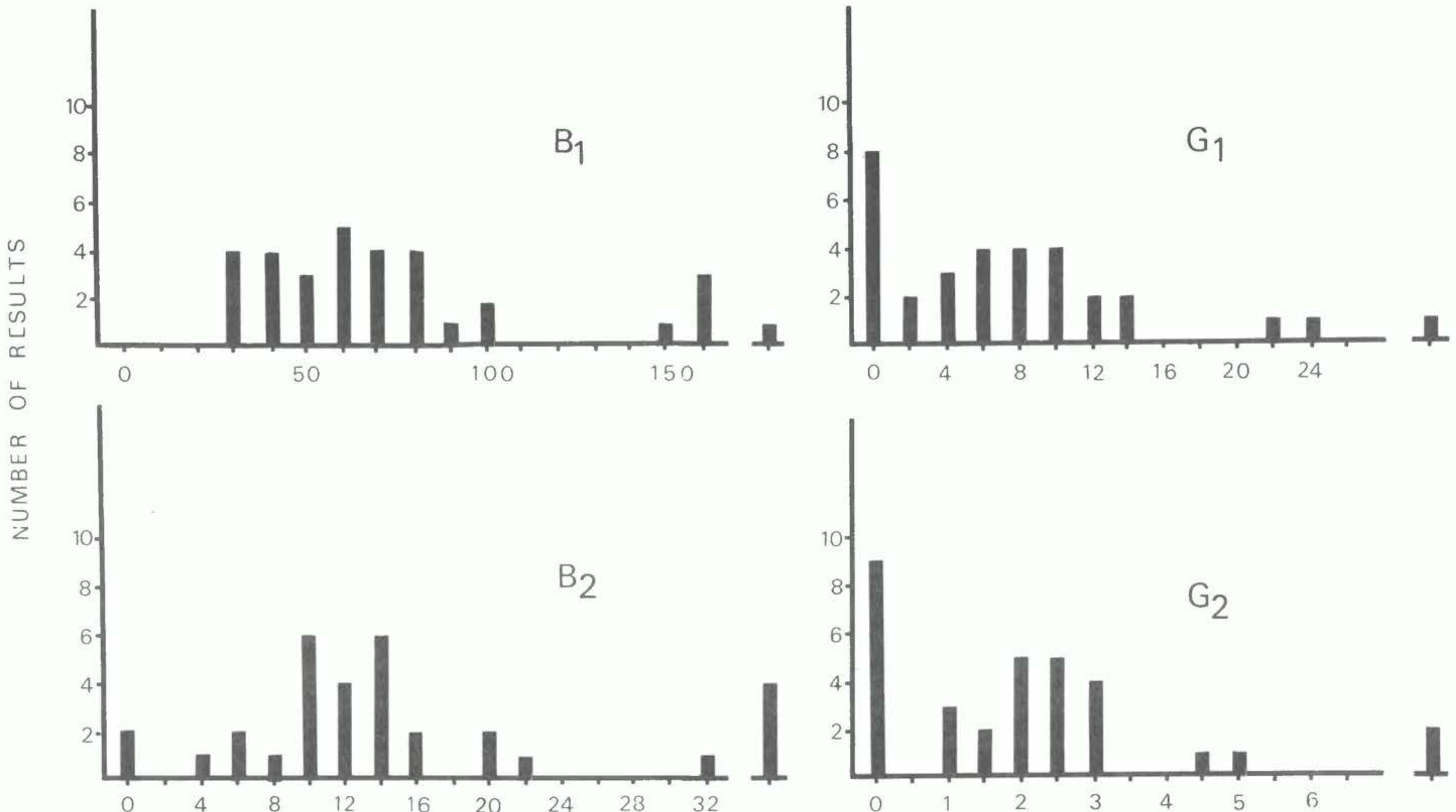

Fig. 2 (b). Distribution of results for the analysis of aflatoxins B_1, B_2, G_1 and G_2 in Deoiled Peanut Meal for the FAO/WHO subgroup.

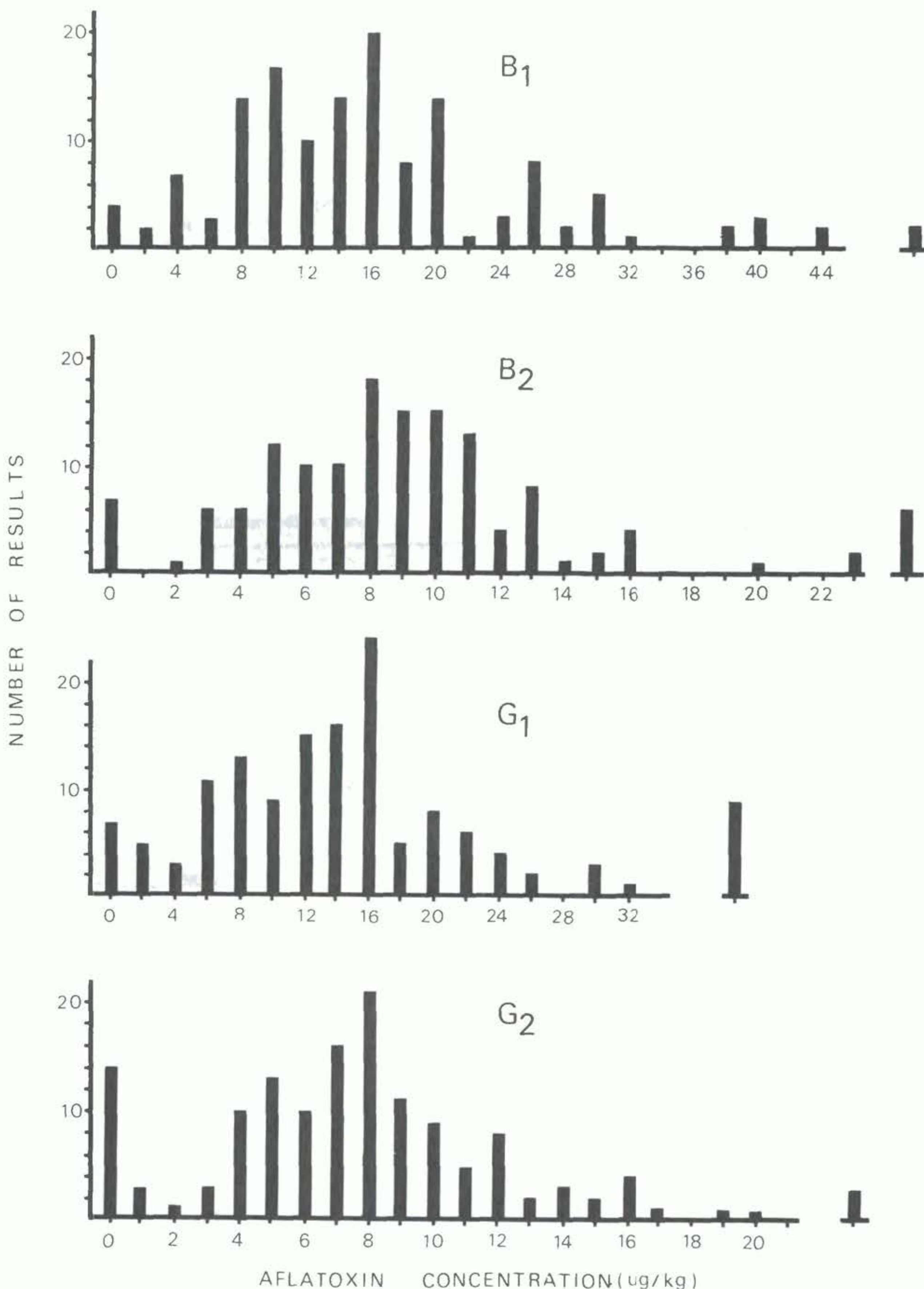

Fig. 3 (a). Distribution of results for the analysis of aflatoxins B_1, B_2, G_1 and G_2 in Yellow Corn Meal.

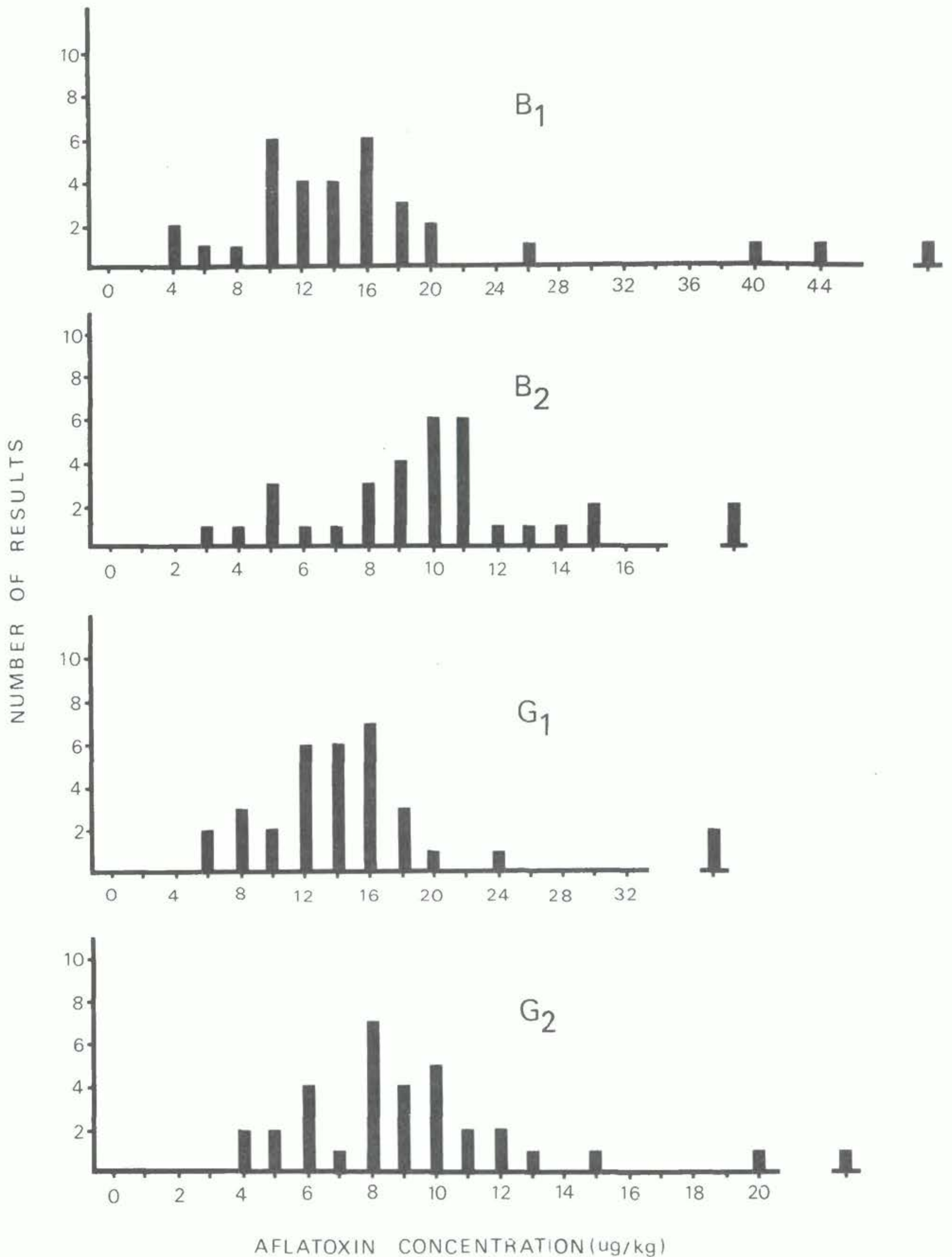

Fig. 3 (b). Distribution of results for the analysis of aflatoxins B_1, B_2, G_1 and G_2 in Yellow Corn Meal for the FAO/WHO Subgroup.

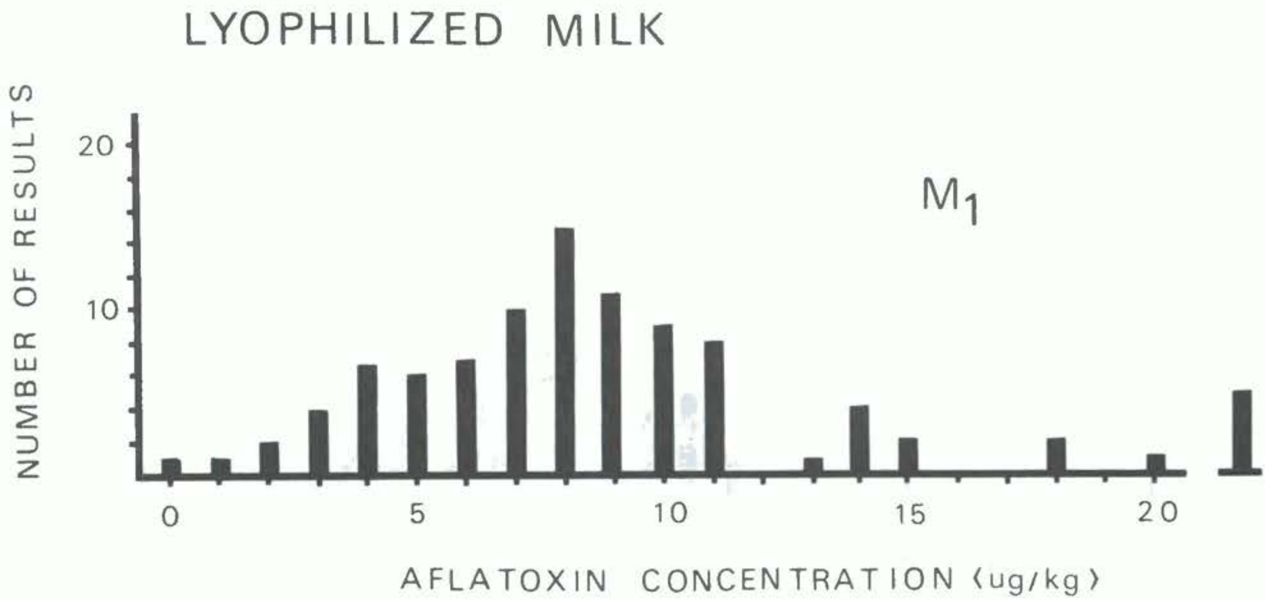

Fig. 4 (a). Distribution of results for the analysis of aflatoxin M_1 in Lyophilized Milk.

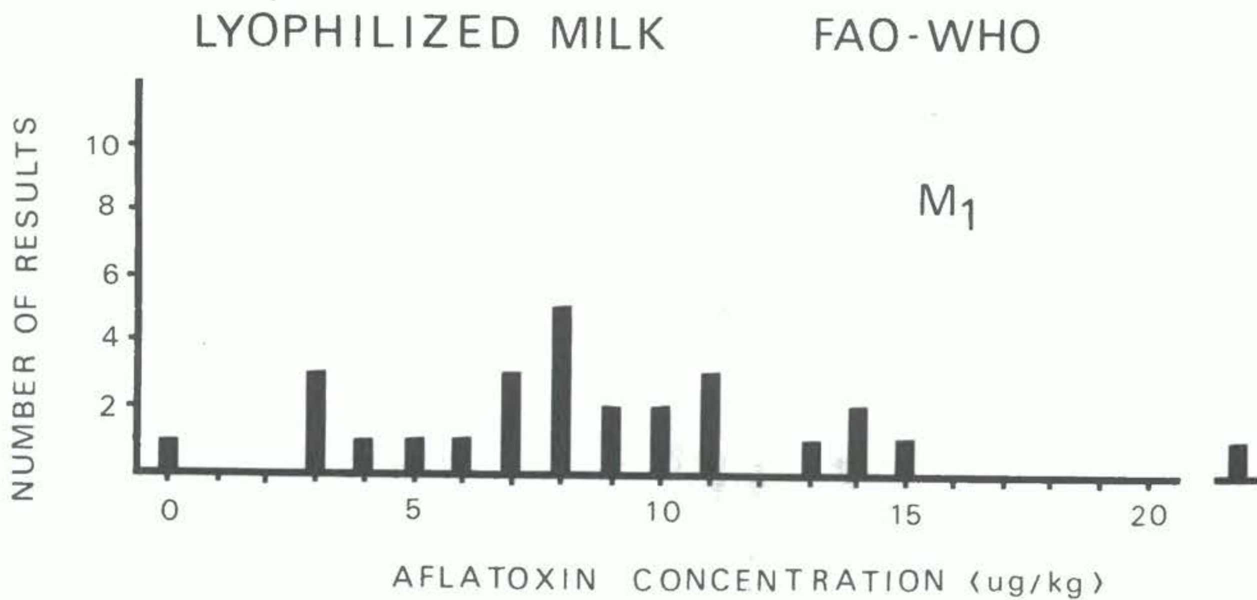

Fig. 4 (b). Distribution of results for the analysis of aflatoxin M_1 in Lyophilized Milk for the FAO/WHO subgroup.

Table 1 (a) Distribution of methods used according to sample analysed for aflatoxins B_1, B_2, G_1 and G_2.

Method	Number of laboratories using method on					
	Raw Peanut Meal		Deoiled Peanut Meal		Yellow Corn Meal	
	FAO/WHO subgroup	all lab.	FAO/WHO subgroup	all lab.	FAO/WHO subgroup	all lab.
CB [1-3]	14	33	13	39	13	42
BF [4]	6	34	4	32	4	27
EEC [5]	3	10	3	10	3	10
HPLC [6-19]	3	21	3	20	3	19
OTHERS [17-36]	11	41	9	41	10	44
TOTAL	37	139	32	142	33	142

Table 1 (b). Distribution of methods used in the analysis of lyophilized milk for aflatoxin M_1.

Method	Number of laboratories	
	FAO/WHO Subgroup	All laboratories
AOAC Method I [37]	5	21
AOAC Method II [38]	7	25
HPLC Methods	4	12
Other Methods [39-43]	11	37
TOTAL	27	95

	Method	Mean	Standard deviation	Coefficient of variation (%)	number of results
B_1	CB	226	121	54	33
	BF	197	98	50	32
	EEC	205	84	41	10
	HPLC	179	87	49	21
	Others	229	118	52	40
	All	211	108	51	136
B_2	CB	45.6	23.7	52	33
	BF	40.8	24.2	59	34
	EEC	34.7	14.4	41	10
	HPLC	38.2	23.1	60	21
	Others	41.9	24.6	59	40
	All	41.4	23.3	56	138
G_1	CB				33
	BF				34
	EEC				10
	HPLC				21
	Others				40
	All				138
G_2	CB				33
	BF				34
	EEC				10
	HPLC				21
	Others				40
	All				138

Table 2 (a). Statistical analysis of results for Raw Peanut Meal.

	Method	Mean	Standard deviation	Coefficient of variation (%)	number results
B_1	CB	262	116	44	14
	BF	264	68	26	5
	EEC	233	73	31	3
	HPLC	183	18	10	3
	Others	210	117	56	11
	All	237	103	43	36
B_2	CB	54.8	23.7	43	14
	BF	59.3	29.9	50	6
	EEC	39.0	14.5	37	3
	HPLC	43.9	7.1	16	3
	Others	36.9	20.1	54	11
	All	48.0	23.1	48	37
G_1	CB				14
	BF				6
	EEC				3
	HPLC				3
	Others				11
	All				37
G_2	CB				14
	BF				6
	EEC				3
	HPLC				3
	Others				11
	All				37

Table 2 (b). Statistical analysis of results for Raw Peanut Meal for the FAO/WHO subgroup.

	Method	Mean	Standard deviation	Coefficient of variation (%)	Number of results
B_1	CB	63.7	41.2	65	38
	BF	36.5	32.7	90	30
	EEC	64.9	21.3	33	10
	HPLC	54.5	35.7	66	20
	Others	56.2	31.8	57	40
	All	54.4	35.8	66	138
B_2	CB	12.1	7.0	58	35
	BF	7.1	4.0	56	31
	EEC	12.4	4.3	35	10
	HPLC	9.7	6.0	62	19
	Others	8.7	7.4	85	37
	All	9.7	6.5	67	132
G_1	CB	6.5	5.9	91	36
	BF	5.1	6.0	118	31
	EEC	7.2	4.3	60	9
	HPLC	4.4	4.6	105	20
	Others	3.3	4.0	121	37
	All	5.0	5.3	106	133
G_2	CB	2.0	1.7	85	36
	BF	1.6	1.7	106	27
	EEC	1.5	0.8	53	8
	HPLC	1.1	1.0	91	20
	Others	0.9	1.5	167	36
	All	1.4	1.6	114	127

Table 3 (a) Statistical analysis of results for Deoiled Peanut Meal

	Method	Mean	Standard deviation	Coefficient of variation (%)	Number of results
B_1	CB	84.0	44.7	53	13
	BF	71.0	68.4	96	3
	EEC	72.3	24.6	34	3
	HPLC	46.2	23.4	51	3
	Others	51.7	19.7	38	9
	All	68.6	39.2	57	31
B_2	CB	14.4	8.2	57	10
	BF	9.6	1.6	17	3
	EEF	16.6	5.0	30	3
	HPLC	10.4	2.7	26	3
	Others	7.7	4.3	56	9
	All	11.5	6.5	57	28
G_1	CB	7.3	6.0	82	12
	BF	9.5	10.5	111	4
	EEC	5.6	4.5	80	3
	HPLC	3.4	5.8	171	3
	Others	4.9	5.0	102	9
	All	6.3	6.1	97	31
G_2	CB	2.3	1.5	65	12
	BF	1.3	1.4	108	3
	EEC	1.2	0.7	58	3
	HPLC	0.6	1.0	167	3
	Others	1.2	1.2	100	9
	All	1.6	1.4	88	30

Table 3 (b) Statistical analysis of results for Deoiled Peanut Meal for the FAO/WHO subgroup

	Method	Mean	Standard deviation	Coefficient of variation (%)	Number of results
B_1	CB	14.8	9.0	61	42
	BF	15.0	11.6	77	26
	EEC	15.2	7.3	48	10
	HPLC	14.1	7.8	55	19
	Others	16.4	8.5	52	43
	All	15.3	9.0	59	140
B_2	CB	8.7	4.0	46	39
	BF	7.6	4.5	59	26
	EEC	8.4	4.0	48	10
	HPLC	8.8	3.0	34	18
	Others	7.8	4.5	58	42
	All	8.2	4.1	50	135
G_1	CB	13.4	6.6	49	41
	BF	11.8	7.1	60	22
	EEC	13.9	6.5	47	10
	HPLC	11.3	6.7	59	18
	Others	11.8	7.4	63	41
	All	12.4	6.9	56	132
G_2	CB	7.6	4.0	53	41
	BF	6.1	3.3	54	25
	EEC	8.7	4.9	56	10
	HPLC	7.1	3.7	52	19
	Others	7.0	5.0	71	43
	All	7.2	4.2	58	138

Table 4 (a). Statistical analysis of results for Yellow Corn Meal.

	Method	Mean	Standard deviation	Coefficient of variation (%)	Number of results
B_1	CB	16.0	8.7	54	13
	BF	23.4	16.5	71	3
	EEC	12.9	6.3	49	3
	HPLC	11.4	3.5	31	3
	Others	11.6	5.0	43	10
	All	14.6	8.4	58	32
B_2	CB	9.3	3.3	35	12
	BF	10.9	2.0	18	3
	EEC	7.6	3.4	45	3
	HPLC	9.4	0.9	10	3
	Others	8.7	3.3	38	10
	All	9.1	3.0	33	31
G_1	CB	13.0	3.6	28	13
	BF	10.0	1.2	12	2
	EEC	15.3	7.4	48	3
	HPLC	10.6	1.4	13	3
	Others	13.3	3.8	29	10
	All	12.9	3.9	30	31
G_2	CB	8.6	4.0	47	13
	BF	8.9	1.7	19	3
	EEC	8.9	5.4	61	3
	HPLC	8.4	1.5	18	3
	Others	8.5	2.7	32	10
	All	8.6	3.3	38	32

Table 4 (b). Statistical analysis of results for Yellow Corn Meal for the FAO/WHO subgroup.

Table 5 (a). Statistical analysis of results for lyophilized milk.

	Method	Mean	Standard deviation	Coefficient of variation (%)	Number of results
M_1	AOAC I	6.6	2.3	35	20
	AOAC II	6.9	4.3	62	23
	HPLC	8.3	1.6	19	12
	Others	9.0	4.0	44	35
	All	7.8	3.6	46	90

Table 5 (b). Statistical analysis of results for lyophilized milk for FAO/WHO participants.

