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Annual Report of the Council for 1985

Presented at the Annual General Meeting of the Association of Public Analysts at Taunton on 24th–26th April, 1986, by the Honorary Secretary, Mr. K. T. Chisnall.

The public analysts laboratories provide an important service to the community. Some of the issues in which public analysts have been involved during the year are described in this report.

House of Lords Select Committee on Science and Technology

The final report of the House of Lords Select Committee on Science and Technology was published on 12th December, 1985. The document contained some 63 recommendations for action by local authorities and many of these recommendations are of considerable importance to the public analysts service.

Whilst concluding that the overall level of scientific and technical expertise in local government is generally good and in some authorities excellent, there are nevertheless warning signals for the future. In particular, the report contains a warning that "local scientific services are now in danger of being adversely affected by financial constraints, by the abolition of the GLC and the metropolitan county councils, and by the fall in direct concern shown by central Government over the past few years".

Among major recommendations for the public analysts service were the following:

1. All local authorities should set up a Science and Technology Sub-Committee of their Policy and Resource Committee.
2. In all Councils with a duty to appoint a public analyst and an agricultural analyst, the analyst should have access to the local authority's management team, if not membership of it, and have chief officer status.
3. An increase in co-operation between local authorities and scientific and technical services is necessary, whether by means of lead authorities or through joint provision. The links between local authority services and those of central authorities and professional bodies must also receive attention.
4. Every local authority should formally consider whether any of its scientific and technical services could now or in the future be better provided jointly or in collaboration with another local authority.
5. The public analysts service should move towards regionalisation of laboratories provided that accessibility to local authorities is maintained.

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6. District and county councils must reduce duplication between their scientific services.
7. Each local authority association should set up a Science and Technology Committee, with functions relating to the information of elected members, the identification of research and applications, and improvement of scientific and technical performance in member authorities.

The Association welcomed the report, in general, and was pleased to note that many of the Select Committee's recommendations were in broad agreement with the proposals made in the Association's recent policy statement. The Association, of course, remains of the opinion that any steps towards a re-organisation of the scientific services should use the existing laboratories as a basis for development.

Local Government Act 1985

The Local Government Act 1985, which received Royal Assent in July, makes sweeping changes in the administration of Greater London and the six Metropolitan Counties. The Greater London Council and the Metropolitan County Councils were abolished at the beginning of April 1986 and their functions dispersed among the Borough and District Councils. Prior to the passing of the Act, public analysts in these areas were answerable to their respective Food and Drug Authorities, and the Association of Public Analysts has consistently expressed the view that the laboratory services which have been developed since 1974 should be retained, because it is both inefficient and uneconomical to run laboratories in smaller units. All local authority matters of a scientific nature should be co-ordinated, and the public analyst, already appointed under the Food and other Acts, is ideally placed to direct this function. The House of Lords Select Committee report was most encouraging in this respect, since it placed great emphasis on the excellence of the existing scientific services in the Metropolitan Counties. The House of Lords Select Committee had already recommended that mechanisms be found to allow retention of the services. At the time of writing this report, arrangements were still under discussion by the Metropolitan Districts in all of the Counties, and there was some reason for optimism that the recommendations of the Select Committee would be heeded.

Agriculture

The most significant piece of legislation in this area of work is the Food and Environment Protection Act which became law in September 1985. Extensive discussions were held on the Bill, but many of the Association's misgivings remain. The Act is intended to allow for the safe storage, distribution, and possible withdrawal of food from sale following major incidents. Several incidents throughout the world have shown the wisdom of anticipating such emergencies and of setting up procedures which will prevent the distribution of contaminated food. The Association maintains

that the inspectorate set up to deal with such emergencies must be drawn from local authorities, which already have the daily duty of monitoring food quality. The expertise and knowledge of local officials could be vital in an emergency.

Members of the Association have long had involvement with pesticide residue analysis, and the results of surveys have been formally published since 1967. Any enforcement action based on the results of analytical investigations would have to be taken under the general provision of the Food Act, using EEC directives and the Codex Alimentarius as sources of guidance on acceptable limits. Ministers now have both the Food Act and the Food and Environment Protection Act as legislation under which limits for pesticide residues in food can be set, but no indication has been given of a time scale for introduction of Regulations to parallel the legislation in other countries.