	Method	Mean	Standard deviation	Coefficient of variation (%)	Number of results
M_1	AOAC I	5.7	2.3	40	5
	AOAC II	5.3	3.5	66	7
	HPLC	8.3	1.6	19	4
	Others	10.4	3.7	36	10
	All	7.8	3.8	49	26

Table 6. Distribution of zero results for analysis of Deoiled Peanut Meal

Method	Number of zeros reported (%)/number of results			
	B_1	B_2	G_1	G_2
CB	1/38 (3)	3/35 (9)	9/36 (25)	10/36 (28)
BF	0/30 (0)	0/31 (0)	12/31 (39)	9/27 (33)
EEC	0/10 (0)	0/10 (0)	0/9 (0)	0/8 (0)
HPLC	0/20 (0)	1/19 (5)	5/20 (25)	5/20 (25)
OTHERS	1/40 (3)	7/37 (19)	17/37 (46)	20/36 (56)
ALL	2/138 (2)	11/132 (8)	43/133 (32)	44/127 (35)

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ANALYTICAL QUALITY ASSURANCE

- CADMIUM AND LEAD -

by

M. E. Knowles and J. A. Burrell
Ministry of Agriculture, Fisheries and Food
Food Laboratory, Norwich UK

CONTENTS

		Page
1.	Summary	29
2.	Introduction	29
3.	Participation	29
4.	Distribution of Samples	29
5.	Submission of Reports	30
6.	Analytical Methodology	31
7.	Results	33
8.	Analysis of Results	33
9.	Appraisal of Results	33
10.	Discussion and Conclusions	36

ANNEX 1:	Results and analytical details	
	Notes and key to Tables 1 and 2	37
	Table 1 - Analysis for cadmium	38 - 44
	Figure 1 - Distribution of cadmium results	45
	Table 2 - Analysis for lead	46 - 51
	Figure 2 - Distribution of lead results	52
ANNEX 2:	Analysis of results	
	Notes and key to Tables 3 and 4	53
	Table 3 - Variation of cadmium results	54 - 55
	Table 4 - Variation of lead results	56 - 57
	Table 5 - Laboratories with results eliminated as outliers	58
	Table 6 - Data eliminated by Cochran's Test	58
ANNEX 3:	AQA Participation	
	(a) National participation	59
	(b) Participating laboratories	60
ANNEX 4:	References quoted by participating laboratories	62
ANNEX 5:	Consideration of principles involved in the statistical analysis of AQA results	63 - 64

1. SUMMARY

Four samples of freeze dried vegetables and shellfish were analysed in duplicate, for cadmium and lead, by 37 laboratories from 14 countries contributing data to the FAO/WHO Food and Animal Feed Contamination Monitoring Programme. The samples, which were not identified to the participants, consisted of the National Bureau of Standards, Standard Reference Materials (SRM) spinach, orchard leaves, tomato leaves and oyster tissue. Full experimental details of methods used, which were to be representative of those normally employed in monitoring programmes, were asked to be fully reported with the results.

Most were analysed by atomic absorption spectrophotometry, with flame and graphite electrothermal atomisation being equally popular. A few participants used anodic stripping voltammetry.

The majority of participants used dry ashing for sample preparation, the remainder using a variety of wet ashing procedures or occasionally low temperature plasma ashing. Intermediate preparations were not related to digestion, the majority (21) simply solubilised the ash, or digest, and measured the solution directly, others separated the metals from the sample digest by complexation and solvent extraction.

The AQA exercise produced results 20% of which were eliminated as outliers. The mean and mode obtained for samples in most instances differed substantially from certified or expected values, intra-laboratory variances were comparable with that of the Association of Official Analytical Chemists (AOAC) standard methods, inter-laboratory error however was excessive. Statistical examination of data did not demonstrate that the variety of analytical methods was the cause of high inter-laboratory error.

Enquiries should be conducted among participants by WHO appointed expert(s) to identify the causes of the problems and where appropriate assist laboratories to overcome them.

2. INTRODUCTION

The Ministry of Agriculture, Fisheries and Food, Food Laboratory in Norwich was requested and agreed to coordinate the Analytical Quality Assurance (AQA) for lead and cadmium. The overall exercise involved the distribution of four National Bureau of Standards reference materials, analysis in duplicate of check samples despatched from the coordinating laboratory in mid-June and submission of completed reports by the autumn. The data received by the coordinating laboratory is presented in this report.

3. PARTICIPATION

Agreements to participate were received from 14 countries. Forty-two sets of samples were requested. Eventually, completed report forms were received from 37 laboratories from 13 countries. Details are given in Annex 3. Unfortunately, samples despatched to Egypt were returned undelivered.

4. DISTRIBUTION OF SAMPLES

The following Standard Reference Materials were obtained from the National Bureau of Standards, Washington, DC, USA.

FAO/WHO AQA Code	SRM	Type	Cadmium mg/kg	Lead mg/kg	Units obtained	Portions distributed g
A	1570	Spinach	(1.5)	1.2 ± 0.2	13	15
B	1571	Orchard leaves	0.11 ± 0.01	45 ± 3	5	7.5
C	1573	Tomato leaves	(3)	6.3 ± 0.3	11	15
D	1566	Oyster tissue	3.5 ± 0.4	0.48 ± 0.04	25	15

NB. Cadmium values in parenthesis are uncertified.

On receipt the individual unit bottles of SRM were thoroughly mixed by manual shaking. Portions were then weighed directly into heavy gauge polythene bags and sealed to exclude as much air as possible. A set of four samples together with two report forms and a sheet of instructions were sealed in a further plastic bag. The requisite number of samples together with a covering letter were sent to the coordinating centre in each participating country for distribution to laboratories. These were packed in padded paper bags and sent by air mail letter-post on 17 June 1980.

The instructions to participants indicated (a) how to dry the samples before analysis (as directed by NBS), (b) requested analysis for lead and cadmium in duplicate by the method normally used for food monitoring programmes, (c) gave directions on the completion of the report and (d) asked laboratories to return completed reports direct to the UK Food Laboratory by the end of September 1980.

Separate report forms were to be completed for cadmium and lead. The element reported upon had to be entered and the duplicate results obtained expressed as mg/kg of dried material. Complete and detailed descriptions of the analytical method were to be given under the headings (a) sample preparation/size, (b) dissolution/separation, (c) measurement, (d) standardisation/calibration and (e) reagent blank/recovery checks/corrections applied. The detailed information required was, ashing temperature, chelating conditions, atomic absorption spectrometer (AA) atomiser type and values for reagent blank and recovery estimates etc.

Finally participants were invited to give any further comments they thought relevant and to make reference to any published methods used.

5. SUBMISSION OF REPORTS

Most reports were received by or immediately after the end of September. Reminders were sent to countries with laboratories that had not reported and a few more results were forthcoming; the last being received in early December. One country was unable to forward completed reports for laboratories coded 34-37. However the collaborating centre was able to send a summary of the results in advance. It was thus possible to include the results in the overall statistical analysis, but not in that analysis for which method details were required. With few exceptions the methodological information supplied on the report forms was highly informative, along the lines requested.

Two laboratories reported results involving two separate analytical methods (see Tables 1 and 3 attached). Laboratory 19 quoted duplicate results for both and statistically they were treated as if they were from separate laboratories. Laboratory 24 quoted single results for each of their two methods, stating that these represented average values. These two sets of figures were examined as if they were replicates from a common method. Laboratory 8 was dissatisfied with the lead replicate results reported for A and D and they analysed two additional replicates. The first results were taken and used for the AQA but the further replicates are mentioned when the results of the exercise are discussed. Two laboratories, 22 and 33, quoted more than two replicate results from the one method. Two were selected for the statistical analysis using random numbers as recommended by the AOAC (1). Laboratory 29 did not report cadmium results. Laboratory 14 was unable to analyse sample D. The end-measurement used by laboratory 34 for cadmium could not be identified from the report. Dithizone complex formation was involved in the analysis and the use of a mixed colour titration with dithizone was reportedly used for lead. A further two laboratories, 27 and 33, reported difficulties solubilising the sample residues after digestion. Laboratory 27 used dry ashing, 33 - wet ashing. Laboratory 33 also mentioned that the levels measured were atypically high and that the method they used was only really suitable for levels less than 0.5 mg/kg. In view of the repeatability of their results they also queried sample homogeneity.

(1) Youden, W.J. and Steiner, E.H., (1974), Statistical Manual of the AOAC.

6. ANALYTICAL METHODOLOGY

Summaries of the analytical methods used by the participating laboratories are given in Table 1 and 2 (Annex 1). A common method for cadmium and lead was used by most laboratories, and is described in detail for cadmium in this Report. Where different methods for lead are used, these will be discussed where appropriate.

Thirty-eight analytical procedures for cadmium are described in Table 1, although 37 laboratories took part in the AQA, laboratory 29 did not report results for cadmium. However laboratories 19 and 24 carried out the analyses using two different methods each of which is described.

6.1 Digestion

Three types of digestion procedure were employed, dry ashing, the most popular, was used by 21 laboratories, 15 laboratories used wet ashing and two employed low temperature ashing in an oxygen plasma.

Dry Ashing

For dry ashing, the analysis sample size ranged from 0.1 g up to 6 g. The results from laboratories employing the smallest sample size, i.e.0.1 g - 0. 25 g, apparently produced less reliable results. The commonest final ashing temperature used was 450°C. Four laboratories used a temperature as high as 550°C but only after pre-treatment of the sample with sulphuric acid, which reputedly converts the metal to less volatile sulphates. The time for which the maximum ashing temperature was held was given by 15 laboratories. Twelve left their samples in the furnace at least overnight, one for as much as 24 hours and another for 48 hours. Four laboratories used much shorter periods ranging from 4-10 hours.

Wet Ashing

For the 15 laboratories using wet ashing, sample sizes of 0.25 to 5 g were employed. Digestion agents consisting of nitric, sulphuric and perchloric acids and hydrogen peroxide were used in variable combination as shown below:-

Digestion Agent	Laboratories
Nitric, sulphuric & perchloric acid	2
Nitric, sulphuric acid & hydrogen peroxide	2
Sulphuric acid & hydrogen peroxide	2
Nitric and sulphuric acid	1
Nitric acid and hydrogen peroxide	4
Nitric acid	2
Sulphuric acid	1
Hydrogen peroxide with ferrous ion	1

Low Temperature Ashing

The two laboratories using low temperature ashing employed sample sizes of 0.5 and 2 g, however, the laboratory ashing 2 g followed this treatment with further digestion using nitric and sulphuric acids.

6.2 Separation

In each method described, digestion is followed by either simple dilution or dissolution of ash, referred to as "direct measurement" or removal of analyte from the bulk of inorganic material by complexation and solvent extraction. The nature of this preparation did not appear to be related to type of digestion employed.

Direct measurement was used in 21 of the 38 methods. For 14 nitric acid was utilized, although for 3, where anodic stripping voltammetry (ASV) was used, acetate buffer was additionally added. In six methods, ash was dissolved in hydrochloric acid, and for one, the ash was dissolved in water.

In 17 of the analytical procedures lead and cadmium were extracted from the digest, or ash solution, most commonly complexed as a dithiocarbamate chelate. The most favoured solvent was 4-methyl pentan-2-one (methyl isobutyl ketone, MIBK). The metal chelates were extracted into MIBK in 11 of the methods described, four after complexation using sodium diethyldithiocarbamate (NaDDC), six using ammonium pyrrolidinedithiocarbamate (APDC) and one using APDC and diethylammonium diethyldithiocarbamate (DDDC) in combination. In addition, laboratory 31 used NaDDC but extracted the complex into butyl acetate and laboratory 5 used DDDC and extracted into xylene. By contrast, laboratory 30 reacted the cadmium ions with iodide ions and extracted the metal iodate anion, using Amberlite resin, into MIBK. This procedure was not used for lead. Finally, for three methods, dithizone separation was involved, though it was not clear from the report form exactly how it was done for laboratory 34. Laboratory 2 and 28 extracted the metals using dithizone dissolved in chloroform and then back extracted the metals into dilute hydrochloric acid for measurement.

6.3 Measurement

Atomic absorption spectrophotometry (AAS) was used to measure cadmium in 33 of the 38 procedures. Anodic stripping voltammetry (ASV) was the main alternative. The measurement used by laboratory 34 was unclear from their report. AAS was used either in the air/acetylene flame atomisation mode or by using electrothermal atomisation by carbon rod or furnace. Flame was exclusively used by 15 laboratories and flameless atomisation by 11. Five further laboratories used either technique. Deuterium background correction was reported as being used by 12 laboratories. Laboratory 12 made background corrections for cadmium at 229 nm using a rhenium lamp. For four laboratories ASV, with or without differential pulse, was employed for measurement. Three of these laboratories reported conducting measurements in dilute nitric acid but with the addition of acetic acid, sodium acetate and tartaric acid.

6.4 Calibration

Comparison with a standard curve was most frequently used to calibrate AAS instruments in either flame or flameless modes. For five of the 33 laboratories, however, the method of standard additions was preferred. Interestingly, laboratory 8 used NBS SRM 1571 orchard leaves and 1577 bovine liver to standardise measurements. The method of standard addition was used for calibration of stripping voltammetric measurements.

6.5 Checks and Corrections

Information on these points was less forthcoming than on others. It was hoped that participants would give full information on the reagent blank and recovery estimates conducted and to mention if corrections were made to results based on these checks. Five laboratories (1, 3, 20, 27, 36) did not report conducting any of the checks. The remaining 33 laboratories all reported reagent blank determinations, but only 13 stated that they made corrections. Many of the reagent blanks were negligible. Twenty-four of the 38 laboratories reported conducting some sort of recovery determination for cadmium mainly as a check on the current analysis. Recoveries from 77 - 113% were reported by 20 laboratories but only one laboratory, No. 32, reported applying recovery factors to the submitted results.

6.6 Lead Analysis

Laboratory 29 used a brief dry ashing technique and conducted AAS flame measurements on dilute nitric acid solutions of the ash. Laboratory 30 employed pulse polarography and ASV measurements on nitric acid solutions of the dry ash. Whilst the method for laboratory 34 was not clearly stated for lead, it appeared that a dithizone complex formation (two step separation) was followed by mixed colour titration with dithizone. This laboratory was the only one not employing AAS or ASV measurement.

6.7 References

Any reference made to published methods in report forms are reproduced in Annex 4; the information is listed as supplied.

7. RESULTS

The AQA results given in Tables 1 and 2 (Annex 1) contain the analytical results for cadmium and lead respectively. The distribution of results is shown by histograms (Figure 1 and 2). The outline histograms in the figures represent the total results, whilst the shaded areas represent those remaining after the statistical elimination of outliers as described below.

8. ANALYSIS OF RESULTS

For this purpose use has been made of statistical procedures described by the American Association of Official Analytical Chemists (2) and of the International Organisation for Standardisation as formalised and published by the British Standards Institution (2).

The procedures described assess the precision of a method of test from the result of an inter-laboratory precision experiment such as a collaborative trial. Their purpose is to assess random error exhibited among and within laboratories carrying out a standardised test on identical material. It is reasonable to assume the uniformity of the test material (NBS Standard) used in the AQA, but not of analytical methodology, (Section 6). However, the aim of the AQA was to assess the comparability of data being submitted to the Monitoring Programme, that is to assess how closely the data resembled that which might have been produced by a standardised test. It was therefore decided to subject the data from the AQA to a treatment appropriate to a method trial. The results obtained, however, should be treated with some caution and only taken as a guide.

An idea of the approximations made, and the validity of some of the assumptions, is perhaps best obtained by a consideration of some of the principles involved in this statistical analysis, shown in Annex 5. The data that remained after elimination of outliers was taken for each sample in turn and the mean, reproducibility R and repeatability r calculated. The results of these caluations are given in Annex 2 at the foot of Tables 3, for cadmium and 4, for lead. Tables 3 and 4 summarise data resulting from a comparison of the variance of results from laboratories using a common method with total variance. The purpose was to determine if the use of a common procedure tended to give more precise results, using analysis of variance. The comparisons were made for intra and inter-laboratory variances. The methodological features so tested were dry ashing, wet ashing, flame AAS, flameless (graphite) AAS, chelation/extraction, and dissolution followed by direct measurement.

9. APPRAISAL OF RESULTS

Table 5 (Annex 2) indicate the laboratory results eliminated as outliers by the statistical analysis of the AQA data. This process removed some 18 and 22% of the data supplied for cadmium and lead respectively. Eighteen out of the 37 participating laboratories (49%)

(2) "Precision of test method, part 1". BS5497: Part 1: 1979, British Standards Institution, London.

had no results eliminated. Details of the data eliminated by Cochran's test, because of wide duplicates, are given in Table 6 of Annex 2. Duplicate results for cadmium from laboratories 5 and 24 and for lead from 6, 8, 14, 24 and 32, appeared to consist of one estimate typical of the general distribution and another a rogue value. Of those that remained most would have been subsequently eliminated by Dixon's test as outliers. Figures 1 and 2, show the spread of results to be broad although the distributions appear reasonably unimodal for samples A, B and D. The modes for the cadmium results for samples A, B and D did not lie as close to the expected values, quoted by the NBS, as expected. From the illustration of cadmium results for sample C, two modes could be postulated, both very different from the expected value. However, it should be noted that NBS on the SRM certificate for tomato leaves quoted a non-certified value for cadmium of 3 mg/kg stating that the cadmium was not sufficiently homogeneous for certification. The spread of the AQA results for cadmium might well indicate the extent of this lack of homogeneity. NBS made the same statement for the uncertified cadmium level quoted for spinach. The spread of AQA results for sample A (spinach) was no worse than for sample B and D. The spread of lead results for A, B and D are unimodal; however the spread for sample C suggests the presence of two modes. The modes of the distributions of B and C are much less than the NBS certified values.

The main parameters which quantify the distribution of the AQA results, the means, the repeatabilities r and reproducibilities R, are tabulated next.

Sample	Expected	Mean AQA	% of Expected	r%	R%
Cadmium					
Orchard leaves B	0.11 $\pm$ 0.01	0.14	127	29.6	181
Spinach A	(1.5)	1.3	87	21.7	78
Tomato leaves C	(3)	2.35	78	20.1	61.7
Oyster tissues D	3.5 $\pm$ 0.4	3.3	94	23.9	88.4
Lead					
Oyster tissues D	0.48 $\pm$ 0.04	0.69	144	50.4	177
Spinach A	1.2 $\pm$ 0.2	1.2	100	28.6	153
Tomato leaves C	6.3 $\pm$ 0.3	5.0	79	20.8	108
Orchard leaves B	45.0 $\pm$ 3	38	84	13.3	50.0

The means for cadmium range from 78 - 127% of the expected values and for lead from 79 - 144%. The results larger than expected were associated with the lowest levels tested, that is 0.11 cadmium and 0.48 mg/kg lead. The repeatability r for cadmium ranged from 20 - 30%. Reproducibility R was very large for the 0.11 mg/kg level at 181%, but less excessive for the remainder ranging from 62 - 88%. For lead results both r and R were larger; r ranged from 13 - 50% and R from 50 - 177%. Reproducibility appeared to decrease as the lead level increased. In absolute terms the per cent reproducibility for lead in orchard leaves of 50% means 19 mg/kg!

Some indication of what has been regarded as acceptable, in terms of r and R can be gained by consideration of the data used to validate two AOAC standard methods; 25.026 (12th Edition) Cadmium, Atomic Absorption Spectrophotometric Method - Official Final Action[3] and 25.060 Lead, Atomic Absorption Spectrophotometric Method - Official First Action[4]. For the cadmium method five laboratories analysed six commotities in duplicate at three concentration levels. At the lowest level, 0.1 mg/Kg fresh weight, r ranged from 19 - 91%, mean 47. At the highest level, 1.5 mg/Kg, r ranged from 9 - 34%, mean 17%. R at 0.1 mg/Kg ranged from 33 - 118%, mean 53%, and at the 1.5 level, 14 - 59%, mean 35%. For the lead method, seven laboratories measured singly eight commodities at three levels. The commodities were paired in terms of type and level present and the data obtained at levels below 5 mg/Kg (drained weight) yielded r values from 20 - 25% and R values of from 35 - 60%. For levels above 10 mg/Kg, r ranged from 19 - 42% and of R from 15 - 45%.

The repeatabilities exhibited by the AQA for cadmium compare favourably to those quoted for the AOAC method, however, reproducibilities are up to two times greater. For lead repeatabilities from the AQA are comparable with those obtained for the AOAC method. Both exercises gave higher repeatability at the lowest concentration levels analysed. Again, reproducibilities are much larger for the AQA by about a factor of 3.

The comparison of variance of methodological features for cadmium, (Table 3) revealed little of significance. Only two groups had a method variance either consistently larger or smaller than total variance and no firm trend could be discerned. An apparently statistically significant difference for inter-laboratory variance was found in the chelation group for only one of the four samples. Means for different methods were reasonably consistent, only one minor trend was indicated for chelation, which gave lower results than direct measurement.

For lead (Table 4) apparently statistical significant differences from total variance were indicated, for intra-laboratory variance, with chelation, sample A, and for inter-laboratory variance for dry washing, sample C, AAS flame, sample C and chelation, samples C and D. For dry ashing the total variances were all larger than those of the group, however, the apparently high precision exhibited for sample C was not reflected for the other samples in the group. For flame AAS the inter-laboratory variance was consistently less than for the total distribution, again, however, data computed for samples A, B and D gave no support for the significance indicated for sample C. Little supporting evidence was also provided for the two samples from the chelation group with apparently smaller variances. The lead means for the separated methods were also reasonably consistent though the tendency shown for chelation to give lower results than direct measurement was stronger.

Bearing in mind the deficiencies of the statistical model used to analyse the AQA data, it would be unwise to draw any firm conclusions as to the relative merits of using any particular analytical method to analyse traces of cadmium or lead in food. However, an impression was obtained that chelation tended to give more precise but lower results.

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(3) "Collaborative study of a method for the Atomic Absorption Spectrophotometric and Polarographic determination of cadmium in food." Gajan, R.J. et al, Journal of the AOAC, 1973, 56, (4),, 876.

(4) "Collaborative study on a method for determining lead in food and animal products." Hoover, W.L. et al, 1972, Journal of the AOAC, 55, (4), 737.

10. DISCUSSION AND CONCLUSIONS

In essence, the FAO/WHO programme[5] is striving to establish and strengthen, where necessary, national monitoring programmes, principally to aid the control and prevention of food contamination, but also to meet the needs of the international programme. The latter has to coordinate the collection and storage of data, to provide, and to disseminate internationally, information to facilitate the evaluation of risk to human health, the setting of standards for contaminants in food and to aid the formulation of plans to reduce, or eliminate, health, or economic risks from food contamination. To make evaluation and standardisation possible for both national and international purposes, monitoring data must be comparable and the extent of comparability required needs to be defined. That which is desirable must be balanced against that which is practicable.

Two aspects arising from the analysis of the AQA data need detailed consideration, namely that some 20% of the data had to be eliminated and the distribution and comparability of the data that remained. Taking the latter first, the findings suggest that although intra-laboratory variance is comparable with those exhibited by recognised standard methods of analysis for lead and cadmium, the inter-laboratory variation is excessive. In addition, the mean calculated by the AQA for the four samples deviated substantially from well authenticated expected values; the situation was worse for lead than for cadmium.

The high inter-laboratory variations might be thought due to the use of non-standardised methods, however the statistical examination of methodological features does not support this hypothesis. When taken separately, results for specific methods give similar broad inter-laboratory distributions about reasonably consistent means, a possible exception being chelation.

It would be imprudent to dismiss method difference as a source of inter-laboratory error. Initially, investigations should be directed to identify other causes, with particular attention paid to the experience of participants. Comparison should also be made between laboratories which performed well and those which performed badly to identify potential significant differences.

One way in which this might be achieved is by informal contact between laboratory personnel and WHO experts followed by limited sample exchange.

The other important feature shown by this AQA is the elimination of 20% of the results as outliers. During normal monitoring/submission of data to WHO these would of course go unnoticed, thus potentially introducing errors in the calculated overall exposure to the particular element. The solution to this particular problem can only come from that described above, i.e. improved training and collaborative trials.

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(5) Guidelines for Establishing or Strengthening National Food Contamination Monitoring Programmes WHO/HCS/FCM/78.1 - WHO, Geneva 1979.

ANNEX I

NOTES AND KEY TO TABLES 1 AND 2

Tables 1 and 2 respectively contain the AQA results for cadmium and lead. They also summarise the analytical method details supplied by participating laboratories.

The results are expressed as mg/kg on the dry weight of the samples supplied.

The "expected" values quoted by the NBS are given at the head of the results columns.

The more significant analytical features, which were subjected to special statistical investigation, are highlighted by the use of block capitals.

The reference letters and symbols indicate the following:

Results

Superfix a.	Eliminated as an outlying laboratory by Rank Sum (95% probability).
Superfix b.	Eliminated because replicates were found to be too wide by Cochran's test (95% probability).
Superfix c.	Eliminated as an outlying result by Dixon's test (95% probability).
Superfix d.	Eliminated as the limit of determination appeared atypically high.
Superfix z.	Eliminated as only single results supplied.
Superfix y.	Result reported as not detectable, taken as 0 for calculation.
Superfix x.	Result reported as <0.1, taken as 0.1 for calculation.
Superfix w.	Result reported as <0.01, taken as 0.01 for calculation.
Superfix v.	Result reported as <0.05, taken as 0.05 for calculation.
≠	No result reported.

Analysis

Superfix *	Deuterium background correction used in AAS measurement.
Superfix **	Background correction for cadmium measurement by AAS conducted using a rhenium lamp at 219 nm.

TABLE 1.

JOINT FAO/WHO FOOD CONTAMINATION MONITORING PROGRAMME:

ANALYTICAL QUALITY ASSURANCE FOR LEAD AND CADMIUM 1980

ANALYSIS FOR CADMIUM

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD					
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE, DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	CORRECTIONS BLANK	CORRECTIONS RECOVERY
EXPECTED	(1.5)	0.11	(3.0)	3.5						
1	2.78[c] 2.62[c]	0.64[c] 0.63[c]	1.64 1.56	2.68 2.56	2 g DRY ash ($450^{o}C/8h$)	Dissolved in $HCl/HNO_3/H_2O$ (2:1:3) Added sodium acetate EXTRACTED APDC/MIBK	AAS FLAME	CURVE (0-1 ppm)		
2	1.72 1.74	0.16 0.12	2.59 2.65	4.17 4.33	0.5-1.0g DRY ash ($450^{o}C/24h$)	Dissolved in IN HCl Added ammonium citrate Adjusted pH to 9.3 EXTRACTED dithizone/chloroform Back extracted 0.1N HCl	AAS FLAMELESS	CURVE (0.01-0.2 ppm) (HCl)	MEASURED APPLIED	MEASURED NOT APPLIED
3	0.85 0.87	0.095 0.090	1.75 1.85	2.9 3.0	0.5g WET ash (H_2SO_4, H_2O_2)	EXTRACTED APDC/MIBK	AAS FLAME	CURVE		
4	1.444 1.355	0.099 0.108	0.761[c] 0.620[c]	1.216 1.268	1g (A, D) 0.5g (B, C) WET ash (HNO_3, H_2SO_4, H_2O_2)	EXTRACTED APDC/MIBK	AAS FLAME	CURVE (0.2-0.5ppm)	MEASURED NOT APPLIED	

TABLE 1. (continued)

LAB NO	Results mg/kg				ANALYTICAL METHOD					
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE, DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	CORRECTIONS BLANK	CORRECTIONS RECOVERY
EXPECTED	(1.5)	0.11	(3.0)	3.5						
5	1.7 2.1	0.16[b] 0.26[b]	2.3 2.9	3.9 4.8	5g WET ash (H_2SO_4, H_2O_2)	EXTRACTED DDDC/XYLENE	AAS FLAME*	CURVE (0.5–2.0ppm)	MEASURED NOT APPLIED	MEASURED NOT APPLIED
6	1.2 1.2	0.5[b] 0.2[b]	0.5[b] 2.0[b]	4.0 3.0	2–5g WET ash (HNO_3+H_2O_2)	Diluted to 10 ml DIRECT MEASUREMENT on aliquot	Reversed POLAROGRAPHY	STANDARD ADDITIONS (1–10ug)		
7	1.39 1.41	0.101 0.122	2.88 2.83	3.40 3.43	0.5g LOW TEMP ash (oxygen plasma)	Dissolved in d.HNO_3 DIRECT MEASUREMENT	AAS FLAME* (A,C,D) FLAMELESS (B) *	CURVE (0.2–0.8ppm) STANDARD ADDITIONS (2–8 ppb)	MEASURED NOT APPLIED	MEASURED NOT APPLIED
8	1.29 1.44	0.092 0.082	2.37 2.35	3.35 3.34	2–3g DRY ash (450°C/night)	Added HCl & dried Dissolved in 0.1 N HNO_3 DIRECT MEASUREMENT	AAS FLAME (A,C,D) * AAS FLAMELESS (B) *	Relative tq NBS STANDARDS	MEASURED NOT APPLIED	
9	1.39 1.39	0.15 0.15	2.56 2.61	3.51 3.41	5g WET ash HNO_3	Diluted to 25 ml DIRECT MEASUREMENT	AAS FLAME *	CURVE (0.01–2.00 ppm)	MEASURED NOT APPLIED	
10	0.82 0.83	0.09 0.08	1.67 1.67	4.33 4.31	1g DRY ash (with H_2SO_4) (550°C)	Dissolved in IN HCl Added ammonium citrate, $NH_3OH.HCl$, NH_4OH (pH 9.5), $(NH_4)S_2O_4$ EXTRACTED NaDDC/MIBK	AAS FLAMELESS	CURVE (5–15ppb)	MEASURED	MEASURED

TABLE 1 (continued)

LAB NO	RESULTS mg/kg				ANALYTIC METHOD				CORRECTIONS	
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE, DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	BLANK	RECOVERY
EXPECTED	(1.5)	0.11	(3.0)	3.5						
11	0.309 0.283	0.029 0.049	1.845 2.387	2.475 2.310	5 g DRY ash (475°C/18h)	Dissolved in c.HCl, dried Dissolved in 2N HCl Diluted to 25 ml DIRECT MEASUREMENT	AAS FLAMELESS	CURVE (0–0.01ppm)	MEASURED NOT APPLIED	MEASURED
12	1.6 1.6	0.2 0.15	2.6 2.6	3.2 3.3	2 g DRY ash (450°C)	Dissolved in d. HCl Diluted to 10 ml & filtered DIRECT MEASUREMENT	AAS FLAME **	CURVE	MEASURED NOT APPLIED	MEASURED NOT APPLIED
13	1.4 1.6	0.07 0.09	2.8 2.9	3.2 3.[illegible]	2 g DRY ash (450°C/night)	Dissolved in d.HCl & centrifuged A,C & D DIRECT MEASUREMENT B treated with ascorbic acid EXTRACTED APDC/MIBK	AAS FLAME AAS FLAMELESS	CURVE CURVE	MEASURED APPLIED	
14	1.4 1.6	0.4 0.4	2.7 2.8	– –	2 g DRY ash (400°C)	Dissolved in d.HCl DIRECT MEASUREMENT	AAS FLAME	CURVE	MEASURED NOT APPLIED	MEASURED NOT APPLIED
15	1.7 1.7	0.15 0.13	2.6 3.0	4.3 4.5	1–2 g WET ash (HNO_3,H_2O_2)	Diluted to 25 ml with d.HNO_3 DIRECT MEASUREMENT	A,C,D AAS FLAME B AAS FLAMELESS	CURVE STANDARD ADDITIONS	MEASURED	
16	23.10[a] 24.20[a]	2.26[a] 1.92[a]	35.50[a] 34.67[a]	62.80[a] 58.20[a]	1 g DRY ash (450°C/16h)	Dissolve in d.HNO_3 DIRECT MEASUREMENT of appropriate dilutions in d.HNO_3	AAS FLAMELESS	CURVE (0–10 ppb)	MEASURED	MEASURED

TABLE 1. (continued)

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD					
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE, DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	CORRECTIONS BLANK	CORRECTIONS RECOVERY
EXPECTED	(1.5)	0.11	(3.0)	3.5						
17	1.31 1.37	0.127 0.127	2.25 2.36	2.77 2.95	0.5 - 1 g WET ash (H_2SO_4, HNO_3)	Added NH_4OH (pH 4) EXTRACTION APDC/MIBK	AAS FLAME	CURVE (0–0.4 ppm)	MEASURED NOT APPLIED	
18	1.34 1.26	0.097 0.103	2.88 2.60	3.52 3.49	0.4–1.4 g DRY ash (with H_2SO_4)	Dissolved in d.HNO_3 added acetate buffer DIRECT MEASUREMENT	Differential pulse ASV	STANDARD ADDITIONS	MEASURED APPLIED	MEASURED NOT APPLIED
19 METHOD 1	1.9 1.9	0.18 0.22	3.3 3.4	4.4 4.1	1.5–2 g WET ash (HNO_3)	DIRECT MEASUREMENT	AAS	STANDARD ADDITIONS	MEASURED	APPLIED
19 METHOD 2	1.4 1.4	0.2 0.2	2.5 2.9	3.4 3.8	1 - 4 g WET ash (HNO_3, H_2O_2)	DIRECT MEASUREMENT	AAS FLAME	STANDARD ADDITIONS	MEASURED	APPLIED
20	1.6 1.7	0.37 0.36	2.6 2.7	3.0 2.8	1 g WET ash (HNO_3, H_2O_2)	Dissolve in d.HNO_3 DIRECT MEASUREMENT	AAS FLAME OR FLAMELESS *	STANDARD ADDITIONS		
21	1.18 1.13	0.11 0.11	1.93 2.10	3.05 2.91	2 g LOW TEMP Asher then WET ash (HNO_3, H_2SO_4)	Dissolved in d.HNO_3 Added ammonium citrate, NH_4OH(pH 7) & $(NH_4)_2SO_4$ EXTRACTED DDTC/MIBK	AAS FLAME *	CURVE (0–0.4 ppm)	MEASURED APPLIED	MEASURED NOT APPLIED
22	1.18 1.14	0.03 0.03	2.13 1.99	3.15 3.17	1 g WET ash (H_2SO_4, HNO_3, $HClO_4$)	EXTRACTED Na DDC/MIBK	AAS FLAME	CURVE (0–0.2 ppm)	MEASURED	MEASURED

TABLE 1 (continued)

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD					
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE, DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	CORRECTIONS BLANK	CORRECTIONS RECOVERY
EXPECTED	(1.5)	0.11	(3.0)	3.5						
23	1.4 1.2	0.08 0.05	1.6 1.9	1.9 1.2	5 g DRY ash (460°C/16h)	Dissolved in d.HCl EXTRACTED APDC/MIBK	AAS FLAME	CURVE	MEASURED	
24 (1)	1.30[b]	<0.01	2.7[b]	3.6[b]	0.2g DRY ashed (H_2SO_4)(550°C)	Dissolved in d.HNO_3 & filtered. Added acetate buffer (pH 4.2) DIRECT MEASUREMENT	ASV	STANDARD ADDITIONS	MEASURED APPLIED	MEASURED NOT APPLIED
24 (2)	0.16[b]	0.03	4.4[b]	5.0[b]	1.0 g WET ash (H_2SO_4)	Filtered, dried & dissolved in water DIRECT MEASUREMENT	AAS FLAMELESS	SINGLE STANDARD	MEASURED APPLIED	MEASURED NOT APPLIED
25	3.2[c] 3.4[c]	0.14 0.19	3.2 3.0	3.9 4.0	4 g DRY ash (500°C/night)	Dissolved in d.HNO_3 DIRECT MEASUREMENT	AAS FLAMELESS *	CURVE (0-2 mg)	MEASURED	MEASURED
26	1.50 1.55	<0.05[v] <0.05[v]	1.36 1.38	2.9 2.7	2 g DRY ash (H_2SO_4) (500°C/4h)	Dissolved in d.HCl. Added citric acid & NH_4OH (pH 4) EXTRACTED APDC/MIBK	AAS FLAME	CURVE	MEASURED NOT APPLIED	MEASURED NOT APPLIED
27	1.39 1.26	0.21 0.19	1.77 1.94	5.18 4.74	1 g DRY ash (400°C/night)	Dissolved in d.HNO_3 filtered DIRECT MEASUREMENT	AAS FURNACE *	SINGLE STANDARD (0.01 ppm)		
28	8.8[a] 9.6[a]	11.4[a] 9.6[a]	20.2[a] 19.2[a]	10.6[a] 12.0[a]	1 g DRY ash (450°C/6h) then WET ash $(HNO_3, HClO_4)$	Dissolved in d.HCl. added ammium citrate and NH_4OH (pH 9.2) EXTRACTED Dithizone/-chloroform Back extracted d.HCl	AAS FLAMELESS*	CURVE	MEASURED	MEASURED

TABLE 1 (continued)

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD					
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE, DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	CORRECTIONS BLANK	CORRECTIONS RECOVERY
EXPECTED	(1.5)	0.11	(3.0)	3.5						
29					No report received for cadmium					
30	1.47 1.53	0.11 0.08	2.58 2.49	3.42 3.29	2–3 g DRY ash (H_2SO_4) (550°C/12hr)	Dissolved in d.HCl Added KI/HCl EXTRACTED Amberlite/MIBK	AAS FLAME *	CURVE (0.02–0.5ppm)	MEASURED APPLIED	
31	1.00 0.81	0.30[b] ND [by]	2.50 1.98	0.40 0.45	1 g DRY ash (475°C/16h)	Dissolved in d.HNO_3 Added citric acid and ammonia (pH 4) EXTRACTED APDC/butyl acetate	AAS FLAMELESS	CURVE (0–1 ppm)	MEASURED APPLIED	MEASURED NOT APPLIED
32	1.97 2.04	0.23 0.23	1.75[b] 3.25[b]	3.78 4.50	0.25–0.5 g WET ash (HNO_3, H_2SO_4, H_2O_2)	Diluted with water EXTRACTED APDC + DDDC/MIBK	AAS FLAMELESS*	CURVE (0–0.2 ppm)	MEASURED APPLIED	MEASURED APPLIED
33	0.59 0.98	<0.01[bw] 0.35 [b]	1.99 1.47	3.82 2.86	0.25 g WET ash (HNO_3, $HClO_4$ H_2SO_4)	Dissolved in acetate buffer DIRECT MEASUREMENT	ASV	STANDARD ADDITIONS	MEASURED APPLIED	
34	1.10 1.15	0.13 0.15	1.90 2.00	4.00 3.90	0.25–0.5 g WET ash (H_2O_2/Fe^{2+})	Dithizone complex formation two step separation	?	CURVE		MEASURED
35	1.06 1.10	0.26 0.26	1.83 1.75	2.24 2.19	1–2 g DRY ash (450°C/10h)	Dissolved in d.HNO_3 DIRECT MEASUREMENT	AAS FLAME	CURVE (0.05–0.40 ppm)	MEASURED	MEASURED

TABLE 1 (continued)

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD				
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE, DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	CORRECTIONS BLANK RECOVERY
EXPECTED	(1.5)	0.11	(3.0)	3.5					
36	1.65 1.33	0.48[b] 0.60[b]	2.25 2.32	4.70 5.40	3–6 g DRY ash (450°C/48h)	Dissolved in d.HNO_3 Neutralised (pH 8.5) Added citric acid EXTRACTED Na DDC/MIBK)	AAS FLAME	STANDARD ADDITIONS	
37	2.09[a] 2.15[a]	1.16[a] 0.82[a]	4.05[a] 3.30[a]	8.56[a] 8.76[a]	0.1 g DRY ash (480°C/5h)	Dissolved in c.HCl DIRECT MEASUREMENT	AAS FLAMELESS *	STANDARD ADDITIONS (0.05–0.1 ng)	MEASURED MEASURED

WHO/FAO AQA 1980. DISTRIBUTION OF CADMIUM RESULTS. FIGURE 1.

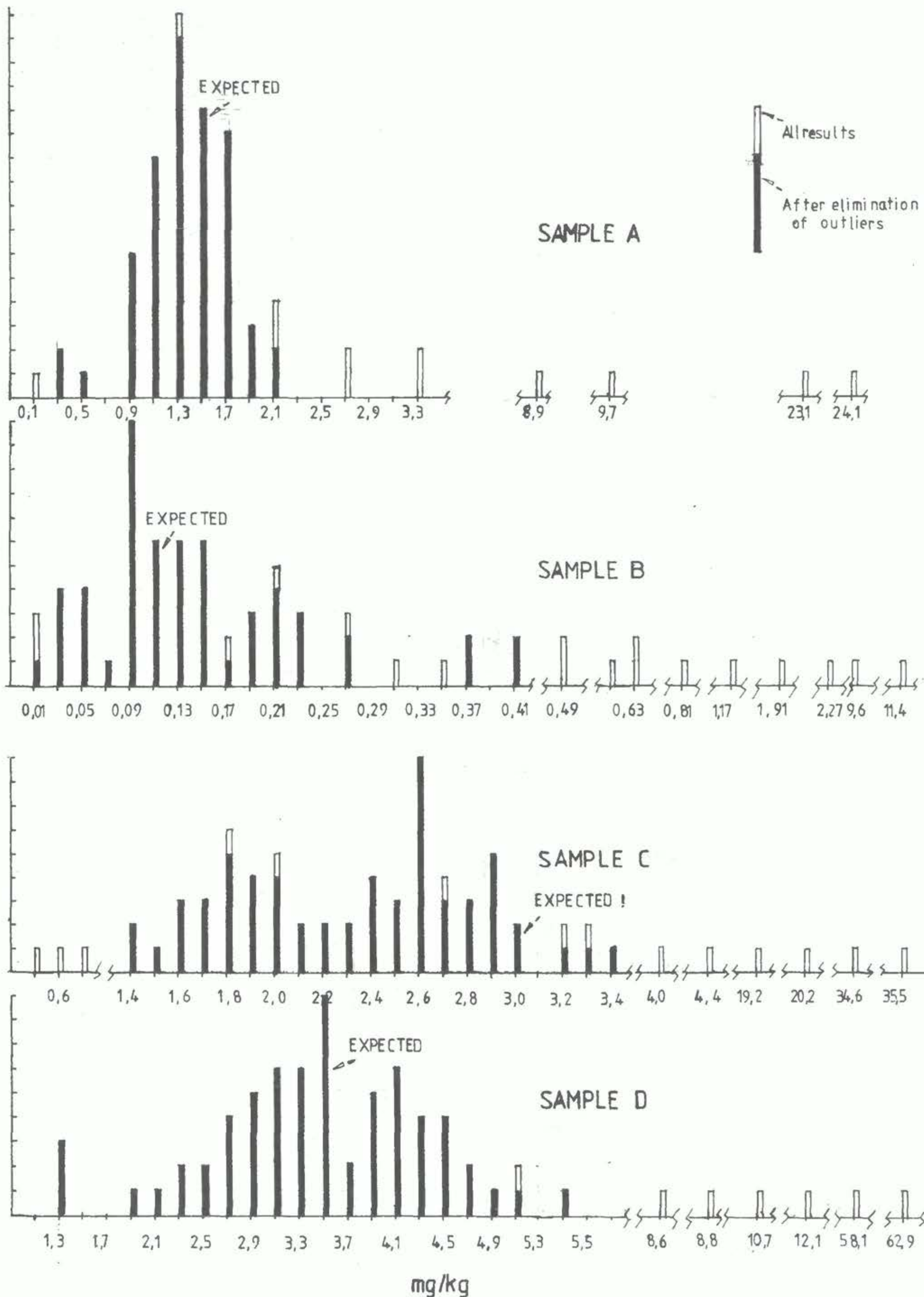


TABLE 2

JOINT FAO/WHO FOOD CONTAMINATION MONITORING PROGRAMME:

ANALYTICAL QUALITY ASSURANCE FOR LEAD AND CADMIUM 1980

ANALYSIS FOR LEAD

	RESULTS mg/kg				ANALYTICAL METHOD					
LAB NO	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE, DIGESTION	SEPARATION	MEASURE-MENT	CALIBRA-TION	CORRECTIONS BLANK	RECOVERY
EXPECTED	1.2	45.0	6.3	0.48						
1	2.77 2.88	7.16[c] 3.26[c]	0.97[c] 0.78[c]	0.49 0.52	2g DRY ash (450°c/8h)	Dissolved in HCl/HNO_3/H_2O (2:1:3) Added sodium acetate EXTRACTED APDC/MIBK	AAS FLAME	CURVE (0-5 ppm)	-	
2	0.72 0.79	34.79 34.62	3.75 4.04	0.25 0.23	0.5-1.0g DRY ash (450°C/24h)	Dissolved in IN HCl Added ammonium citrate adjusted pH to 9.3 EXTRACTED dithizone/chloroform Back extracted 0.1N HCl	AAS FLAMELESS	CURVE (0.01 - 0.2ppm HCl)	MEASURED APPLIED	MEASURED NOT APPLIED
3	1.0 0.95	36 37	4.5 4.4	0.5 0.75	0.5g WET ash (H_2SO_4 + H_2O_2)	EXTRACTED APDC/MIBK	AAS FLAME	CURVE	-	-
4	1.298 1.340	30.380 29.914	2.986 3.510	1.180 1.140	1g (A,D) 0.5g (B,C) WET ash (HNO_3 H_2SO_4, H_2O_2)	EXTRACTED APDC/MIBK	AAS FLAME	CURVE (02-0.5ppm)	MEASURED NOT APPLIED	-
5	0.4[a] 0.4[a]	25.5[a] 22.9[a]	0.6[a] 0.5[a]	0.0[a] 0.0[a]	5g WET ash (H_2SO_4,H_2O_2)	EXTRACTED DDC/XYLENE	AAS FLAME*	CURVE (1.0-10ppm)	MEASURED NOT APPLIED	MEASURED NOT APPLIED

TABLE 2 (continued)

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD					
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	CORRECTIONS BLANK	CORRECTIONS RECOVERY
EXPECTED	1.2	45.0	6.3	0.48						
6	2.1 2.5	41.0[b] 61.7[b]	3.2 3.9	0.4 <0.1[x]	2-5g WET ash ($HNO_3+H_2O_2$)	Diluted to 10 ml DIRECT MEASUREMENT	Reversed POLARO-GRAPHY	STANDARD ADDITIONS (1 - 10µg)	-	-
7	1.18 1.12	44.1 45.6	6.32 6.12	0.490 0.495	0.5g LOW TEMP ash (oxygen plasma)	Dissolved in d.HNO_3 DIRECT MEASUREMENT	A A S FLAME (B,C) * FLAMELESS (A,D) *	CURVE (0.5-2.0ppm) STANDARD ADDITIONS (20-80ppb)	MEASURED NOT APPLIED	MEASURED NOT APPLIED
8	0.69 0.91	33.8 32.8	3.72 3.72	1.43[b] 0.43[b]	2-3g DRY Ash (450°C/night)	Added HCl & dried Dissolved in 0.1N HNO_3 DIRECT MEASUREMENT	AAS FLAME (B,C) * AAS FLAMELESS (A,D) *	Relative to NBS Standards	MEASURED NOT APPLIED	
9	0.90 0.82	40.3 39.4	5.37 5.38	0.34 0.42	5g WET ash HNO_3	Diluted to 25 ml DIRECT MEASUREMENT	AAS FLAME *	CURVE (0.01-2.00 ppm)	MEASURED NOT APPLIED	-
10	0.48 0.38	37.8 35.3	3.59 3.87	0.19 0.10	1g DRY ash (with H_2SO_4) (550°C)	Dissolved in 1N HCl Added ammonium citrate, $NH_3OH.HCL$, NH_4OH (pH9.5) & $(NH_4)_2SO_4$ EXTRACTED NaDDC/MIBK	AAS FLAMELESS	CURVE (10-300ppb)	MEASURED -	MEASURED -
11	0.163[a] 0.155[a]	3.145[a] 2.849[a]	0.516[a] 0.471[a]	0.399[a] 0.433[a]	5g DRY ash (475°C/18h)	Dissolved in c,HCl, dried Dissolved in 2N HCl Diluted to 25ml DIRECT MEASUREMENT	AAS FLAMELESS	CURVE (0-0.5ppm)	MEASURED NOT APPLIED	MEASURED
12	0.8 0.9	47 47	5.8 6.0	1.1 1.3	2g DRY ash (450°C)	Dissolved in d,HCl Diluted to 10ml and filtered DIRECT MEASUREMENT	AAS FLAME	CURVE	MEASURED NOT APPLIED	MEASURED NOT APPLIED

TABLE 2 (continued)

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD				CORRECTIONS	
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	BLANK	RECOVERY
EXPECTED	1.2	45.0	6.3	0.48						
13	0.9 1.0	38 42	5.5 5.5	0.27 0.27	2g Dry ash (450°/night)	Dissolved in d.HCl & centrifuged A, C & D DIRECT MEASUREMENT B treated with ascorbic acid EXTRACTED APDC/MIBK	AAS FLAME * AAS FLAMELESS *	CURVE CURVE	MEASURED APPLIED	-
14	ND[by] 1.8[b]	43 42	9.5[b] 5.9[b]	ND[yd] ND[yd]	2g DRY ash ($400^{o}C$)	Dissolved in d.HCl DIRECT MEASUREMENT	AAS FLAME	CURVE	MEASURED NOT APPLIED	MEASURED NOT APPLIED
15	1.6 1.5	39 43	5.8 5.4	0.45 0.65	1-2g WET ash (HNO_3+ H_2O_2	Diluted to 25ml with d.HNO_3 DIRECT MEASUREMENT	A,C.D AAS FLAME B AAS FLAMELESS	CURVE STANDARD ADDITIONS	MEASURED -	-
16	1.25 1.39	49.7 50.8	5.54 5.04	1.17 0.98	1g DRY ash ($450^{o}C$/16h)	Dissolve in d.HNO_3 DIRECT MEASUREMENT of approp. dilutions in d. HNO_3	AAS FLAMELESS	CURVE (0-100ppb)	MEASURED	MEASURED
17	0.55 0.56	41.9 39.6	4.41 4.14	0.41 0.36	0.5 - 1g WET ash (H_2SO_4+ HNO_3)	Added NH_4OH (pH4) EXTRACTION ADDC/MIBK	AAS FLAME	CURVE (0-4ppm)	MEASURED NOT APPLIED	-
18	1.08 1.16	44.2 45.6	5.74 5.46	0.536 0.558	0.4 - 1.4g DRY ash (with H_2SO_4)	Dissolved in d.HNO_3 added acetate buffer DIRECT MEASUREMENT	Differential pulse ASV	STANDARD ADDITIONS	MEASURED	MEASURED