Although there was a slight drop in the annual number of samples analysed for pesticide residues, this was compensated by sampling to a more definitive pattern. The results show that seven samples or 1·3 per cent of the products analysed exceeded the maximum residue limits, although 23·7 per cent contained detectable amounts of residues, that is, those above the reporting limits. The reporting limit is the figure which the relevant sub-committee has set and is usually one-tenth of the maximum residue limits.

Products	No. of samples	Samples above reporting limits		Samples above MRL's	
		No.	*%*	*No.*	*%*
Flour & Cereal Products	107	32	29·9	2	1·9
Fruit	74	10	13·5	1	1·4
Meat & Meat Products	222	61	27·5	1	0·5
Vegetables	121	21	17·3	3	2·5
1984 Foods Total	524	124	23·7	7	1·3
1983 Foods Total	615	81	13·2	7	1·1

There is no justification for complacency, and there is need for more resources to be given for the purpose of residue analysis, especially as the range and type of pesticide products are continuously changing, and there is a need to remain up to date.

Milk and Dairy Products

Following the various reports on nutrition, there has appeared on the market a number of products having the nature of imitation cream, reconstituted cream and synthetic cream. In many cases, the labelling of these products seems to do nothing for consumer education. For example, one product describes itself as "A non-dairy cream" while containing protein derived from milk, so that it could and did give rise to an allergic response in a sensitive individual who assumed it contained no milk ingredients.

In some areas, milk still contains antibiotic residues, and about 50 per cent of pasteurised milk contains 2 per cent or more of added water. The Association has been looking anew at the composition and freezing-point of both cow's and goat's milk, since both analytical approaches are used in the detection of added water. The Association is also working with the British Standards Institution in reviewing methods of analysis of dairy products.

Meat and Fish Products

The Meat Products and Spreadable Fish Products Regulations 1984 come fully into effect on 1st July 1986. The extremely long gestation period for these Regulations reflects the complexity of control of this range of products. Already the APA has recommended amendments to MAFF including, in particular, a definition for lean meat which excludes any possiblity of using mechanically recovered meat (MRM) or unacceptable parts of the carcass as part of the lean meat content. Frozen fish products contain varying amounts of glaze, and in some cases this can amount to incorporation of water. The Association has made recommendations to the Ministry about compositional standards and labelling of fish and fish products.

Other Foods

The year 1985 will probably be best remembered for the scandal resulting from the adulteration of Austrian, German and Italian wines with diethylene glycol. The Association has insisted that the presence of any detectable diethylene glycol renders the wine unsatisfactory, while some of the Trade would like acceptability to be based on a limit of detection.

The EEC proposal for the labelling of spiritous beverages was considered during the year, and the Association recommended improvements in the labelling of under-strength spirits.

In January 1985 an article in the Grocer magazine indicated concern by importers regarding enforcement of quality standards for tomato produce at the ports of entry. The Association has joined U.K. importers in strongly resisting the Italian proposal that puree made from mouldy tomatoes should qualify for EEC aid.

More detailed information about the type of fat in foods will probably be required soon, reflecting the need to reduce the amount of fat, and particularly saturated fats, in the diet. The Association has therefore been investigating the analytical methods that will be needed to examine samples in this respect.

Work has been done towards developing a standard for pizzas, particularly with respect to cheese content. Consumers do not always appreciate that cheese analogues (synthetic cheese substitutes) do exist and are used in some pizzas.

Dietary Supplements and Other Special Foods

The comments in our report two years ago about so-called "health foods" are still pertinent. Some dietary supplements intended for slimmers or those engaged in sport bear exaggerated claims on the labels. The number of unsatisfactory samples of this type is not falling as fast as it should. The public interest in organically-grown foods is not at present justified by the analytical evidence.