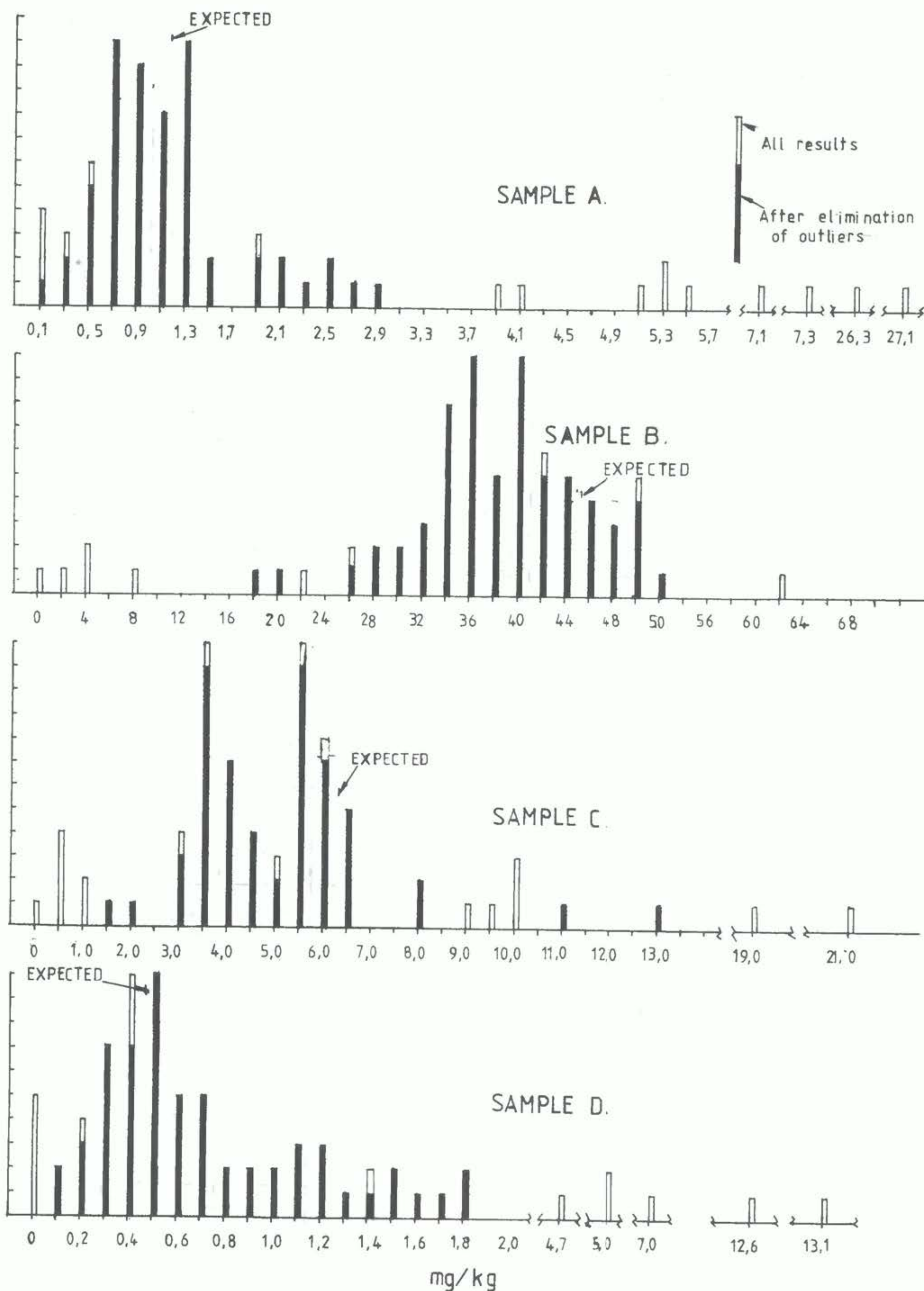
Table 2. (continued)

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD					
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	CORRECTIONS BLANK	RECOVERY
EXTRACTED	1.2	45.0	6.3	0.48						
19 METHOD 1	1.3 1.1	48 50	6.0 6.5	0.8 1.1	1.5-2g WET ash (HNO_3)	DIRECT MEASUREMENT	AAS	STANDARD ADDITIONS	MEASURED	-
19 METHOD 2	1.3 1.8	40 39	6.3 6.7	0.7 0.9	1-4g WET Ash ($HNO_3+H_2O_2$)	DIRECT MEASUREMENT	AAS FLAME	STANDARD ADDITIONS	APPLIED	-
20	5.5 c 5.3 c	41 41	11 13	1.5 1.4	1g WET Ash ($HNO_3+H_2O_2$)	Dissolve in d.HNO_3 DIRECT MEASUREMENT	AAS FLAME or FLAMELESS*	STANDARD ADDITIONS	-	-
21	0.77 0.74	41.0 37.3	3.13 3.30	0.52 0.45	2g LOW TEMP Asher then WET ash ($HNO_3+H_2SO_4$)	Dissolved in d. HNO_3 Added ammonium citrate $_1$.NH_4OH(pH7) $(NH_4)_2SO_4$ EXTRACTED DDTC/MIBK	AAS FLAME*	CURVE (0-4ppm)	MEASURED APPLIED	MEASURED NOT APPLIED
22	0.86 0.89	31.87 33.74	5.39 5.62	0.65 0.70	1g WET ash (H_2SO_4, HNO_3, $HClO_4$)	EXTRACTED NaDDC/MIBK	AAS FLAME	CURVE (0 - 0.2ppm)	MEASURED -	MEASURED -
23	0.9 1.1	44.9 45.2	3.5 3.3	0.6 0.6	5g DRY ash (460°C/16h)	Dissolved in d. HCl EXTRACTED APDC/MIBK (DIRECT MEASUREMENT)	AAS FLAME	CURVE	MEASURED	-
24 (1)	1.30	26.00	5.10[b]	0.34	0.2g DRY ash (H_2SO_4) (550°C)	Dissolved in d.HNO_3 & filtered. Added acetate buffer (pH 4.2) DIRECT MEASUREMENT	ASV	STANDARD ADDITIONS	MEASURED	MEASURED
24 (2)	1.25	27.30	3.20[b]	0.85	1.0g WET ash (H_2SO_4)	Filtered, dried and dissolved in water DIRECT MEASUREMENT	AAS FLAMELESS	SINGLE STANDARD	APPLIED	NOT APP.

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD				CORRECTIONS	
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	BLANK	RECOVERY
EXTRACTED	1.2	45.0	6.3	0.48						
25	1.1 1.3	47.5 49.2	6.0 6.3	0.65 0.65	4g DRY ash (500°C/night)	Dissolved in d.HNO_3 DIRECT MEASUREMENT	AAS FLAMELESS*	CURVE (0-12ng)	MEASURED APPLIED	MEASURED -
26	0.75 0.63	39 38	3.8 3.3	0.31 0.56	2g DRY ash (H_2SO_4) (500°C/4h)	Dissolved in d. HCl added citric acid, & NH_4OH(pH4) EXTRACTED APDC/MIBK	AAS FLAME	CURVE	MEASURED NOT APPLIED	NOT APPLIED
27	1.81 2.07	36.03 39.59	5.82 5.67	1.63 1.53	1g DRY ash (400°C/night)	Dissolved in d. HNO_3 filtered DIRECT MEASUREMENT	AAS FLAMELESS *	SINGLE STANDARD (0.05ppm)	-	-
28	7.5[c] 7.0[c]	33.4 35.0	9.9[c] 9.0[c]	5.0[b] 7.0[b]	1g DRY ash (450°C/6h) Then Wet ash (HNO_3,$HClO_4$)	Dissolved in d. HCL added ammium citrate and NH_4OH (pH9·2) EXTRACTION Dithizone/ chloroform Back extraction HCl	AAS FLAMELESS *	CURVE	MEASURED -	MEASURED -
29	0[z]	0[z]	0[z]	1.81[z]	10g DRY Ash (3h)	Dissolved in d. HNO_3 DIRECT MEASUREMENT	AAS FLAME	CURVE	-	-
30	0.51 0.52	36.3 33.9	3.72 3.42	0.28 0.51	2g DRY ash (450°C/2h)	Dissolved in d. HNO_3, filtered diluted to volume DIRECT MEASUREMENT	A & D pulse POLAROGRAPHY B & C ASV	CURVE (0.1-3.5ppm)	MEASURED NOT APPLIED	- -
31	0.61 0.60	32.6 28.0	5.1 4.3	0.20[d] ND[vd]	1g DRY ash (475°C/16h)	Dissolved in d. HNO_3 added citric acid and ammonia (pH.4) EXTRACTED APDC/ butyl acetate	AAS FLAMELESS	CURVE (0-4ppm)	MEASURED APPLIED	MEASURED APPLIED

Table 2. (continued)

LAB NO	RESULTS mg/kg				ANALYTICAL METHOD				CORRECTIONS	
	A SPINACH	B ORCHARD LEAVES	C TOMATO LEAVES	D OYSTER TISSUE	SAMPLE SIZE DIGESTION	SEPARATION	MEASUREMENT	CALIBRATION	BLANK	RECOVERY
EXTRACTED	1.2	45.0	6.3	0.48						
32	1.02 1.12	35.52 43.20	3.55[b] 5.39[b]	0.44 0.44	0.25 - 0.5g WET ash (HNO_3, H_2SO_4 H_2O_2)	Diluted with water EXTRACTED APDC+DDDC/MIBK	AAS FLAMELESS *	CURVE (0-0.2) ppm	MEASURED APPLIED	MEASURED APPLIED
33	0.36 0.62	20.3 17.8	1.49 2.10	0.25 0.26	0.25g WET ash (HNO_3, $HClO_4$ H_2SO_4	Dissolved in acetate buffer DIRECT MEASUREMENT	ASV	STANDARD ADDITIONS	MEASURED APPLIED	- -
34	5.0[a] 5.2[a]	52.0[a] 50.0[a]	10.0[a] 10.2[a]	4.7[a] 5.0[a]	0.25 - 0.5g WET ash (H_2O_2/Fe^{2+})	Dithizone complex formation two-step separation	Mixed colour TITRATION with dithizone	CURVE	-	MEASURED
35	3.9[c] 4.0[c]	36.1 34.8	7.8 7.9	1.7 1.8	1 - 2g DRY ash (450°C/10h)	Dissolved in d. HNO_3 DIRECT MEASUREMENT	AAS FLAME	CURVE (0.5 - 0.40 ppm)	MEASURED APPLIED	MEASURED APPLIED
36	2.4 2.3	36.1 39.4	4.1 4.2	1.0 1.2	3 - 6g DRY ash (450°C/48h)	Dissolved in d. HNO_3 Neutralised (pH8.5) Added citric acid EDTRACTED NaDDC/MIBK	AAS FLAME	STANDARD ADDITIONS	-	-
37	26.4[c] 27.1[c]	35.9 33.5	18.6[b] 20.9[b]	12.6[c] 13.1[c]	0.1g DRY ash (480°C/5h)	Dissolved in d. HCl DIRECT MEASUREMENT	AAS FLAMELESS *	STANDARD ADDITIONS (0.05-0.1ng)	MEASURED	MEASURED


WHO/FAO AQA 1980. DISTRIBUTION OF LEAD RESULTS. FIGURE 2.
EXPECTED
SAMPLE A.
All results
After elimination of outliers
0,1 0,5 0,9 1,3 1,7 2,1 2,5 2,9 3,3 3,7 4,1 4,5 4,9 5,3 5,7 7,1 7,3 26,3 27,1
SAMPLE B.
EXPECTED
0 4 8 12 16 20 24 28 32 36 40 44 48 50 56 60 64 68
EXPECTED
SAMPLE C.
0 1,0 2,0 3,0 4,0 5,0 6,0 7,0 8,0 9,0 10,0 11,0 12,0 13,0 19,0 21,0
EXPECTED
SAMPLE D.
0 0,2 0,4 0,6 0,8 1,0 1,2 1,4 1,6 1,8 2,0 4,7 5,0 7,0 12,6 13,1
mg/kg

ANNEX 2

NOTES and KEY to TABLES 3 and 4

Tables 3 and 4 summarise the information obtained from the statistical examination of the cadmium and lead results respectively.

The first column, of both tables, specifies the group considered statistically. Data included in these groups are results that remained after the elimination of outliers as described in section 8. Additionally, the analytical method for laboratories 24 to 37 had not been reported when statistical analysis commenced and had to be excluded from all but the Total group. The numbers of laboratories involved are given in column 3. Statistical analysis was conducted for each sample as indicated in column 2.

The mean for the sample, in the group under consideration, is given at column 4.

Columns 5 - 10 refer to consideration of within laboratory variance.

Columns 5 and 6 give repeatability r and percent repeatibility. Columns 7 - 10 refer to the two way analysis of variance which involved testing if results from a group, dry ashing say, had a significantly different spread than the results as a whole. Column 7 indicates the variances being compared, the larger variance indicated on the left. Column 8 gives their ratio. This Fr ratio, calculated from the appropriate repeatability, can be regarded as a variable with a probability distribution. The probability associated with the Fr ratio can be calculated and is given in column 10. If this probability is less than, or equal, 0.05, a situation only likely to occur five in 100 times, it is reasonable to assume the variances ratioed are different, that is significantly different at the 95% probability. The significance is indicated in column 9.

Reproducibility R and per cent reproducibility are given in columns 11 and 12. Reproducibility is related to the among laboratories variance and includes the within laboratory component.

Columns 13 to 16 contain analagous information to columns 7 and 10 for between laboratory variance. FB in column 14 is calculated from both reproducibility R and repeatability r.

The abbreviation used in the table indicates the following:

No.	Number of Laboratories
Fr.	Ratio of within laboratory and total variance
FB.	Ratio of between laboratory and total variance
Sig.	Statistical significance
S.	Statistically significant, probability ≤ 0.05
NS.	Not statistically significant, probability > 0.05

The abbreviation for the groups are defined in column 1 of the table.

TABLE 3. VARIATION OF CADMIUM RESULTS. COMPARISON BETWEEN METHODOLOGICAL FEATURES AND TOTAL VARIATION USING ANALYSIS OF VARIENCE

	Samp	No	Mean	Within Laboratories						Among Labs		Between Laboratories			
				Repeatability		Ratio	Fr	Sig	Pr	Reproducibility		Ratio	FB	Sig	PR
				r	%					R	%				
Dry Ashed (DA)	A	13	1.28	0.254	19.8	T vs DA	1.3490	NS	0.60	1.12	87.4	DA vs T	1.1247	NS	0.76
	B	13	0.134	0.054	40.1	DA vs T	1.6176	NS	0.29	0.268	201	DA vs T	1.0813	NS	0.82
	C	15	2.28	0.474	20.8	DA vs T	1.0053	NS	0.95	1.52	67.0	DA vs T	1.1132	NS	0.78
	D	14	3.15	0.513	16.3	T vs DA	2.3688	NS	0.10	3.32	106	DA vs T	1.3222	NS	0.51
Wet Ashed (WA)	A	13	1.42	0.321	22.6	WA vs T	1.1875	NS	0.67	1.13	79.8	WA vs T	1.1303	NS	0.75
	B	11	0.159	0.028	17.8	T vs WA	2.2051	NS	0.19	0.252	159	T vs WA	1.0348	NS	1.0
	C	11	2.44	0.610	21.8	WA vs T	1.3493	NS	0.51	1.50	55.2	T vs WA	1.1131	NS	0.77
	D	14	3.38	1.01	29.9	WA vsT	1.6329	NS	0.26	2.48	73.4	T vs WA	1.4633	NS	0.47
Graphite AAS (GF)	A	6	1.18	0.799	16.8	T vs GF	2.2045	NS	0.39	1.79	152	GF vs T	2.9214	NS	0.06
	B	9	0.133	0.051	38.6	GF vs T	1.4928	NS	0.41	0.173	130	T vs GF	2.2713	NS	0.23
	C	6	2.27	0.652	28.8	GF vs T	1.9040	NS	0.25	1.55	68.4	GF vs T	1.1014	NS	0.76
	D	7	3.49	0.667	19.1	T vs GF	1.4036	NS	0.72	4.45	127	T vs GF	3.7663	NS	0.10
AAS Flame (FS)	A	16	1.42	0.268	18.9	T vs FS	1.2091	NS	0.71	0.717	50.4	T vs FS	2.2803	NS	0.10
	B	13	0.122	0.083	23.9	FS vs T	3.8670	S	0.003	0.250	205	T vs FS	1.1027	NS	0.90
	C	17	2.33	0.458	19.7	FS vs T	2.0513	NS	0.09	1.39	59.5	T vs FS	1.1786	NS	0.75
	D	18	3.09	0.631	20.4	T vs FS	1.5443	NS	0.35	2.24	72.6	T vs FS	1.7101	NS	0.26
Chelation (CE)	A	13	1.36	0.302	22.2	CE vs T	1.0465	NS	0.87	1.10	80.5	CE vs T	1.0676	NS	0.85
	B	12	0.102	0.032	31.6	T vs CE	1.7087	NS	0.35	0.144	141	T vs CE	3.2280	S	0.05
	C	13	2.11	0.502	23.8	CE vs T	1.1311	NS	0.86	1.34	63.8	T vs CE	1.1821	NS	0.68
	D	15	2.96	0.744	25.2	T vs CE	1.1282	NS	0.65	3.31	112	CE vs T	1.2963	NS	0.53

TABLE 3. continued

	Samp	No	Mean	Within Laboratories						Among Labs		Between Laboratories			
				Repeatability r	%	Ratio	Fr	Sig	Pr	Reproducibility R	%	Ratio	FB	Sig	PR
Dissolution & direct measurement (DM)	A	14	1.34	0.267	19.9	T vs DM	1.2205	NS	0.73	1.14	85.1	DM vs T	1.1660	NS	0.70
	B	13	0.179	0.051	28.5	DM vs T	1.4745	NS	0.39	0.292	163	DM vs T	1.2888	NS	0.56
	C	13	2.67	0.489	18.3	DM vs T	1.0702	NS	0.84	1.16	43.5	T vs DM	1.6148	NS	0.38
	D	14	3.60	0.83	23.1	DM vs T	1.1058	NS	0.78	1.94	53.8	T vs DM	2.4166	NS	0.09
Total	A	31	1.34	0.292	21.7					1.05	78.0				
	B	28	0.142	0.042	29.6					0.257	181				
	C	30	2.35	0.472	20.1					1.45	61.7				
	D	32	3.31	0.79	23.9					2.93	88.4				
Expected	A		(1.5)												
	B		0.11												
	C		(3)												
	D		3.5												

TABLE 4. VARIATION OF LEAD RESULTS. COMPARISON BETWEEN METHODOLOGICAL FEATURES AND TOTAL VARIATION, USING ANALYSIS OF VARIANCE

	Samp	No	Mean	Within Laboratories						Among Labs		Between Laboratories			
				Repeatability r	%	Ratio	Fr	Sig	Pr	Reproducibility R	%	Ratio	FB	Sig	PR
Dry Ashed (DA)	A	15	1.04	0.275	26.5	T vs DA	1.5042	NS	0.42	1.78	172	T vs DA	1.0341	NS	0.99
	B	16	40.0	4.44	11.1	T vs DA	1.3097	NS	0.61	17.0	42.6	T vs DA	1.2683	NS	0.64
	C	14	4.65	0.698	15.0	T vs DA	2.2492	NS	0.13	2.91	62.6	T vs DA	3.5381	S	0.02
	D	13	0.617	0.257	41.7	T vs DA	1.8276	NS	0.27	1.20	194	T vs DA	1.0143	NS	1.0
Wet Ashed (WA)	A	10	1.17	0.452	38.5	WA vs T	1.7231	NS	0.26	1.51	129	T vs WA	1.4685	NS	0.56
	B	10	35.8	6.37	17.8	WA vs T	1.5713	NS	0.31	20.1	56	WA vs T	1.0791	NS	0.81
	C	10	5.12	1.50	29.4	WA vs T	2.0644	NS	0.14	8.02	157	WA vs T	2.1706	NS	0.11
	D	11	0.640	0.306	47.7	T vs WA	1.2895	NS	0.27	1.08	169	T vs WA	1.2756	NS	0.71
Graphite AAS (GF)	A	9	1.03	0.301	29.2	T vs GF	1.2539	NS	0.78	1.29	126	T vs GF	1.9693	NS	0.28
	B	8	38.9	7.28	18.7	GF vs T	2.0480	NS	0.16	20.3	52.2	GF vs T	1.0927	NS	0.78
	C	6	4.92	0.882	17.9	T vs GF	1.4099	NS	0.76	2.86	58.0	T vs GF	3.7479	NS	0.15
	D	8	0.647	0.217	33.5	T vs GF	2.5422	NS	0.20	1.34	207	GF vs T	1.2352	NS	0.63
AAS Flame (FS)	A	12	1.16	0.341	29.5	FS vs T	1.0225	NS	0.90	1.75	151	T vs FS	1.0668	NS	0.96
	B	14	38.7	4.21	10.9	T vs FS	1.4615	NS	0.47	42.5	32.2	T vs FS	1.6064	NS	0.37
	C	14	5.18	1.22	23.5	FS vs T	1.3546	NS	0.49	6.47	125	T vs FS	5.0749	S	0.004
	D	12	0.647	0.253	39.1	T vs FS	1.8852	NS	0.27	0.993	154	T vs FS	1.5010	NS	0.48
Chelation (CE)	A	11	1.02	0.157	15.3	T vs CE	4.6317	S	0.01	1.83	179	CE vs T	1.0361	NS	0.89
	B	10	35.3	6.48	18.3	CE vs T	1.6250	NS	0.30	10.5	29.8	T vs CE	3.9894	S	0.03
	C	8	4.03	0.873	21.6	T vs CE	1.4400	NS	0.50	2.35	58.2	T vs CE	5.6650	S	0.02
	D	10	0.512	0.273	53.4	T vs CE	1.6106	NS	0.46	0.784	153	T vs CE	2.4798	NS	0.15

TABLE 4 continued

	Samp	No	Mean	Within Laboratories						Among Labs		Between Laboratories			
				Repeatability		Ratio	Fr	Sig	Pr	Reproducibility		Ratio	FB	Sig	PR
				r	%					R	%				
Dissolution &	A	15	1.15	0.441	38.5	DM vs T	1.7103	N	0.22	1.51	132	T vs DM	1.4707	NS	0.45
direct	B	17	40.6	4.18	10.3	T vs DM	1.6647	NS	0.28	20.4	50.2	DM vs T	1.14[illegible]7	NS	0.72
measurement	C	17	5.31	1.18	22.2	DM vs T	1.2621	NS	0.58	6.14	116	DM vs T	1.2747	NS	0.56
(DM)	D	16	0.752	0.287	38.1	T vs DM	1.4645	NS	0.44	1.29	171	DM vs T	1.1261	NS	0.76
Total	A	29	1.18	0.338	28.6					1.81	153				
(T)	B	32	38.3	5.08	13.3					19.1	50.0				
	C	28	5.03	1.05	20.8					5.44	108				
	D	29	0.689	0.347	50.4					1.22	177				
Expected	A		1.2												
	B		45												
	C		6.3												
	D		0.48												

TABLE 5

LABORATORIES WITH RESULTS ELIMINATED AS OUTLIERS

Sample	Expected Value	Rank Sum	Cochran	Dixon	Other	Total No.
CADMIUM						*
A	(1.5)	16, 28, 37	24	1, 25		6
B	0.11	16, 28, 37	5, 6, 31, 33, 36	1		9
C	(3)	16, 28, 37	6, 24, 32	4		7
D	3.5	16, 28, 37	24		14	5
LEAD						†
A	1.2	5, 11, 34	14	20, 28, 35, 37	29	9
B	45	5, 11, 34	6	1	29	6
C	6.3	5, 11, 34	14, 24, 32, 37	1, 28	29	10
D	0.48	5, 11, 34	8, 28	37	14, 29, 31	9

*Out of 38 † Out of 37

TABLE 6

DATA ELIMINATED BY COCHRAN'S TEST

	A		B		C		D	
	Lab	Data	Lab	Data	Lab	Data	Lab	Data
CADMIUM Expected (mg/kg)		(1.5)		0.11		(3)		3.5
	24	2.7, 4.0	5	0.16, 0.26	6	0.5, 2.0	24	3.6, 5.0
			6	0.2, 0.5	24	2.7, 4.4		
			31	0.3, ND	32	1.75, 3.75		
			33	<0.01, 0.35				
			36	0.48, 0.60				
LEAD Expected (mg/kg)		1.2		45.0		6.3		0.48
	14	ND, 1.8	6	61.7, 41.0	14	9.5, 5.9	8	0.43, 1.43*
					24	3.2, 5.10	28	5.0, 7.0
					32	3.55, 5.39		
					37	18.6, 20.9		

*Laboratory 8 repeated their analysis of sample D and obtained 0.40 and 0.41 mg/kg.

ANNEX 3

COUNTRIES AND LABORATORIES PARTICIPATING IN THE LEAD AND CADMIUM ANALYTICAL QUALITY ASSURANCE 1980

(a) National Participants

COUNTRY	NO OF LABS AGREEING TO PARTICIPATE	NO OF LABS SUBMITTING REPORTS
Austria	2	1
Brazil	2	2
Egypt	2	0*
Germany, Fed. Rep. of	3	3
Guatemala	2	1
Hungary	4	4
Ireland	3	3
Japan	5	5
Kenya	2	1
New Zealand	3	3
Sweden	1	1
Switzerland	4	4
UK	4	4
USA	5	5
TOTAL	42	37

*Unfortunately, Egypt did not receive the 2 samples sent her. The packet was returned to sender, undelivered in January 1981.

ANNEX 3

b) Participating Laboratories

Austria	Forsch, Inst. de Ern. Wirtschaft, Vienna
Brazil	Instituto Adolfo Lutz, Sao Paulo
	CIWBRA, Sao Paulo
Germany, Fed.Rep. of	Inst. F. Biochemie, U. Analutik., Schutzenburg
	Chemischg Landesuntersuchungsansalt, Stuttgart
	Institut fur Hygiene der Bundesanstalts fur
	Milchforschung Kiel
Guatemala	United Food Control Laboratory, Guatemala City
Hungary	Hygienic and Epidemiological Station, Szolnak
	Central Laboratory of Control Service for Food Hygiene, Min. of Agr. Budapest
	Institute of State Food Control and Chemistry, Kecskemet
	Institute of Nutrition, Budapest
Ireland	Public Analysts Laboratory, Galway
	Public Analysts'Laboratory, Dublin
	Fisheries Research Centre, Castle Knock
Japan	Prefectual Institute of Public Health, Osaka
	City Health Research Institute, Nagoya
	National Institute of Hygienic Sciences, Tokyo
	Prefectural Institute of Public Health, Miyagi, Sendai
	Metropolitan Research Lab. of Public Health, Tokyo
Kenya	NPHLS, Nairobi
New Zealand	Dept. of Scientific and Industrial Research, (D.S.I.R.), Petone
	D.S.I.R., Auckland
	D.S.I.R., Christchurch
Sweden	The Swedish National Food Administration, Uppsala
Switzerland	Kantonales Laboratorium, Basel
	Kantonales Laboratorium, Zurich
	Laboratoire Cantonal, Epalinges
	Eidgendssische Forschungsanstalt fur Agriculurchemic und Umweithygien, Ligbgfeld

ANNEX 3. (continued)

U.K.	Food Preservation Research Association, Campden, Gloucester
	Marine Laboratory, Aberdeen
	M.A.F.F. Fisheries Laboratory, Burnham on Crouch
	M.A.F.F. Food Laboratory, Norwich
U.S.A.	Carnation Research Laboratories, Van Nuys, California
	Elemental Analysis Research Centre, USFDA, Ohio
	Del Monte Corp. Research Center, Walnut Creek, California
	Field Service Laboratory, USDA, Russel Research Center Athens, Georgia
	National Food Processors Association, Washington D.C.

ANNEX 4

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ANNEX 5

CONSIDERATION OF PRINCIPLES INVOLVED IN THE STATISTICAL ANALYSIS OF AQA RESULTS

A statistical model is assumed in which the result of a single test is the sum of three components:

$$y = m + B + e$$

where for the material tested

m is the general average
B is the difference between the laboratories
and e is a random error occurring in every test.

m is the value sought by the Monitoring Programme.

B is a term which should be constant on an occasion when a laboratory conducts an analysis under repeatability conditions, that is the analysis is conducted by the same analyst, correctly using a standard method and using the same equipment within a short period of time. B, however, is regarded as a random variable in a series of standard tests conducted by several laboratories, i.e. using different personnel, different equipment on different occasions, that is using reproducibility conditions. The distribution of this variable is assumed to be normal, but in practice it is sufficient that it is unimodal. In fact, the variable B can be regarded as made up of the sum of two components. B_o is a random component that arises from changes of condition within a laboratory, while B_s represents permanent systematic differences between laboratories. B can only be treated as a random variable, either if the differences B_s are comparatively small or if the trial results were selected at random from laboratories likely to use the method. While it is considered unlikely that the first condition will apply, it is hoped that the second condition was approximated by the AQA.

e represents the random error occurring in every single analysis. This again is assumed to be approximately normal but in practice it is sufficient that the distribution is unimodal. For a standard method trial the differences in e between laboratories is assumed to be small and a common average repeatability variance is assumed and can be estimated. The statistical analysis conducted on the AQA data is performed making the assumption that this situation is approximated - an assumption which is open to criticism.

Data from a standard method trial performed on an identical sample is characterised by the calculation of the mean and the parameters reproducibility R and repeatability r.

Reproducibility R is a critical value below which the absolute difference between two single test results, obtained under reproducibility conditions, already described, may be expected to lie with a specified probability; in the absence of other indications, the probability is 95%. Reproducibility R is linearly related to the square root of the reproducibility variance, that is the variance which includes the variance of the random variable B, the between or inter-laboratory variance, and the variance of e, the within or intra-laboratory variance.

Repeatability r is defined in the same manner as reproducibility except that it pertains to repeatability test conditions, which were also described earlier. It is linearly related to the square root of the common average repeatability variance of the variable e.

The statistical analysis was conducted on each level (sample) tested. Firstly, the data was reduced to a common form, that is, duplicate results from each laboratory for each level. Where more than two replicates were supplied they were reduced to two by random selection. If only one result was submitted the data was excluded.

The results were then checked as described below. Those that did not clearly form part of the assumed normal random distribution of the results were rejected as outliers. A 95% probability limit was used throughout.

To begin with, a test was made for the presence of outlying laboratories using the Rank Sample Technique. Reference (1) gives details. This technique eliminates laboratories that show consistently high or low results for all samples analysed. Those laboratories in the AQA whose results were so eliminated are marked in Tables 1 and 2 with the superfix a.

The homogeneity of the variation between replicates was assessed by applying Cochran's test to the wider duplicates. One effect of this test was to eliminate a pair of results in which one replicate might be a good estimate, that is part of the actual distribution, and the other a completely spurious result. Duplicates eliminates as a result of this test are marked in the tables with a superfix b. Dixon's test was used to eliminate oulying individual results. Those are marked in the tables with superfix c.

For the comparison of method with total variance Tables 3 and 4, the within laboratory variances were calculated from the appropriate repeatability r, and inter-laboratory variance was derived from the appropriate reproducibility R after removal of the repeatability component. The ratio of the methodological variance with that of the total variance is calculated, the larger number as numerator, and the significance of the ratio, at the 95% probability level, was assessed using F Tables.

ANALYTICAL QUALITY ASSURANCE

- ORGANOCHLORINE COMPOUNDS -

by

S. A. Slorach and G. Ekström
National Food Administration, Uppsala, Sweden

CONTENTS

		Page
1.	Introduction	66
2.	Matrices used	66
3.	Organochlorine Compounds studied	66
4.	Preparation of samples	66
5.	Shipment of samples	67
6.	Instructions to the participating laboratories	67
7.	Time schedule	67
8.	Results on the solutions in iso-octane	67
9.	Results on soya bean oil samples	68
10.	Results on the butter fat samples	69
11.	Conclusions	70
	Table 1 - 4	71 - 78
	Appendices 1 - 3	79 - 87
	Figures 1 - 15	88 - 102

1. Introduction

At present 21 countries are participating in the Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme. Data are collected via the Collaborating Centre which has been designated in each country. In March 1980 WHO wrote to all the Collaborating Centres inviting them to take part in the AQA exercise. For practical reasons a maximum of five laboratories in each country were invited to particpate. In the organochlorine compounds component, a total of 34 laboratories in 13 different countries have submitted results in the AQA exercise (for further details see Appendix 1).

2. Matrices used

At the Consultation held in Geneva in February 1980, it was decided that for the organochlorine compounds component, two types of sample would be used in the AQA exercise:

(a) Solutions of mixtures of organochlorine compounds in organic solvent (iso-octane)

The results of the analysis of these samples show the ability of the analyst to correctly identify and quantitate organochlorine compounds after the extraction and clean-up phases of the analytical procedure. This exercise was intended as a test of the gas-liquid chromatographic (GLC) part of the analysis although one laboratory used thin-layer chromatography (TLC) for qualitative and quantitative analysis of organochlorine compounds.

(b) Mixtures of organochlorine compounds in soya bean oil or butter fat (butter oil)

Analysis of these samples tests the ability of the laboratory to identify and quantitate the organochlorine compounds in fats/oils of animal and vegetable origin. In other words, it is a test of the whole analytical procedure, including the extraction, clean-up and GLC/TLC stages.

3. Organochlorine compounds studied

Taking into consideration the organochlorine compounds on which data are being collected in the Monitoring Programme, it was decided at the Consultation held in Geneva in February 1980 to choose the organochlorine compounds to be studied from the following list:

(a) DDT-complex (including p,p'-DDT, p,p'-DDE, o,p'-DDT and TDE (DDD))
(b) alpha-, beta-, and gamma-hexachlorocyclohexane (HCH)
(c) heptachlor and beta-heptachlor epoxide
(d) aldrin and dieldrin
(e) hexachlorobenzene (HCB)
(f) polychlorinated biphenyls (PCBs)

The compounds actually included in the AQA exercise are shown in Table 1.

Except for the PCBs, all the organochlorine compounds used to prepare the samples were standard reference substances kindly provided by the US Environmental Protection Agency. The PCB mixture used (Aroclor 1260) was kindly provided by the US Food and Drug Administration.

4. Preparation of samples

The compositions of the solutions of mixtures of organochlorine compounds in iso-octane (Samples 1A and 1B) are shown in Table 1. These solutions were shipped in sealed glass ampules.

The soya bean oil samples consisted of unspiked soya bean oil (Sample 2A) and the same oil spiked with a mixture of organochlorine compounds (Sample 2B). Samples 3A and 3B consisted of unspiked and spiked butter oil (butter fat), respectively. Soya bean oil and

butter oil were kindly supplied by a dairy products company (Arla) in Stockholm. The levels of organochlorine compounds in the samples are shown in Table 1.

The soya bean oil and butter oil samples were prepared in the following way. The oil (500 g) was weighed into a 2.5 l flask and heated to 40°C. The organochlorine compounds were then added in a small volume of iso-octane. The same volume of pure iso-octane (ca 10 ml) was added to the unspiked oils. After adding the iso-octane solutions, the flask was shaken in a water-bath at 40°C for about 1 hr. The oils were then transferred to screw-capped tubes. Before screwing up the caps tightly, the headspace was flushed with nitrogen.

5. Shipment of samples

The well-packed samples were shipped by special air freight direct to the Collaborating Centre in each country. Each centre was requested to confirm delivery of the samples. No reports of samples damaged in transit or samples failing to reach their destination were received by the National Food Administration.

6. Instructions to the participating laboratories

When the samples were sent out they were accompanied by relevant information and instructions to the participating laboratories concerning reporting of results (Appendix 2). Each laboratory was also provided with a form on which the results were to be submitted (Appendix 3). Each laboratory was asked to report the results on the iso-octane solutions before proceeding to analyse the soya bean oil and butter oil samples. This was done in order that laboratories that performed poorly in the relatively simple exercise of identifying and quantitating mixtures of organochlorine compounds in a pure organic solvent could improve their performance before attempting the more difficult task of analysing the oil samples. When a laboratory reported a result which deviated by more than 20% from the actual formulation it was requested to repeat the analysis before analysing the oil samples.

7. Time schedule

The samples were despatched from the National Food Administration (NFA) to the Collaborating Centres on 6 June 1980, except for one or two cases where they were sent later because of a late request to joint the AQA exercise. Results on the iso-octane samples were requested by the NFA not later than 30 September 1980 and results on the oil samples by 30 November 1980. However, several laboratories were late in sending in their results. The present report contains all the results which reached the NFA by 1 February 1981.

8. Results on the solutions in iso-octane

Results on the solutions of mixtures of compounds in iso-octane (Samples 1A and 1B) were obtained from a total of 34 laboratories in 13 countries. They are shown in detail in Table 2. The names and addresses of the participating laboratories are shown in Appendix 1. The laboratories are not listed in the same order as the results in Tables 2 to 4.

(a) Identification of organochlorine compounds

Sample 1A contained hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs) (Aroclor 1260). Two laboratories failed to identify HCB in this sample and four laboratories did not report or were not able to analyse PCBs.

Sample 1B contained HCB, gamma-HCH, dieldrin and p,p'-DDT. One laboratory (using TLC) failed to identify both HCB and gamma-HCH. Two laboratories failed to identify dieldrin and one of these reported p,p'-DDE in sample 1B. All the laboratories identified p,p'-DDT. One laboratory reported the presence of endrin in sample 1B.

(b) Quantitation

Table 2 shows the results obtained in all 34 laboratories. For each result the percentage deviation from the spiked level is shown immediately after the result. In addition, the mean percentage deviation of all the results for samples 1A and 1B has been calculated for each laboratory for the compounds which were identified correctly.

The distribution of the percentage deviations of the results from the spiked level for all the laboratories (which identified the compound correctly) is shown for each organochlorine compound in Figs 1-6. Data on these figures also indicate the arithmetical mean, the median and the range of results reported.

The following may be concluded concerning the results of the quantitation of the organochlorine compounds in iso-octane.

There were large differences between the performances of the different participating laboratories. The mean percentage deviation in the results for the compounds identified was 10% or less for 14 of 34 laboratories, 11-20% for 12, 21-30% for four and more than 30% for four laboratories. (These figures do not take into account the improvement in results which was obtained when analysis of the samples was repeated following a poor result the first time.)

Fig. 1 shows that the results concerning HCB in sample 1A from about half the laboratories (17 of 32) were within ±10% of the spiked level and the results from 23 of the 32 laboratories were within ±20%. Fig. 2 shows that only 11 of the 28 laboratories which reported results for PCBs in Sample 1A obtained values within ±10% of the spiked level. 21 of the 28 laboratories obtained results within ±20% of the spiked level. The results from the two laboratories reporting 5.7 and <0.5 ng/μl, respectively have not been included in this calculation. In the majority of cases the levels were underestimated.

Fig. 3 shows that 18 of 33 laboratories reported results for HCB in Sample 1B which were within ±10% of the spiked level. 24 of the 33 laboratories obtained results within ±20% of the spiked level. As would be expected, the results of analyzing Samples 1A and 1B for hexachlorobenzene are very similar (cf. Table 2 and Figs 1 and 3).

Fig. 4 shows that 23 of the 33 laboratories obtained results for gamma-HCH in sample 1B within ±10% of the spiked level and 28 of the 33 laboratories had results within ±20% of it.

Fig. 5 shows that 18 of 32 laboratories obtained results for dieldrin in Sample 1B within ±10% of the spiked level and 24 of 32 were within ±20% of it.

Fig. 6 shows that 17 of 34 laboratories reported results on p,p'-DDT that were within ±10% of the spiked level and 25 of 34 were within ±20% of it.

9. Results on soya bean oil samples

Results of the analyses of the unspiked and spiked soya bean oil samples (Samples 2A and 2B) were submitted by 23 laboratories and are shown in Table 3.

Apart from one laboratory which reported 0.196 mg PCBs (Aroclor 1260)/kg in sample 2A (and also 0.06 mg PCBs/kg in sample 2B), all laboratories reported that the levels of organochlorine compounds in the unspiked sample were below the limit of detection.

(a) Identification of organochlorine compounds

In Sample 2B one laboratory failed to identify α-HCH and no less than four laboratories failed to report p,p'-DDT.

(b) Quantitation

Fig. 7 shows that nine of 22 laboratories reported results for α-HCH in Sample 2B which were within ±10% of the spiked level and 15 of 22 were within ±20% of it.

Fig. 8 shows that nine of 23 laboratories reported results for γ-HCH in Sample 2B which were within ±10% of the spiked level and 19 of 23 were within ±20% of it. The majority of the laboratories underestimated the level.

Fig. 9 shows that only eight of 23 laboratories submitted results for dieldrin in Sample 2B which were within ±10% of the spiked level and 14 of 23 were within ±20% of it.

Fig. 10 shows that 11 of 18 laboratories submitted results for p,p'-DDT in Sample 2B which were within ±10% of the spiked level. Only one laboratory reported a level outside the ±20% range from the spiked level.

10. Results on the butter fat samples

Results on the analysis of the unspiked and spiked butter fat samples (Samples 3A and 3B) were submitted by 23 laboratories and are shown in Table 4.

(a) Unspiked butter oil sample

The unspiked butter fat sample (3A) contained trace amounts of HCB, α-HCH, γ-HCH and p,p'-DDE. The levels shown in brackets under this sample in Table 4 are the levels found on analysis carried out by the National Food Administration (NFA). The levels of several of the organochlorine compounds in the unspiked butter sample were below the limit of detection of the methods used in some laboratories. On the other hand, some laboratories reported other substances in trace amounts in this sample.

The majority of the laboratories reporting levels of HCB in sample 3A reported levels somewhat lower than the level found by the NFA. The levels of α-HCH reported varied from 0.019 to 0.04 mg/kg (level found by the NFA ca 0.03 mg/kg). The levels of γ-HCH reported varied from <0.003 to 0.02 mg/kg (NFA result: 0.01 mg/kg).

(b) Identification of organochlorine compounds in Sample 3B

The results in Table 4 show that four laboratories did not report beta-HCH in this sample and one did not identify heptachlor epoxide. All the laboratories identified p,p'-DDE but one failed to identify dieldrin. Five laboratories did not report levels of PCBs.

(c) Quantitation

Fig. 11 shows that 12 of 19 laboratories reported results for β-HCH in Sample 3B which were within ±10% of the spiked level and 16 of the 19 were within ±20% of it.

Fig. 12 shows that 12 of 22 laboratories submitted results for heptachlor epoxide in Sample 3B which were within ±10% of the spiked level and 15 of 22 were within ±20% of it.

Fig. 13 show that only nine of the 23 laboratories reported results for p,p'-DDE in Sample 3B which were within ±10% of the spiked level. However, 20 of 23 were within ±20% of it. The majority of laboratories underestimated the DDE level.

Fig. 14 shows that half (11 of 22) of the laboratories submitted results for dieldrin in Sample 3B which were within ± 10% of the spiked level, and only 12 of 22 were within ±20% of it.

Finally, Fig. 15 shows that eight of 16 laboratories reported levels of PCBs in Sample 3B which were within ±10% of the spiked level and only nine of 16 were within ±20% of it. The results from the two laboratories reporting ≤0.1 and <0.5 mg/kg, respectively, have not been included in this figure.

11. Conclusions

Although it is not possible from the results of the present exercise to draw far-reaching conclusions on the validity of the data on organochlorine compounds being reported in the FAO/WHO Food Monitoring Programme the following can be noted.

The present exercise revealed large differences between laboratories as regards analytical capability. Several laboratories were unable to correctly identify certain organochlorine compounds even when present in a pure organic solvent. The data provided by these laboratories is thus of questionable value.

Other laboratories were able not only to identify all the substances correctly but also to quantitate the levels in the samples accurately.

One of the reasons for errors in quantitation was the use of standard solutions which were too old or which for other reasons did not contain the expected concentration of the organochlorine compound concerned. In most cases in which a large error in quantitation was reported initially much improved results were obtained on repeating the analysis, often with a freshly-prepared standard solution.

It is encouraging to find that most of the laboratories in participating countries which have submitted a large amount of data on organochlorine compounds performed well in the present AQA exercise. In most cases the mean deviation of their results from the spiked levels was within the range ±20%, in many cases within the range ±10%.

Table 1. Composition of samples

Sample	Type of sample	Organochlorine compounds
1A	Iso-octane solution	HCB 0.0985 ng/µl PCBs 0.350 "-
1B	Iso-octane solution	HCB 0.0985 ng/µl γ-HCH 0.0990 "- Dieldrin 0.241 "- p,p'-DDT 0.248 "-
2A	Soya bean oil, unspiked	No organochlorine compounds detected by the NFA
2B	Soya bean oil (same as 2A), spiked	α-HCH 0.0786 mg/kg added γ-HCH 0.0792 "- Dieldrin 0.0481 "- p,p'-DDT 0.0594 "-
3A	Butter fat (butter oil), unspiked	HCB 0.01 mg/kg α-HCH 0.03 "- γ-HCH 0.01 "- p,p'-DDE 0.01 "- } Levels found by the NFA
3B	Butter fat (same as 3A), spiked	β-HCH 0.100 mg/kg added Heptachlor epoxide 0.152 "- p,p'-DDE 0.198 "- Dieldrin 0.144 "- PCBs 0.320 "-

HCB = hexachlorobenzene

HCH = hexachlorocyclohexane

PCBs = polychlorinated biphenyls (Aroclor 1260)

NFA = National Food Administration

Notes on Tables 2-4 : Δ = percentage deviation from the spiked level (calculated as $100 \times \frac{\text{reported level}}{\text{spiked level}} - 100$)

ΣΔ = sum of the absolute values of the percentage deviations

* = result obtained when the analysis was repeated

\- = no result reported

Table 2

SUMMARY OF RESULTS ON SOLUTIONS OF ORGANOCHLORINE COMPOUNDS IN ISO-OCTANE

Laboratory Code number ↓	Organochlorine Compounds Reported in Nanograms per Microlitre													
	Sample nr 1 A				Sample nr 1 B									
	HCB		PCB		HCB		γ-HCH		dieldrin		p,p'DDT			
Spike concentration →	0.0985	Δ	0.350	Δ	0.0985	Δ	0.0990	Δ	0.241	Δ	0.248	Δ	Σ|Δ|	Mean deviation
1.	0.091	- 8	0.243	-31	0.095	- 4	0.109	+10	0.240	0	0.267	+ 8	61	10
2.	0.12	+22	0.37	+ 6	0.14	+42	0.11	+11	0.24	0	0.27	+ 8	89	15
3.	0.110	+12	0.26	-26	0.099	+ 1	0.094	- 5	0.236	- 2	0.173	-30	76	13
											0.19*	-23*	69*	12*
4.	0.124	+26	0.360	+ 3	0.118	+20	0.106	+ 7	0.252	+ 5	0.253	+ 2	63	11
	0.0986*	0*			0.0938*	- 5*							22*	4*
5.	0.09	- 9	0.31	-11	0.088	-11	0.098	- 1	0.21	-13	0.19	-23	68	11
6.	0.10	+ 2	0.19	-46	0.10	+ 2	0.10	+ 1	0.25	+ 4	0.25	+ 1	56	9
			0.36*	+ 3*									13*	2*
7.	0.099	+ 1	0.370	+ 6	0.099	+ 1	0.101	+ 2	0.241	0	0.253	+ 2	12	2
8.[1/]	0.064	-35	0.3	-14	0.062	-37	0.072	-27	0.096	-60	0.175	-29	202[1/]	34
	0.095*	- 4*			0.100*	+ 2*	0.100*	+ 1*	0.135*	-44*	0.220*	-11*	76*	13*
9.	0.10	+ 2	0.39	+11	0.10	+ 2	0.10	+ 1	0.25	+ 4	0.25	+ 1	21	3
10.	0.103	+ 5	0.29	-17	0.090	- 9	0.090	- 9	0.225	- 7	0.228	- 8	55	9
11.	0.096	- 3	0.33	- 6	0.10	+ 2	0.10	+ 1	0.24	0	0.25	+ 1	13	2

[1/] This lab also reported the presence of endrin 0.2 ng/µl in sample 1 B

Table 2.