The Association is of the view that "organically grown" means that pesticides and artificial fertilisers have not been used at any stage of the production process. There is evidence that some products so labelled do contain pesticide residues, perhaps in some cases derived from applications of previous seasons.

Labelling of Foods

The Food Labelling Regulations 1984 have brought improvements, but there are still many labels with fancy names and devices of presentation which obscure the true nature of the food. The label is often the only source of information for the consumer about the product he has purchased. Consumers are becoming more discerning, and this trend is to be encouraged. One of the most important aspects of the work of the public analyst is to compare the composition of a product with claims and statements on the label. Manufacturers can find that zealous marketing of a product brings them into conflict with the Food Labelling Regulations. The ingenious attempts to resolve these difficulties often give rise to semantic problems. For example, the Association drafted a LACOTS Code of Practice so that the words "low" and "reduced" can be used in a way that educates rather than confuses the consumer.

The Association has commented during the year on draft guidelines on nutritional labelling, labelling of fat in foodstuffs, and the EEC proposals to amend the labelling directive.

Imported Foods

Imported food samples still present PA's with a variety of problems. There is only a short time available because goods cannot be held for more than six days, and the possibility of contamination, and of the presence of non-permitted additives and pesticide residues among other things, means that rapid enforcement analysis may miss something, so that further reliance must be placed on the "longstop" of retail sampling.

Since the highly-publicised "dates" case of about two years ago, importers and Air and Seaport Health Authorities have drawn up a procedure for sampling. An acceptance level for infestation in dates has been agreed. This is the first attempt in the U.K. to quantify insect infestation.

Low bacterial counts in frozen prawns led to the suspicion that disinfectants were being used in processing. Trade literature from S.E. Asia confirmed that Chloramine-T was being sold for this purpose, and it has been detected in imported frozen prawns. Analysis for this non-permitted additive will continue.

Environment

The apparent reluctance of central government to support the public analyst service still gives cause for concern. For example, the Department of the Environment refused to specify the public analyst as the person to carry out the analysis for fluoride in water under the Water (Fluoridation) Act 1985.

The possibility of uniform mandatory methods of analysis for water is being discussed within the EEC. The Association welcomes reference methods to resolve doubt or dispute, but opposes mandatory methods as these become outdated and relatively inefficient, adding to the costs of enforcement. The legislative process is too slow for the advances in technology that occur ever more rapidly.

The absurdity to which EEC legislation can now give rise is well illustrated by the Natural Mineral Water Regulations 1985 and the Drinking Water Directive 778/80/EEC. Consumers have to learn that the product labelled as Natural Mineral Water comes out of the ground in such a state of purity that it requires no treatment, while apparently similar products labelled slightly differently, e.g. as spring water, may have needed chlorination and other treatment before becoming fit to drink.

London public analysts have been involved in a survey of lead in fruit and vegetables and a comparison of levels at different distances from main roads and a central reference point. This work was done in response to a publication by the Campaign for Lead-free Air.

Consumers Hazards

Concern over asbestos continues, and the Association plays its full part in providing a service to the community, identifying asbestos and monitoring it's removal. The Association has commented on the proposed new Regulations and particularly welcomes the attempts of the Health and Safety Executive to ensure that all laboratories involved in this work are accredited and of proven ability.

The Association is very critical of the field test recommended by the Department of Trade and Industry to environmental health officiers for lead in surma, the Asian cosmetic. The test is not safe and reliable as claimed by the Department, and public analysts have recommended that it be discarded.

The laudable desire to further reduce the amounts of toxic metals absorbed by children has given rise to fresh activity by the EEC on toys, and a new draft directive is in prospect. The Association has been very active in ensuring that the methods of test are adequate and reasonable, and combine the twin objectives of protecting the consumer's health and also his pocket by not placing an undue burden on industry.

Public analysts have also been investigating the amounts of toxic metals in cosmetics and, for example, conclude that reputable manufacturers keep the level of lead in cosmetics acceptably low, generally below about 5 milligrams per kilogram.

The Association contines to comment on medicines legislation and continues to press for enforcement of the relevant parts of this by local authorities.

Analytical Quality Assurance

Formalised procedures for Analytical Quality Assurance within and between Public Analyst Laboratories were adopted by the Association at its Annual General Meeting in 1984, for internal purposes. This anticipated the recommendation of the House of Lords Select Committee, which the Association therefore supports.

Conclusion

Public analysts serve as experts on many external bodies concerned with a wide range of scientific matters, as described in Appendix II. Nevertheless, the scientific service provided by public analysts laboratories is neither properly utilised nor adequately financed by local authorities. For example, only about £2·5 million (under 5 pence per person per year) is spent auditing the nation's food supply, an amount woefully inadequate to protect the consumer and legitimate industry, considering that the nation's food bill is £28 billion and that over £300 million is spent on advertising food and drink each year.

APPENDIX I

STATISTICAL SUMMARY

Food including complaint samples and those submitted under the Imported Food Regulations	93264
Milk (including those examined for antibiotics and those submitted under the Milk (Special Designation) Regulations	19947
Drinking Waters	12124
Mineral Waters (Natural Mineral Waters Regulations 1985)	56
Swimming Pool waters	40341
Environmental pollution (water, trade effluents, tip leachates, ground waters, etc.)	42333
Environmental pollution (Waste disposal, reclaimed land, etc.)	44902
Feeding Stuffs (Agriculture Act and Medicines Act) and feeding supplements	4208
Fertilizers (all kinds)	2149
Consumer Protection and Trade Description Act samples	6469
Miscellaneous (Health Authorities, HM Coroner, other Local Authority Departments)	20509
Health and Safety at Work Act	22943
TOTAL	309800

APPENDIX II

REPRESENTATION OF PUBLIC ANALYSTS ON OUTSIDE ORGANISATIONS

A. Local Authorities

1. Local Authorities' Co-ordinating Body on Trading Standards (LACOTS).
   (a) Quality Standards Panel.
   (b) Labelling Sub-panel.
   (c) Working Party on Sampling Techniques.
   (d) Working Party on Meat Products.
   (e) Safety Panel.
2. Association of County Councils (ACC).
   (a) Consumer Services Committee, Public Analyst Adviser.
3. Association of Metropolitan Authorities (AMA).
   (a) General Services Committee, Public Analyst Advisers.
   (b) ACC/AMA Joint Waste Disposal Panel.
4. Convention of Scottish Local Authorities (COSLA).
   (a) Scottish Food and Drugs Coordinating Committee.
   (b) Public Analyst Adviser.

B. Central Government

1. Ministry of Agriculture, Fisheries and Food (MAFF).
   (a) Food Advisory Committee.
   (b) Steering Group on Food Surveillance, Heavy Metals Working Party.
   (c) Steering Group on Food Surveillance, Quality Assurance Sub-committee.
   (d) Steering Group on Food Surveillance, Sub-group on Mycotoxins.
   (e) Advisory Committee on Pesticides.
   (f) Veterinary Products Committee, Feeding Stuffs Sub-Committee.
   (g) Veterinary Products Committee, Feeding Stuffs Sub-Committee, Methods of Analysis Panel.
   (h) Working Party on Due Diligence.
   (i) Committee on Analytical Methods for Pesticides and Veterinary Products.
   (j) Working Party on Pesticide Residues, Sub-group on Bromide Residues.
   (k) Working Party on Pesticide Residues, Sub-group on pesticides in Animal Products.
   (l) Working Party on Pesticide Residues, Sub-group on Cereals.
   (m) Working Party on Pesticide Residues, Sub-group on Fruit and Vegetables.
   (n) Analytical Panel on Mycotoxins.

2. Department of the Environment.
   (a) Radioactive Waste Management Advisory Committee.
   (b) Advisory Committee on Safe Transport of Radioactive Materials.
   (c) Steering Committee on Environmental Lead Monitoring.
   (d) Working Party on Pesticides in Drinking Water.
   (e) Standing Committee of Analysts (water analysis).

3. Department of Trade & Industry.
   (a) National Measurement Accreditation Service/National Testing Laboratory Accreditation Scheme (NAMAS/NATLAS), Chemical Technical Committee.