SUMMARY OF RESULTS ON SOLUTIONS OF ORGANOCHLORINE COMPOUNDS IN ISO-OCTANE (Continued)

Laboratory Code number ↓	Organochlorine Compounds Reported in Nanograms per Microlitre													
	Sample nr 1 A				Sample nr 1 B									
	HCB		PCB		HCB		γ-HCH		dieldrin		p,p'DDT			
Spike concentration →	0.0985	Δ	0.350	Δ	0.0985	Δ	0.0990	Δ	0.241	Δ	0.248	Δ	Σ|Δ|	Mean deviation
12.	0.10	+ 2	0.30	-14	0.10	+ 2	0.09	- 9	0.19	-21	0.19	-23	71	12
									0.209*	-13*	0.203*	-18*	58*	10*
13.	0.080	-19	0.36	+ 3	0.09	- 9	0.08	-19	0.15	-38	0.16	-35	123	21
									0.242*	0*	0.241*	- 3*	53*	9*
14.	0.07	-29	0.23	-34	0.12	+22	0.10	+ 1	0.38	+58	0.27	+ 9	153	26
	0.12*	+22*	0.36*	+ 3*					0.30*	+24*			81*	14*
15.	0.101	+ 3	0.351	0	0.099	+1	0.100	+ 1	0.244	+ 1	0.246	- 1	7	1
16.	0.06	-39	0.33	- 6	0.07	-29	0.07	-29	0.21	-13	0.26	+ 5	121	20
17.	0.095	- 4	0.366	+ 5	0.103	+ 5	0.105	+ 6	0.250	+ 4	0.240	- 3	27	4
18.	0.096	- 3	0.20	-43	0.10	+ 2	0.099	0	0.24	0	0.25	+ 1	49	8
			0.4*	+14*									20*	3*
19.	0.111	+13	0.313	-11	0.103	+ 5	0.099	0	0.238	- 7	0.225	- 4	40	7
20.	0.081	-18	0.336	- 4	0.081	-18	0.092	- 7	0.262	+ 9	0.246	- 1	57	9
21.	0.146	+48	0.353	+ 1	0.122	+24	0.100	+ 1	0.242	0	0.255	+ 3	77	13
	0.116*	+18*			0.103*	+ 5*							28*	5*

Table 2. SUMMARY OF RESULTS ON SOLUTIONS OF ORGANOCHLORINE COMPOUNDS IN ISO-OCTANE (Continued)

Laboratory Code number ↓	Organochlorine Compounds Reported in Nanograms per Microlitre													
	Sample nr 1 A				Sample nr 1 B									
	HCB		PCB		HCB		γ-HCH		dieldrin		p,p'DDT			
Spike concentration →	0.0985	Δ	0.350	Δ	0.0985	Δ	0.0990	Δ	0.241	Δ	0.248	Δ	Σ|Δ|	Mean deviation
22.	0.100	+ 2	0.344	- 2	0.104	+ 6	0.103	+ 4	0.233	- 3	0.278	+12	29	5
23.	0.10	+ 2	0.3	-14	0.10	+ 2	0.10	+ 1	0.3	+24	0.15	-40	83	14
24.	-		- [1/]		0.11	+12	0.11	+11	0.285	+18	0.291	+17	58	15
25.	0.062	-37	0.31	-11	0.051	-48	0.088	-11	0.158	-34	0.192	-23	164	27
26.	0.1	+ 2	0.4	+14	0.1	+ 2	0.1	+ 1	0.3	+24	0.2	-19	62	10
27.	0.115	+17	<0.5 [2/]		0.120	+22	0.113	+14	0.242	0	0.239	- 4	57	11
28.	0.09	- 9	-		0.1	+ 2	0.09	- 9	0.2	-17	0.17	-31	68	14
29. [3/]	0.4	+306			-		-		0.2	-17	0.2	-19	342	114
30.	0.122	+24	5.7 [4/]		0.118	+20	0.150	+52	0.300	+24	0.290	+17	137	27
31.	0.11	+12	0.84	+140	0.11	+12	0.1	+ 1	0.28	+16	0.2	-19	200	33
32. [5/]	0.100	+ 2	-		0.112	+14	0.073	-26	-		0.336	+35	77	19
33.	-		0.257	-27	0.256	+160	0.261	+164	-		0.275	+11	362	90
34.	0.10	+ 2	0.28	-20	0.10	+ 2	0.10	+ 1	0.24	0	0.22	-11	36	6

1/ This lab does not carry out PCB analyses
2/ Limit of quantitation for this laboratory
3/ TLC method used for quantitation
4/ Mean value. Value not included in calculation of mean deviation
5/ This lab also reported the presence of p,p'-DDE in sample 1B

Table 3

SUMMARY OF RESULTS ON UNSPIKED AND SPIKED SOYA BEAN OILS

Laboratory Code number ↓	Organochlorine Compounds Reported in mg/kg						
	Sample No 2A (unspiked soya bean oil)	Sample No 2B (spiked soya bean oil)					
		α-HCH	γ-HCH	dieldrin	p,p'DDT		
Spike concentration →	("Pesticide-free")	0.0786 Δ	0.0792 Δ	0.0481 Δ	0.0594 Δ	Σ|Δ|	Mean deviation
1. 1/	Arochlor 1260 0.196 mg/kg	0.068 -13	0.081 + 2	0.074 +54	-	69	23
2.		0.11 +40	0.095 +20	0.033 -31	0.059 - 1	92	23
3.		0.07 -11	0.05 -37	0.04 -17	0.08 +35	100	25
			0.08* + 1*		0.06* + 1*	30*	7*
4.		0.081 + 3	0.076 - 4	0.056 +16	0.054 - 9	32	8
5.		0.066 -16	0.069 -13	0.047 - 2	0.055 - 7	38	9
6.		0.078 - 1	0.081 + 2	0.056 +16	0.059 - 1	20	5
7.		0.10 +27	0.07 -12	0.04 -17	0.07 +18	74	19
8.		0.055 -30	0.065 -18	0.025 -48	0.050 -16	112	28
9.		0.080 + 2	0.078 - 2	0.051 + 6	0.071 +20	30	7
10.		0.077 - 2	0.076 - 4	0.050 + 4	0.053 -11	21	5
11.		0.072 - 8	0.070 -12	0.059 +23	0.060 + 1	44	11
13.		0.08 + 2	0.07 -12	0.04 -17	0.06 + 1	32	8
14.		0.09 +15	0.07 -12	0.03 -38	-	65	22
					0.05* -16*	81*	20*
15.		0.072 - 8	0.074 - 7	0.052 + 8	0.06 + 1	24	6

1/ This lab also reported 0.068 mg/kg of PCB in sample 2B

Table 3 — SUMMARY OF RESULTS ON UNSPIKED AND SPIKED SOYA BEAN OILS (continued)

Laboratory Code number ↓	Organochlorine Compounds Reported in mg/kg										
	Sample No 2A (unspiked soya bean oil)	Sample No 2B (spiked soya bean oil)									
		α-HCH		γ-HCH		dieldrin		p,p'DDT			
Spike concentration →	("Pesticide free")	0.0786	Δ	0.0792	Δ	0.0481	Δ	0.0594	Δ	Σ|Δ|	Mean deviation
16.		0.11	+40	0.09	+14	0.06	+25	-		79	26
17.		0.07	-11	0.08	+ 1	0.06	+25	0.07	+18	55	14
18.		0.061	-22	0.069	-13	0.057	+19	0.050	-16	70	18
19.		0.05	-36	0.06	-24	0.05	+ 4	-		64	21
20.		0.069	-12	0.061	-23	0.059	+23	0.059	- 1	59	15
21.		0.085	+ 8	0.070	-12	0.046	- 4	0.062	+ 4	28	7
24.		-		0.062	-22	0.049	+ 2	0.055	- 7	31	10
27.		0.10	+27	0.08	+ 1	0.05	+ 4	<0.2		32	11
34.		0.08	+ 2	0.08	+ 1	0.11	+129	0.06	+ 1	133	33

Table 4 SUMMARY OF RESULTS ON UNSPIKED AND SPIKED BUTTER OILS

Laboratory Code number ↓	Organochlorine Compounds Reported in mg/kg															
	Sample No 3A (unspiked butter oil)				Sample No 3B (spiked butter oil)											
	HCB	α-HCH	γ-HCH	DDE	β-HCH		heptachlor-epoxide		DDE		dieldrin		PCB			
Spike concentration →	(0.01)	(0.03)	(0.01)	(0.01)	0.100	Δ	0.152	Δ	0.198	Δ	0.144	Δ	0.320	Δ	Σ|Δ|	Mean deviation [1/]
1. [2/]	0.004	0.026	0.004	-	-		0.195	+28	0.232	+17	0.201	+40	0.086	-73	158	40
2.		0.024	0.003	0.011	0.11	+10	0.15	- 1	0.18	- 9	-		0.42	+31	51	13
3.		0.03			0.10	0	0.19	+25	0.17	-14	0.18	+25	<0 .1		64	16
4.	0.005	0.034	0.004	0.006	0.108	+ 8	0.157	+ 3	0.204	+ 3	0.159	+10	0.355	+11	35	7
5.	0.006	0.028	0.008	0.006	0.085	-15	0.16	+ 5	0.18	- 9	0.13	-10	0.35	+ 9	48	10
6.	0.006	0.019	0.004	0.008	0.091	- 9	0.166	+ 9	0.178	-10	0.144	0	0.403	+26	54	11
7.	-	0.03	-	-	0.09	-10	0.16	+ 5	0.16	-19	0.13	-10	0.29	- 9	53	11
8.	0.009	0.023	0.006	0.010	0.048	-52	0.120	-21	0.220	+11	0.088	-39	-		123	31
9.	<0.006	0.031	<0.005	<0.010	0.103	+ 3	0.147	- 3	0.172	-13	0.132	- 8	0.460	+44	71	14
10.	0.007	0.032	0.004	0.006	0.096	- 4	0.153	+ 1	0.185	- 7	0.139	- 3	0.25	-22	37	7
11.	0.013	0.028	0.005	0.007	0.10	0	0.16	+ 5	0.20	+ 1	0.15	+ 4	0.34	+ 6	16	3
13.	-	0.02	-	-	0.08	-20	0.11	-28	0.19	- 4	0.09	-37	-		89	23
14.	<0.01	0.03	<0.01	-	0.09	-10	0.07	-54	0.11	-44	0.08	-44	-		152	38

1/ Calculated for sample 3B only
2/ This lab also reported 0.134 mg/kg of PCB in sample 3A

Table 4 SUMMARY OF RESULTS ON UNSPIKED AND SPIKED BUTTER OILS (Continued)

Laboratory Code number ↓	Organochlorine Compounds Reported in mg/kg															
	Sample No 3A (unspiked butter oil)				Sample No 3B (spiked butter oil)											
	HCB	α-HCH	γ-HCH	DDE	β-HCH		heptachlor-epoxide		DDE		dieldrin		PCB			
Spike concentration →	(0.01)	(0.03)	(0.01)	(0.01)	0.100	Δ	0.152	Δ	0.198	Δ	0.144	Δ	0.320	Δ	Σ|Δ|	Mean deviation[1/]
15.	0.005	0.030	0.005	0.006	0.079	-21	0.150	- 1	0.179	-10	0.145	+ 1	0.331	+ 3	36	7
16.	-	0.05	0.02	-	-		-		0.17	-14	0.15	+ 4	-		18	9
17.	0.005	0.03	<0.005	0.006	0.11	+10	0.17	+12	0.23	+16	0.15	+ 4	0.32	0	42	8
18.	0.005	0.030	<0.005	<0.010	0.088	-12	0.156	+ 3	0.162	-18	0.154	+ 7	0.250	-22	62	12
19.	-	-	-	<0.03	-		0.07	-54	0.13	-34	0.06	-58	0.4	+25	171	43
20.		0.04			0.120	+20	0.158	+ 4	0.165	-17	0.178	+24	0.339	+ 6	71	14
21.	0.008	0.031	0.005	0.007	0.095	- 5	0.134	-12	0.182	- 8	0.082	-43	0.305	- 5	73	15
											0.148*	+ 3*			33*	7*
24.	0.021	-	0.006	0.005	-		0.180	+18	0.168	-15	0.103	-28	-		61	20
27.	<0.01	0.03	<0.01	<0.05	0.09	-10	0.15	- 1	0.22	+11	0.12	-17	<0.5		39	10
34.	0.02	0.04	0.01	<0.02	0.14	+40	0.21	+38	0.28	+41	0.21	+46	0.30	- 6	171	34

1/ Calculated for sample 3B only

APPENDIX I

LIST OF PARTICIPATING LABORATORIES

Name and address of laboratory

Food and Drug Administration
Attn: Dr M. T. Jeffus
3032 Bryan Street
DALLAS
Texas 75204
USA

Kantonales Laboratorium
Attn: Hr. Tuor
Vonmattstr. 16
CH-6000 LUZERN
Switzerland

National Institute of Hygienic Sciences
Division of Food
Attn: Dr M. Takeda
18-1 Kamiyoga 1-chome
Setagaya-ku
TOKYO 158
Japan

Kantonales Laboratorium
Basel-Stadt
Attn: Dr H. Egli
Postfach
CH-4012 BASEL
Switzerland

National Food Administration
Attn: Mr Ö. Andersson
Box 622
S-751 26 UPPSALA
Sweden

Chemische Landesuntersuchungsanstalt
Attn: Dr Frank
Postfach 1219
D-700 STUTTGART-1
Germany, Fed. Rep. of

CICOBRA
Attn: Dr Osvaldo Marmo
Ave. da Consolacao 896
Laboratorio
SAO PAULO, CEP 01302
Brazil

APPENDIX I (continued)

Name and Address of Laboratory

Hygienic and Epidemiological Station in Nógrád
Attn: Dr Kádár Jánosné
Bem u. 7-9
SALGÓTARJAN
Hungary

Veterinary Station in Budapest
Attn: Dr Keresztessy Árpád
Lehel utca 43-47
1389 BUDAPEST
Hungary

Hygienic Control for Food Industry
Attn: Dr Simonffy Zoltán
Ministry of Agriculture and Food
Mester u. 81
1095 BUDAPEST
Hungary

Institute of Nutrition
Attn: Dr Soós Katalin
Gyáli ut 3/a
BUDAPEST, IX
Hungary

Hygienic and Epidemiological Station in Hajdu-Bihar
Laboratory for Food Chemistry
Attn: Dr Ditrói Ferencné
Pf.115
4001 DEBRECEN
Hungary

Institut für Hygiene
Bundesanstalt für Milchforschung
Attn: Dr A. Blüthgen
Postfach 1649
2300 KIEL 1
Germany, Fed. Rep. of

Rijks Instituut voor de Volksgezondheid
Attn: Dr P. A. Greeve
Postbus 1
BILTHOVEN
Netherlands

Gemeinschaftslaboratorium
Attn: Dr A. Litscher
Galactina AG/VM Bern AG
Postfach
CH-3123 BELP
Switzerland

APPENDIX I (continued)

Name and address of Laboratory

Laboratorio Unificado de Control de Alimentos
Attn: Dr Marit de Campos
INCAP
Apartado Postal 1188
GUATEMALA, C.A.
Guatemala

Meat Monitoring Laboratory
Attn: Dr J.W. Lock
Wallaceville Research Laboratory
Private Bag
UPPER HUTT
New Zealand

Miyagi Profectural Institute of Public Health
Attn: Dr Kei-ichi Sakai
4-7-2, Saiwaicho
SENDAI, 983
Japan

Institut für Biochemie und Analytik
Attn: Dir. u. Prof. H-D Ocker
Bundesforschungsanstalt für Cetreide-und Kartoffelverarbeitung
Am Schützenberg
D-4930 DETMOLD
Germany, Fed. Rep. of

Kochi Prefectural Public Health Laboratory
Attn: Dr M. Uyeta
2-4-1, Marunouchi
KOCHI, 780
Japan

Mississippi State Chemical Laboratory
Attn: Dr L.G. Lane
Box CR
MISSISSIPPI STATE, Miss. 39762
USA

Food and Drug Administration
Attn: Dr G.M. Doose
1521 West Pico Boulevard
LOS ANGELES
California 90015
USA

State Laboratory
Attn: Dr J. Quigley
Upper Merrion Street
DUBLIN 2
Ireland

APPENDIX I (continued)

Name and address of Laboratory

Lebensmitteluntersuchungsanstalt der Stadt Wien
Attn: Dr Psota
Viehmarktgasse 1
A-1030 WIEN
Austria

Ministry of Agriculture, Fisheries and Food
Tolworth Laboratory
Attn: Dr H. P. Wardall
Hook Rise
South Tolworth
SURBITON
Surrey KT6 7NF
United Kingdom

Department of Scientific and Industrial Research
Chemistry Division/Lower Hutt
Attn: Dr I.R.C. McDonald
PETONE
New Zealand

Food and Drug Administration
New York Regional Laboratory
Attn: Dr Ted M. Hopes
850 Third Avenue
Brooklyn
NEW YORK 11232
USA

Instituto Adolfo Lutz
Attn: Dr W.H. Lara
Av. Dr. Arnaldo, 355
Caixa Postal 7027
SAO PAULO
Brazil

Nagoya City Health Research Institute
Attn: Dr Y. Sakabe
1-11 Hagiyamacho
Mizuho-ku
NAGOYA CITY
Japan

Ministry of Agriculture, Fisheries and Food
Central Veterinary Laboratory
Attn: Dr D.F. Waddell
New Haw
WEYBRIDGE
Surrey KT15 3NB
United Kingdom

APPENDIX 1 (continued)

Name and address of Laboratory

Osaka Prefectural Institute of Public Health
Attn: Dr T. Kashimoto
1-3-69 Nakamichi
Higashinari-ku
OSAKA
Japan

Laboratoire Cantonal de Chimie
Attn: Dr Cl. Corvi
Case Postal 109
CH-1025 GENEVE
Switzerland

The Agricultural Institute
Pesticide Residue Laboratory
Attn: Dr J. F. Eades
Oak Park Research Centre
CARLOW
Ireland

Food and Drug Administration
Attn: Dr J. E. Westfall
1560 E. Jeffersson Avenue
DETROIT, Michigan 48207
USA

APPENDIX 2

Information and instructions sent together with the samples to the Collaborating Centres in the Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme

Samples for analytical quality assurance - organochlorine compounds component

We have today despatched to you by air, batches of analytical quality assurance (AQA) samples for analysis by the participating laboratories in your country.

Each batch consists of the following six samples:

1. A solution of certain organochlorine compounds in iso-octane (sample labelled 1 A).

2. A second solution of certain organochlorine compounds in iso-octane (sample labelled 1 B).

3. Unspiked soyabean oil (sample labelled 2 A).

4. Spiked soyabean oil (sample labelled 2 B).

5, Unspiked butterfat (sample labelled 3 A).

6. Spiked butterfat (sample labelled 3 B).

The organochlorine compounds used for spiking the soyabean oil and butterfat samples and for preparing the iso-octane solutions have been chosen from the following list:

(a) DDT-complex (including p,p'-DDT, p,p'-DDE, o,p'-DDT and DDD or TDE)
(b) alpha-, beta- and gamma-hexachlorocyclohexane (HCH)
(c) heptachlor and beta-heptachlor epoxide
(d) aldrin and dieldrin
(e) hexachlorobenzene (HCB)
(f) polychlorinated biphenyls (PCBs)

You are asked to instruct the participating laboratories in your country to first analyse <u>only the iso-octane samples</u> and to report the results directly to us on the form supplied as soon as possible and <u>not later than 30 September 1980</u>. This step is being taken to check that the ability of the laboratories to identify and quantify pure organochlorine compounds without an extraction stage or interference from other compounds. We will assess the results of the analysis of the iso-octane solutions and if they are far from the actual levels we will advise them (with a copy to you). At the same time as we report the outcome of the analysis of the iso-octane solutions we will provide instructions regarding the analysis of the soyabean oil and butterfat samples.

The main object of the AQA exercise is to assess quality of the data being collected in the food contamination monitoring programme. Therefore we ask you to instruct the participating laboratories to analyse the soyabean oil and butterfat samples in the way they would routinely analyse the corresponding food samples. Thus duplicate analyses should <u>not</u> be performed unless this is done routinely.

APPENDIX 2 (continued)

The results are to be reported on the attached forms (copies of the form are enclosed with each batch of samples). A single result should be reported for each compound. A detailed description (in English) of the methods used to analyse the soyabean oil and butterfat samples should be sent with the results. In the case of PCBs it is important to state which PCB standard has been used. Please ask the laboratories to send us the original chromatograms together with each set of results (the chromatograms will be returned if this is requested).

Please note that if the level of a compound in a soyabean oil or butterfat sample is below the level that the laboratory is able to quantify the result should be reported as "less than x mg/kg", where x is the lowest level that the laboratory routinely quantifies. Concentrations below the limit of detection or quantification should NOT be reported as "not detectable", "trace" or the like.

It is only necessary to report the levels of the organochlorine compounds listed above but each laboratory may, if it wishes, report the levels of other organochlorine compounds found in the sample.

We wish to receive the results of the analysis of the soyabean oil and butterfat samples by 30 November 1980.

Please acknowledge receipt of the enclosed batches of samples by completing the attached form and return it immediately by airmail to :

Dr Stuart Slorach
National Food Administration
Box 622
S-751 26 UPPSALA
Sweden

We look forward to hearing from you in the near future and thank you for your cooperation in this work.

Yours sincerely,

(Signed) Stuart A. Slorach (Signed) Georg Ekström

APPENDIX 3

FORM FOR REPORTING RESULTS

Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme -
Analytical Quality Assurance: Organochlorine Compounds Component

REPORT OF RESULTS

Country:

Name and address of
laboratory:

Name of responsible
analyst:

Sample number:

Date received:

Date analysed:

Are results corrected for recovery? YES / NO (circle)

Do you want your chromatograms back?: YES / NO (circle)

Please report the results overleaf. A single result should be reported for each compound. A detailed description (in English) of the methods used to analyse the soyabean oil and butterfat samples should be sent with the results. In the case of PCBs it is important to state which PCB standard has been used. Please send the original chromatograms together with each set of results (the chromatograms will be returned if this is requested).

Please note that if the level of a compound in a soyabean oil or butterfat sample is below the level that the laboratory is able to quantify the result should be reported as "less than x mg/kg", where x is the lowest level that the laboratory routinely quantifies. Concentrations below the limit of detection of quantification should NOT be reported as "not detectable", "trace" or the like.

APPENDIX 3 (continued)

Analytical results

Organchochlorine compound	Concentration[x)]

x) Concentrations should be expressed in mg/kg for soyabean oil and butterfat samples and in ng/μl for iso-octane solutions.

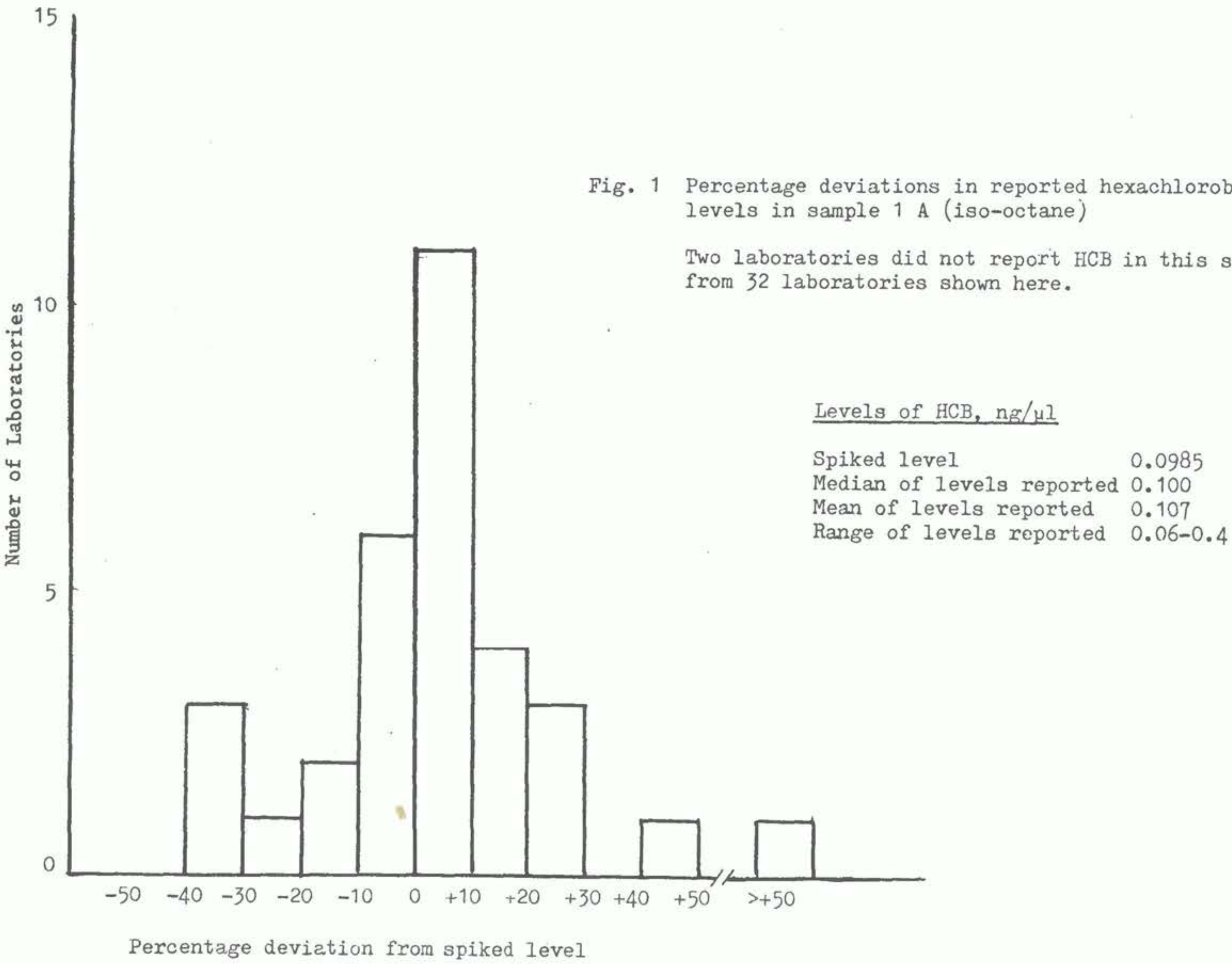


Fig. 1 Percentage deviations in reported hexachlorobenzene (HCB) levels in sample 1 A (iso-octane)

Two laboratories did not report HCB in this sample. Results from 32 laboratories shown here.

Levels of HCB, ng/µl

Spiked level	0.0985
Median of levels reported	0.100
Mean of levels reported	0.107
Range of levels reported	0.06-0.4

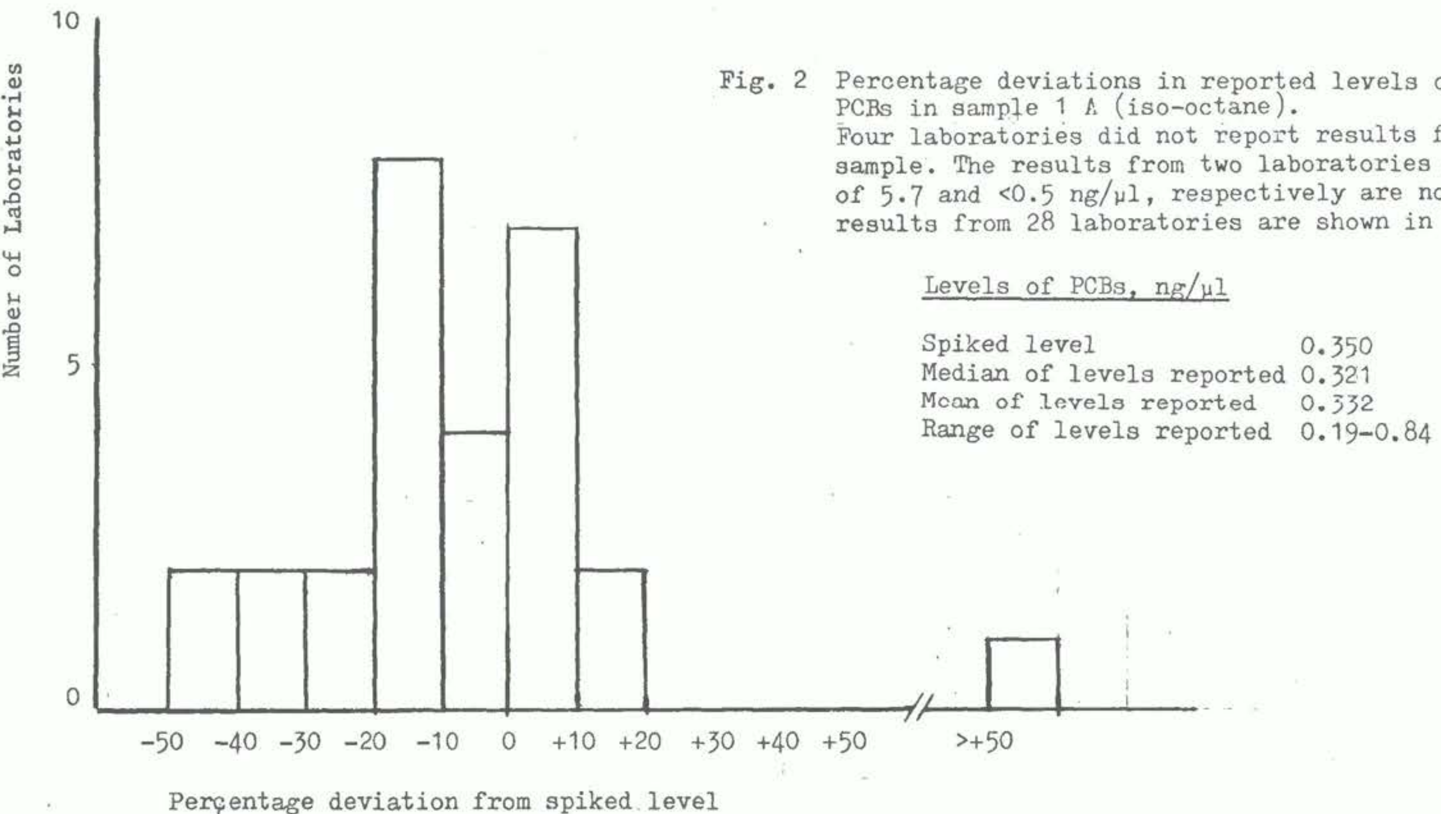


Fig. 2 Percentage deviations in reported levels of PCBs in sample 1 A (iso-octane).
Four laboratories did not report results for PCBs in this sample. The results from two laboratories reporting levels of 5.7 and <0.5 ng/µl, respectively are not shown here. The results from 28 laboratories are shown in this figure.

<u>Levels of PCBs, ng/µl</u>

Spiked level	0.350
Median of levels reported	0.321
Mean of levels reported	0.332
Range of levels reported	0.19-0.84

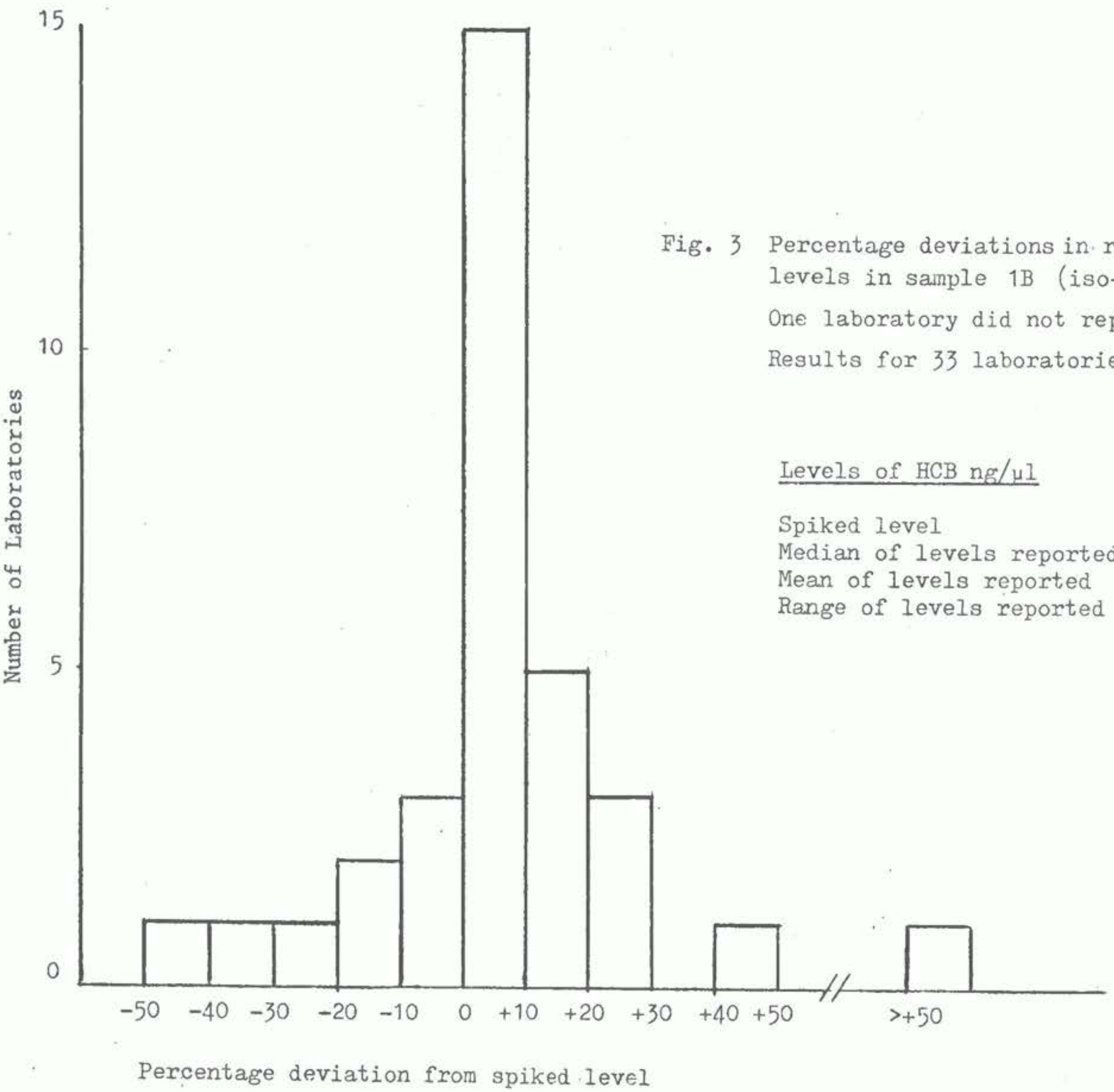
Fig. 3 Percentage deviations in reported hexachlorobenzene (HCB) levels in sample 1B (iso-octane)

One laboratory did not report HCB in this sample.

Results for 33 laboratories are shown.

<u>Levels of HCB ng/µl</u>

Spiked level	0.0985
Median of levels reported	0.100
Mean of levels reported	0.105
Range of levels reported	0.051-0.256



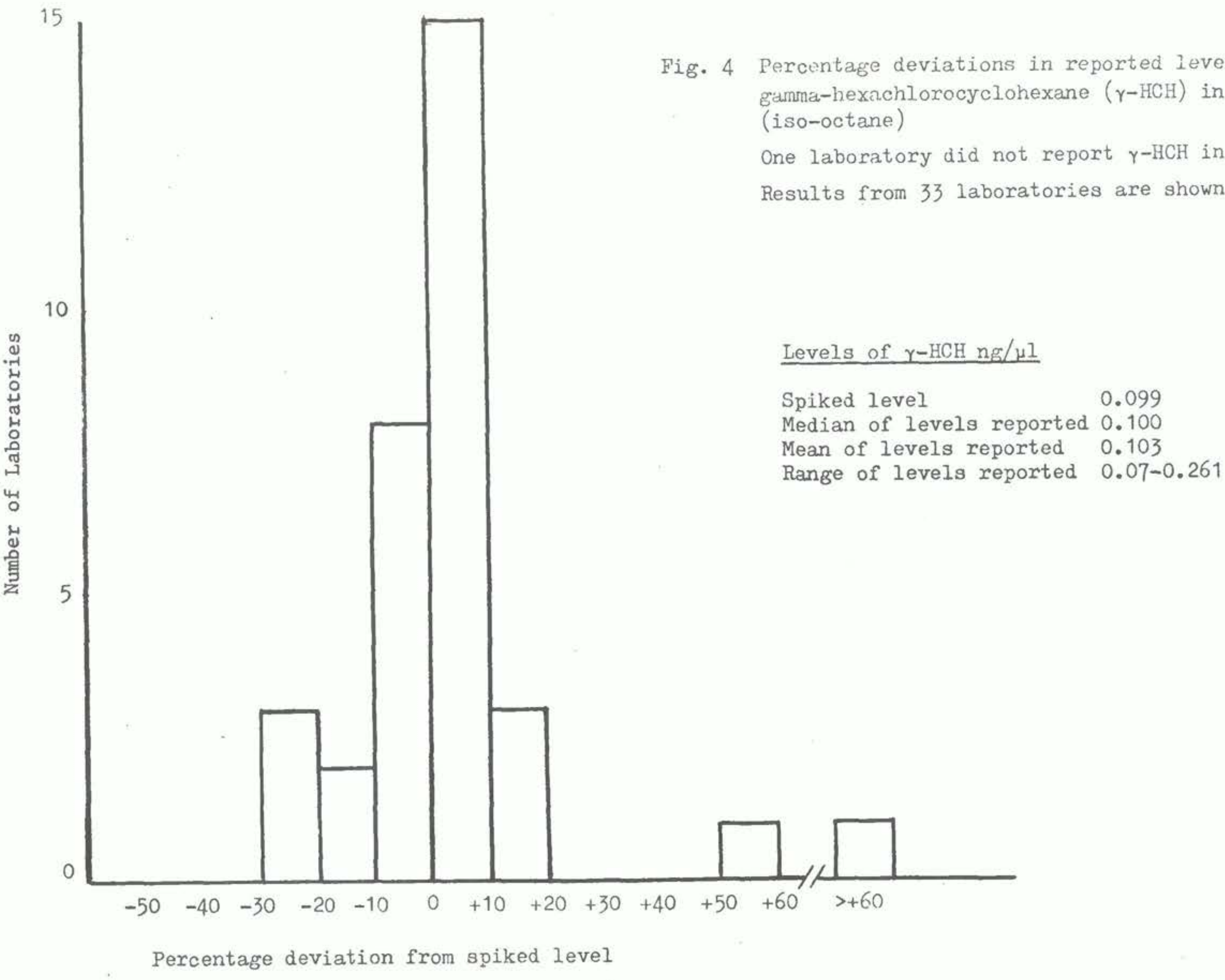


Fig. 4 Percentage deviations in reported levels of gamma-hexachlorocyclohexane (γ-HCH) in sample 1B (iso-octane)

One laboratory did not report γ-HCH in this sample .

Results from 33 laboratories are shown.

<u>Levels of γ-HCH ng/µl</u>

Spiked level	0.099
Median of levels reported	0.100
Mean of levels reported	0.103
Range of levels reported	0.07-0.261

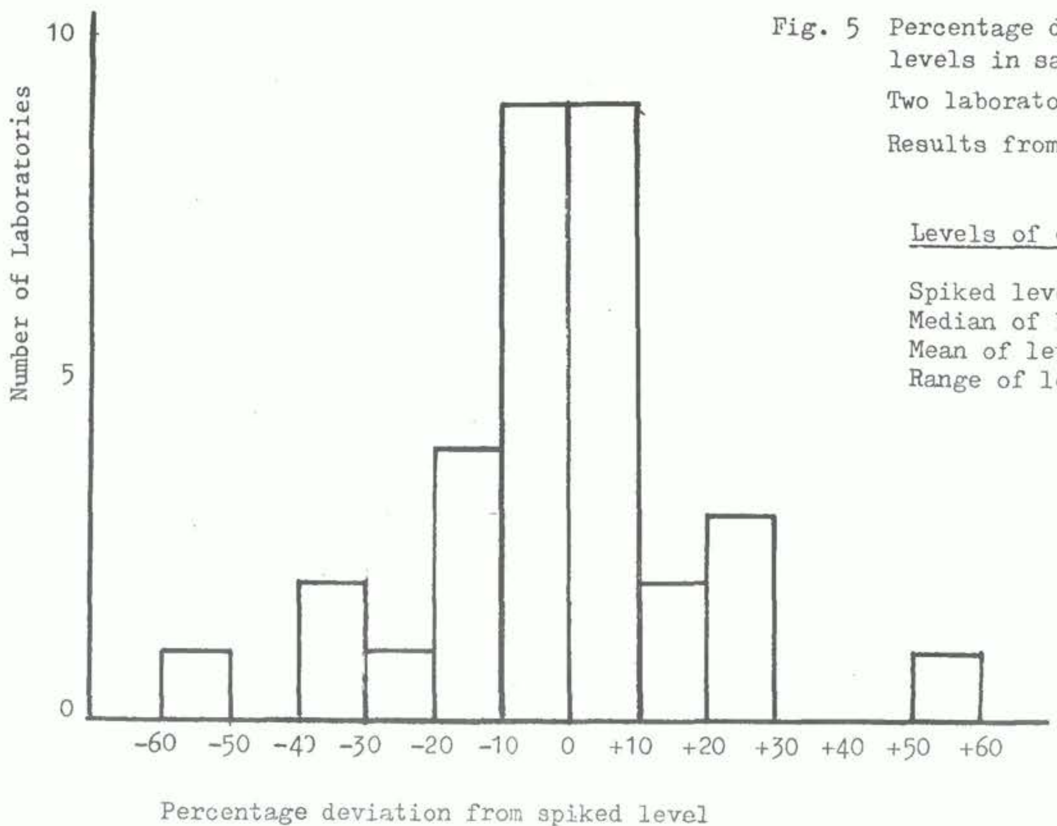


Fig. 5 Percentage deviation in reported dieldrin levels in sample 1 B (iso-octane).

Two laboratories did not report dieldrin in this sample.

Results from 32 laboratories are shown.

<u>Levels of dieldrin, ng/µl</u>

Spiked level	0.241
Median of levels reported	0.24
Mean of levels reported	0.238
Range of levels reported	0.096-0.38

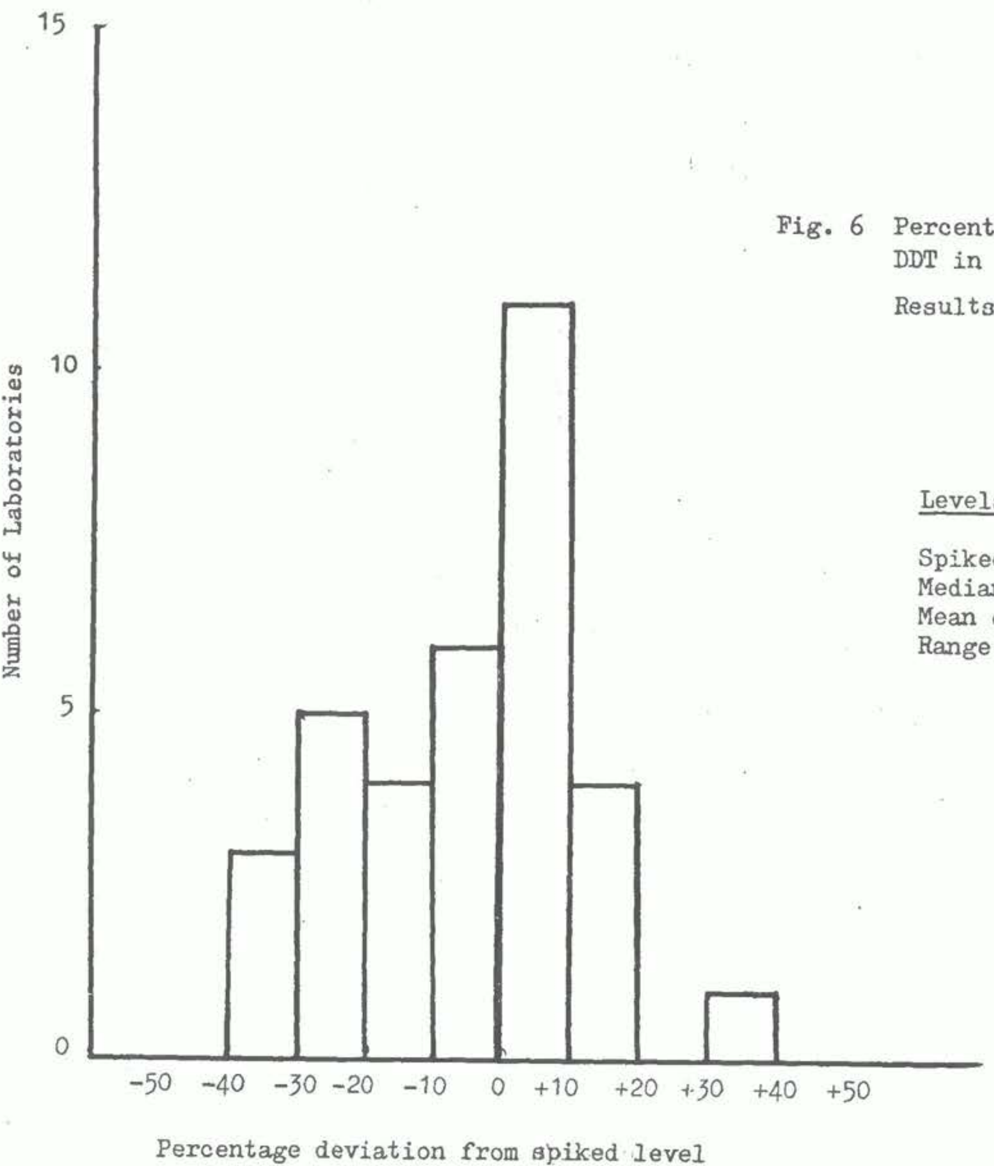


Fig. 6 Percentage deviation in reported levels of p,p'-DDT in sample 1 B (iso-octane).

Results from 34 laboratories are shown.

<u>Levels of p,p'-DDT ng/µl</u>

Spiked level	0.248
Median of levels reported	0.246
Mean of levels reported	0.234
Range of levels reported	0.15-0.336

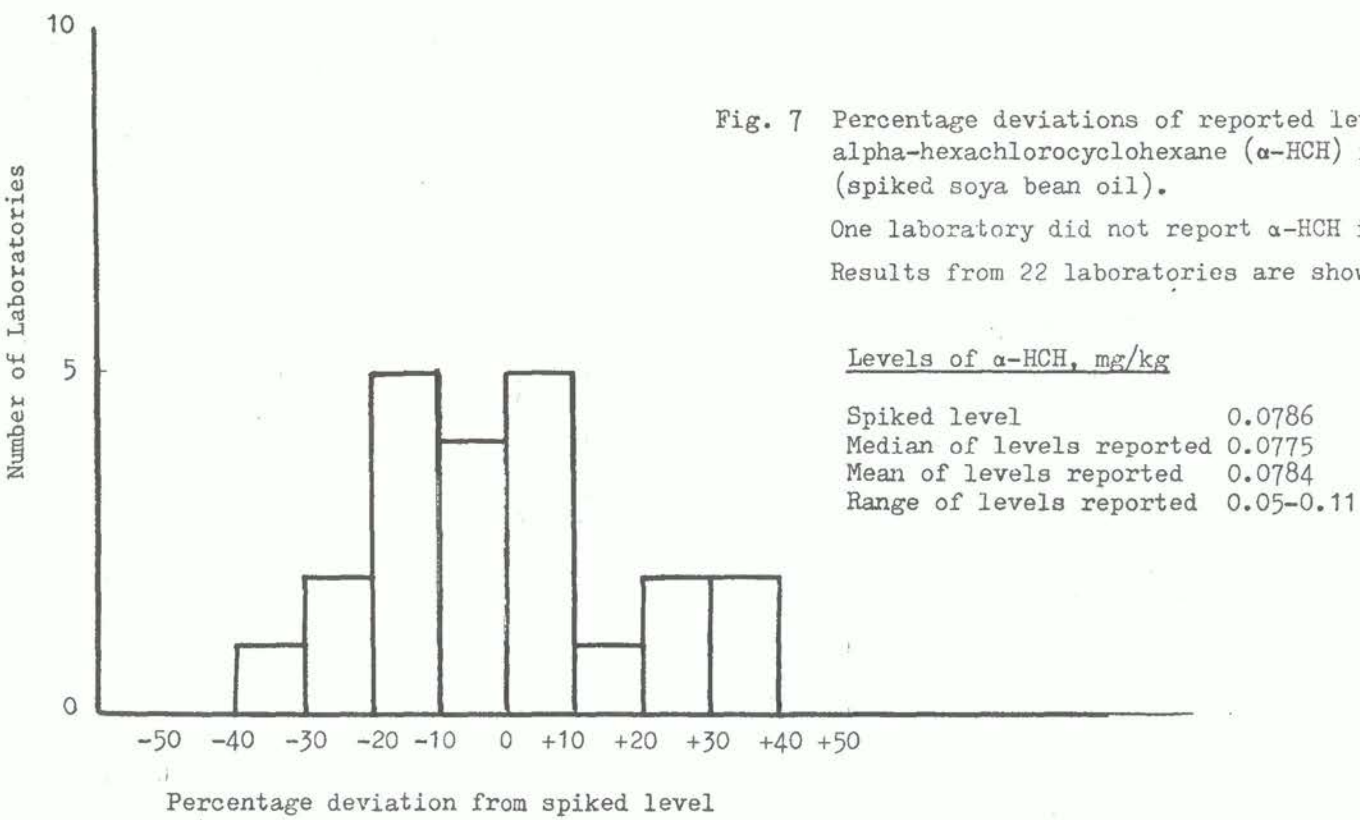


Fig. 7 Percentage deviations of reported levels of alpha-hexachlorocyclohexane (α-HCH) in sample 2B (spiked soya bean oil).

One laboratory did not report α-HCH in this sample.

Results from 22 laboratories are shown.

<u>Levels of α-HCH, mg/kg</u>

Spiked level	0.0786
Median of levels reported	0.0775
Mean of levels reported	0.0784
Range of levels reported	0.05-0.11

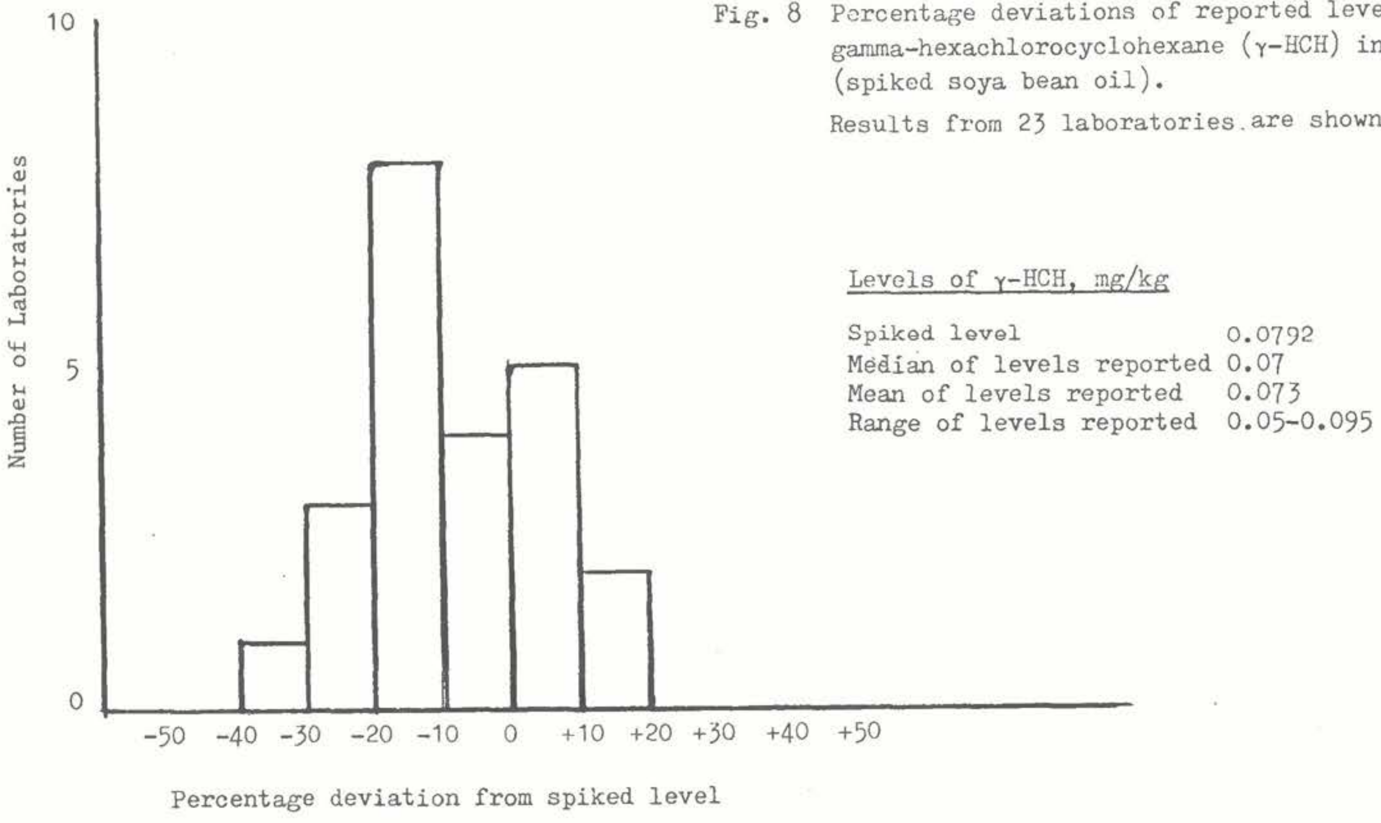


Fig. 8 Percentage deviations of reported levels of gamma-hexachlorocyclohexane (γ-HCH) in sample 2B (spiked soya bean oil).

Results from 23 laboratories are shown.

Levels of γ-HCH, mg/kg

Spiked level	0.0792
Median of levels reported	0.07
Mean of levels reported	0.073
Range of levels reported	0.05-0.095

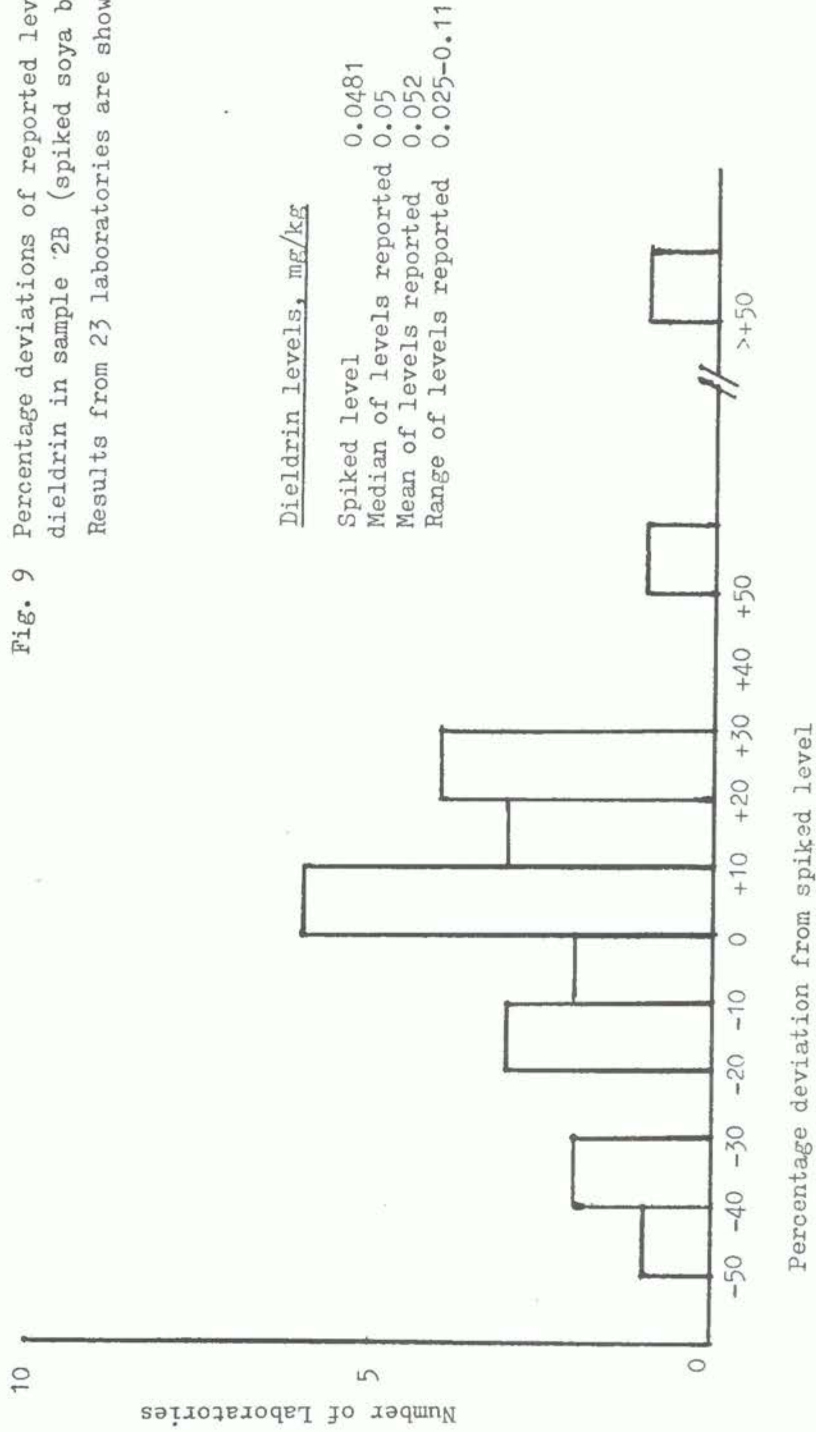


Fig. 9 Percentage deviations of reported levels of dieldrin in sample 2B (spiked soya bean oil). Results from 23 laboratories are shown.

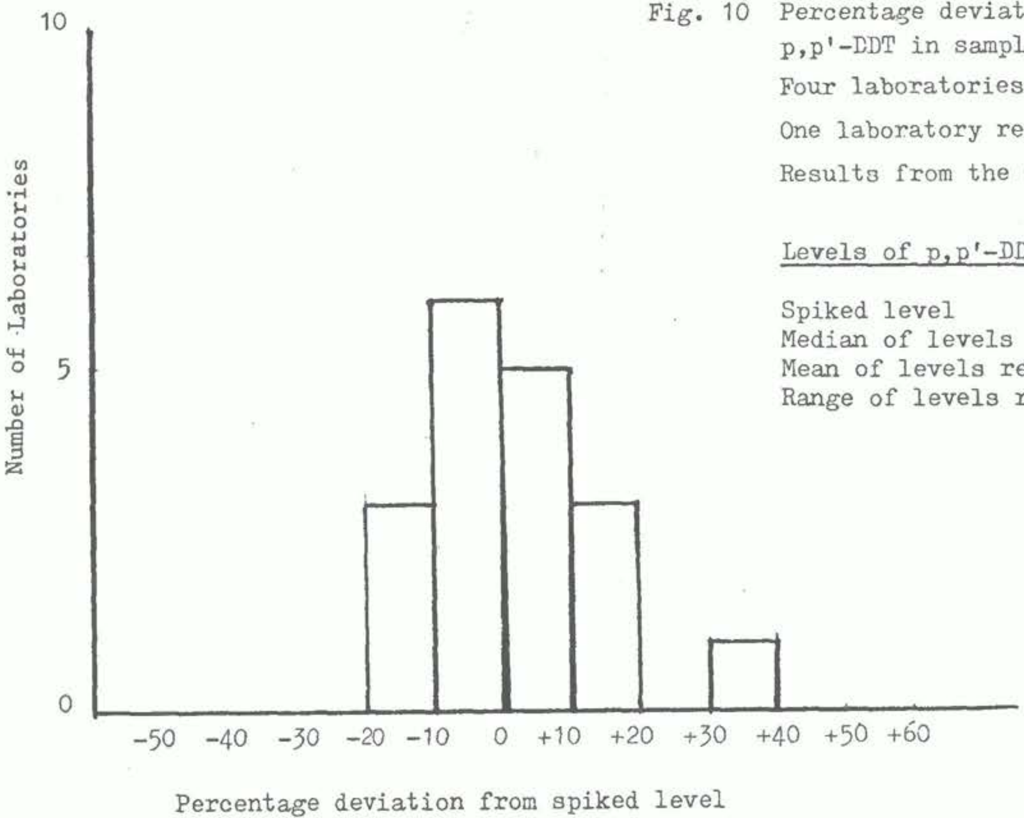


Fig. 10 Percentage deviations of reported levels of p,p'-DDT in sample 2B (spiked soya bean oil).

Four laboratories did not report DDT in this sample.

One laboratory reported result as <0.2 ng/µl.

Results from the other 18 laboratories are shown.

<u>Levels of p,p'-DDT. mg/kg</u>

Spiked level	0.0594
Median of levels reported	0.0595
Mean of levels reported	0.060
Range of levels reported	0.05-0.08

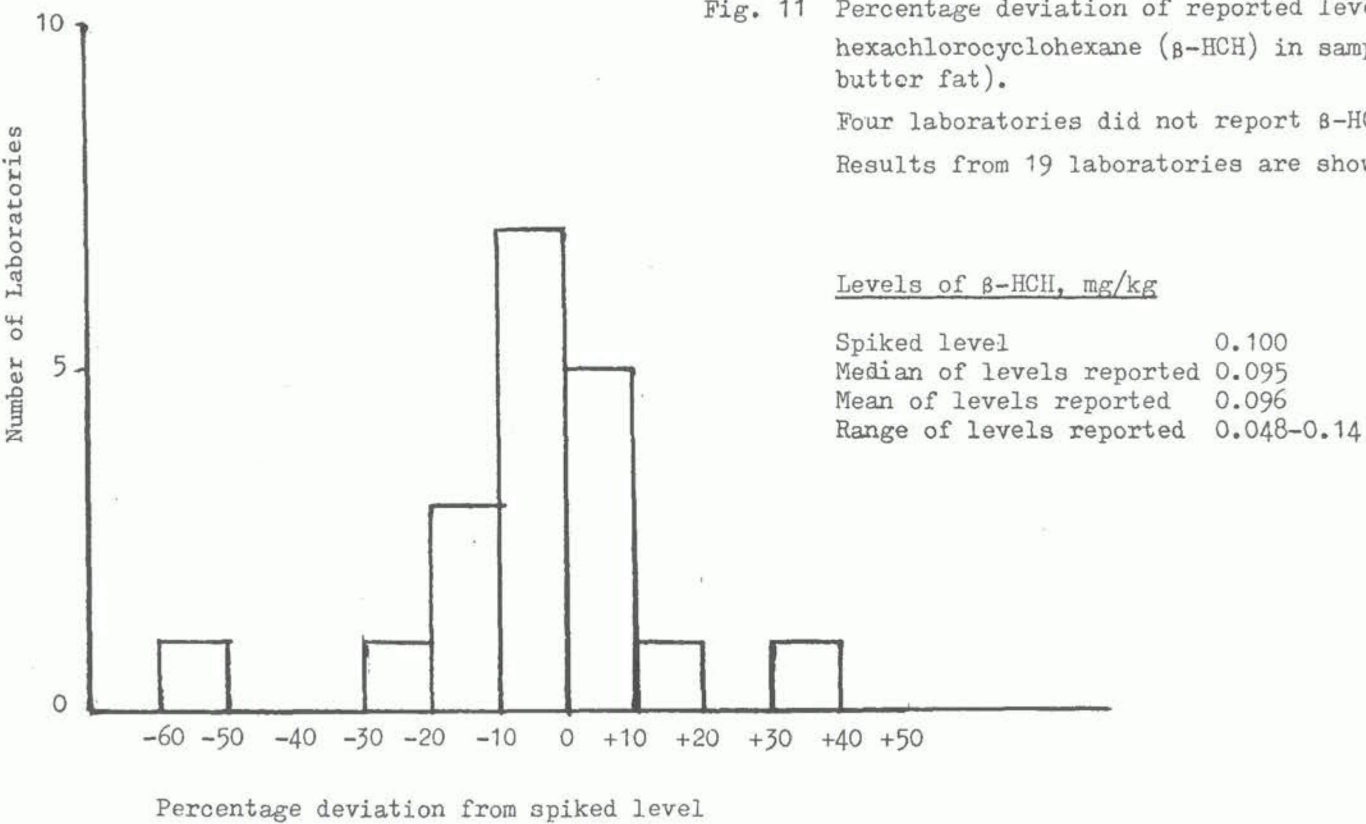


Fig. 11 Percentage deviation of reported levels of beta-hexachlorocyclohexane (β-HCH) in sample 3B (spiked butter fat).

Four laboratories did not report β-HCH in this sample.

Results from 19 laboratories are shown.

<u>Levels of β-HCH, mg/kg</u>

Spiked level	0.100
Median of levels reported	0.095
Mean of levels reported	0.096
Range of levels reported	0.048-0.14

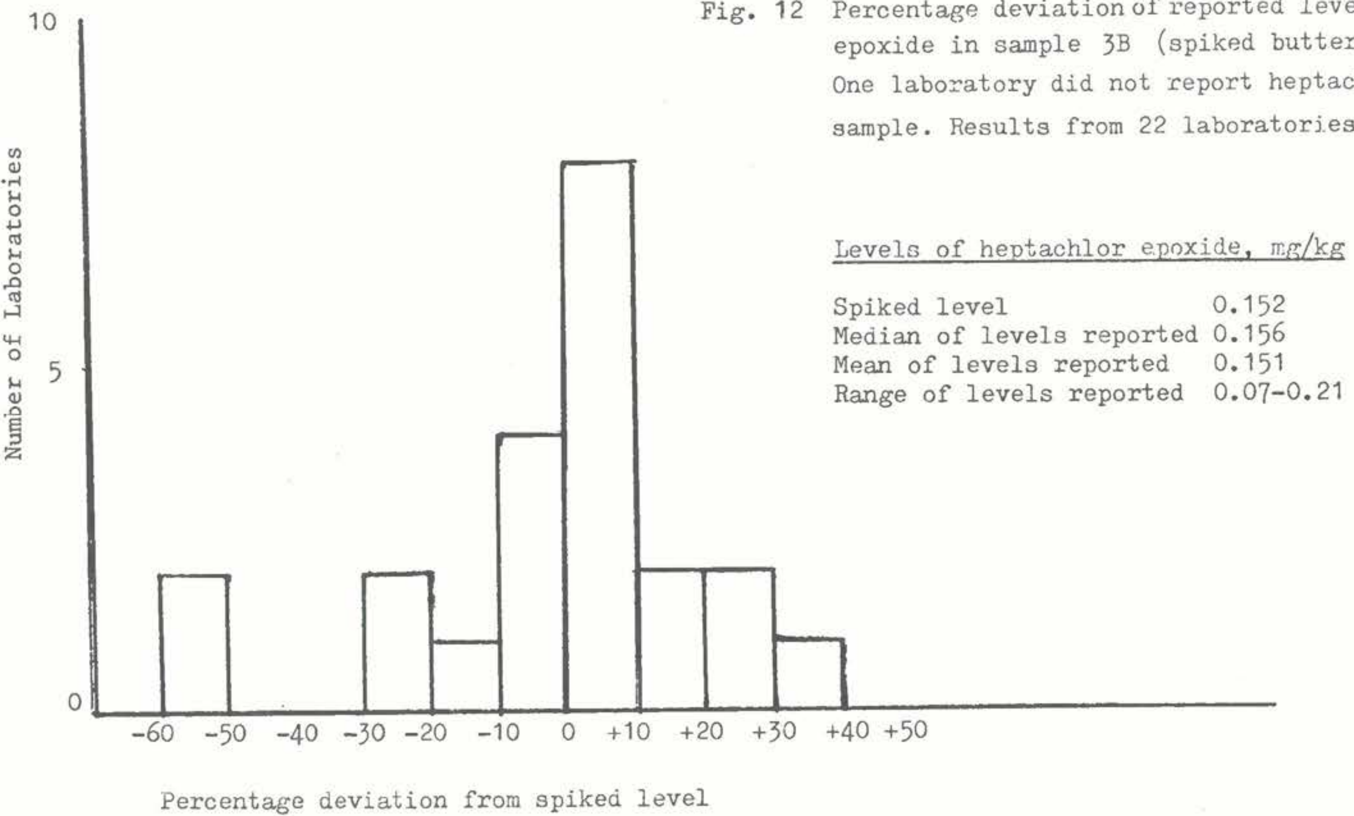


Fig. 12 Percentage deviation of reported levels of heptachlor epoxide in sample 3B (spiked butter fat).
One laboratory did not report heptachlor epoxide in this sample. Results from 22 laboratories are shown.

<u>Levels of heptachlor epoxide, mg/kg</u>

Spiked level	0.152
Median of levels reported	0.156
Mean of levels reported	0.151
Range of levels reported	0.07-0.21

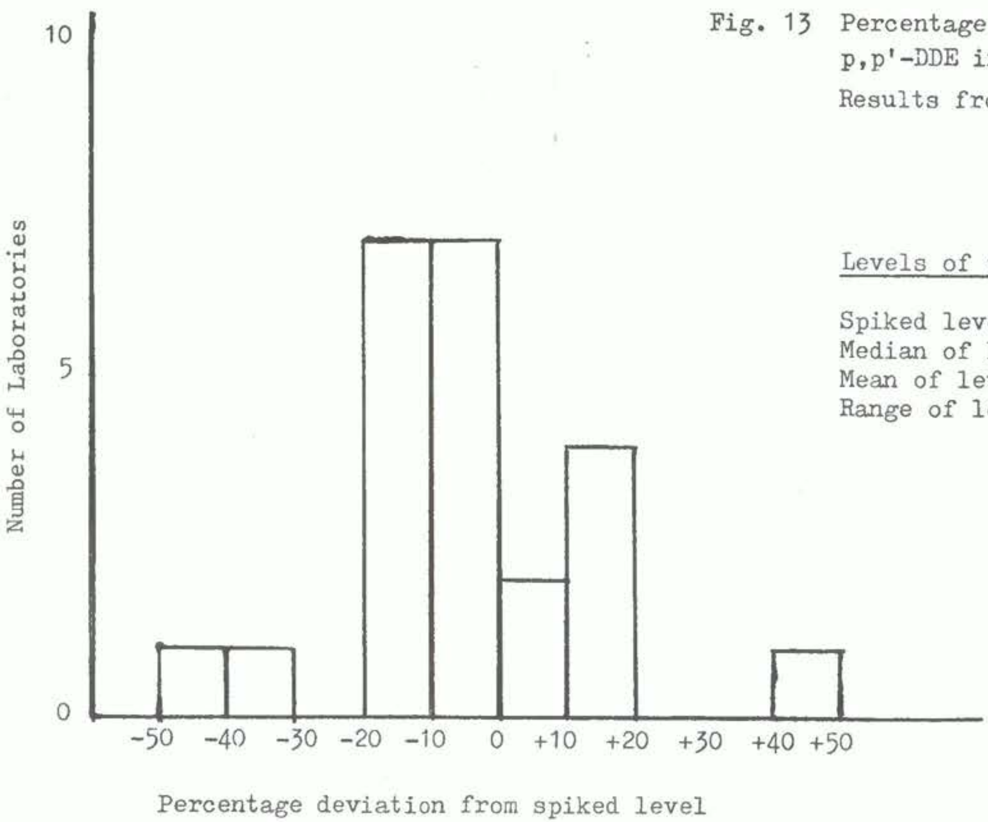


Fig. 13 Percentage deviation of reported levels of p,p'-DDE in sample 3B (spiked butter fat). Results from 23 laboratories are shown.

Levels of p,p'-DDE, mg/kg

Spiked level	0.198
Median of levels reported	0.18
Mean of levels reported	0.186
Range of levels reported	0.11-0.28

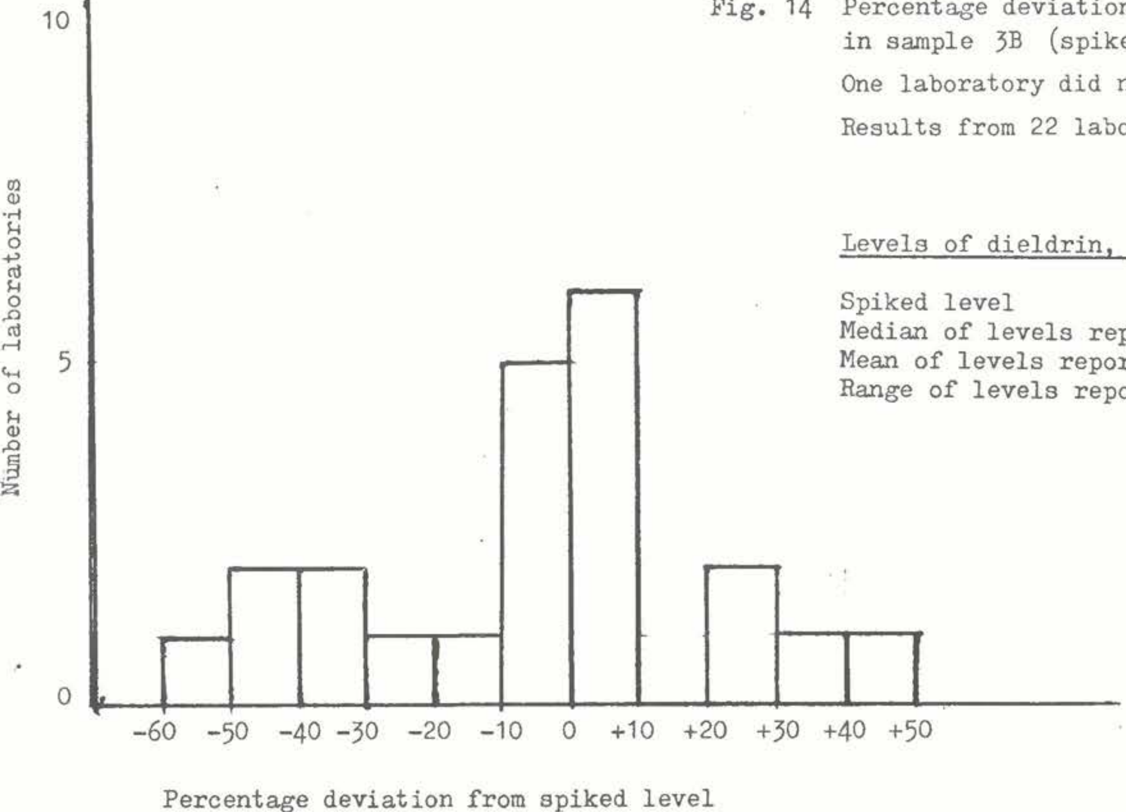


Fig. 14 Percentage deviation of reported levels of dieldrin in sample 3B (spiked butter fat).

One laboratory did not report dieldrin in this sample.

Results from 22 laboratories are shown.

<u>Levels of dieldrin, mg/kg</u>

Spiked level	0.144
Median of levels reported	0.141
Mean of levels reported	0.135
Range of levels reported	0.06-0.21

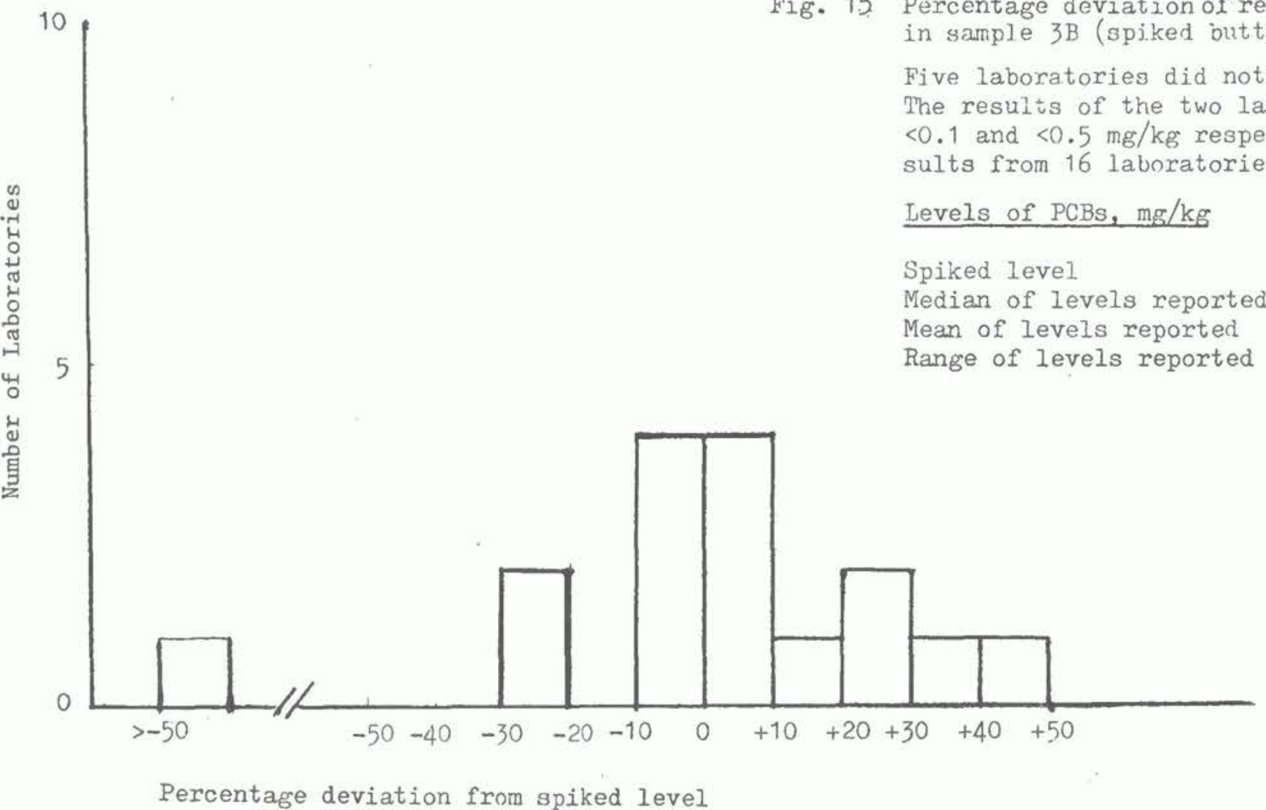


Fig. 15 Percentage deviation of reported levels of PCBs in sample 3B (spiked butter fat).

Five laboratories did not report PCB levels in this sample. The results of the two laboratories reporting levels of <0.1 and <0.5 mg/kg respectively are not shown here. Results from 16 laboratories are shown here.

Levels of PCBs, mg/kg

Spiked level	0.320
Median of levels reported	0.335
Mean of levels reported	0.325
Range of levels reported	0.086-0.46