C. European Community

1. The Community Bureau of Reference: Consultative Committee on Reference Materials for Food Analysis.
2. Working Party on Cosmetic Products.
3. European Committee for Standardisation (CEN), Committee on Safety of Toys.

D. Royal Society of Chemistry

1. National Council.
2. Law and Parliamentary Committee.
3. Professional Affairs Board.
4. Qualifications and Education Board.
5. Benevolent Fund.
6. Examinations and Institutions Committee.
7. Liaison Committee (with APA).
8. Applications Committee.
9. Disciplinary Committee.
10. Ethical Practices Committee.
11. Working Party on Mastership in Chemical Analysis Qualification (M.Chem.A).
12. Analytical Division Council.
13. Registration Committee.
14. Health, Safety and Environment Committee.
15. M.Chem.A. Examination Board.
16. Analytical Methods Committee (AMC), Chairman.
    (a) AMC Meat Factors Sub-committee.
    (b) AMC Veterinary Residues Sub-committee.
    (c) AMC Animal Feeds Sub-committee.
    (d) AMC Antibiotics in Animal Feeds Sub-committee.
    (e) AMC Statistics Sub-committee.
    (f) AMC Metallic Impurities in Organic Matter Sub-committee.

E. British Standards Institution

1. Local Government Liaison Officer—Disinfectants.
2. Disinfectants Standards Committee, DIC/–.
3. Disinfectants, Test Methods, DIC/11.
4. Disinfectants, Specifications, DIC/12.
5. Chemical and Health Council, C/–.
6. Toy Safety Committee, TCM/15.
7. Fertilisers Committee, CIC/37.
8. Technical Committee (TC) for Ceramic Ware, Glassware and Glass Ceramic Ware in Contact with Food, FHM/28/29.
9. TC on Land Quality.
10. Fillings (Rag Flock, etc.) TCM/10.
11. Meat and Meat Products FAC/6.
12. Freezing-point of Milk DAC/2.
13. Dairy Apparatus DAC/3/12.
14. Dairy Products DAC/3.
15. Land Control EBC/47.
16. Cereals and Cereal products, FAC/4.
17. Spices and Condiments FAC/7.
18. Tinned and Metallic Cooking Ware FHM/12.
19. Analysis and Sampling of Iron, Manganese and Chrome Ores.
20. Sampling and Analysis of Fluorspar, Bauxite and Aluminium Ores.
21. Committee on Laboratory Ovens.

F. Other Organisations

1. European Food Law Association, Council.
2. International Committee for Uniform Methods of Sugar Analysis (ICUMSA), National Committee.
3. Meat and Livestock Commission, Serological Testing Committee.
4. Committee of Polytechnic Heads of Analytical Chemistry Departments —ACOT Scheme.
5. Nottinghamshire College of Agriculture, Food Science Advisory Panel.
6. International Standards Organisation (ISO) Committee for Analysis and Sampling of Iron, Manganese and Chrome Ores.
7. ISO Committee for the Sampling and Analysis of Fluorspar, Bauxite and Aluminium Ores.
8. International Union of Pure and Applied Chemistry, (IUPAC), Applied Chemistry Division (Secretary).
9. IUPAC, Food Chemistry Commission (U.K. National Representative).
10. IUPAC, "Pure and Applied Chemistry" Editorial Advisory Board.

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Arachis Oil: A Re-investigation of the Evers Test

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The fatty acids which give rise to the turbidity of the Evers test have been identified and the effects of varying their amounts in test solutions have been studied. An amended procedure for the turbidity temperature measurement is recommended. A survey has been made of the turbidity temperature of a number of authentic arachis oils and a new specific lower limit for the turbidity temperature is now proposed.

Arachis oil is the fixed oil obtained from groundnuts (peanuts), *Arachis hypogaea* L. It has some pharmaceutical uses and is the subject of a current British Pharmacopoeia (B.P.) monograph[1]. The sole B.P. identification test for the oil is that due to Evers[2,3]. This test consists in saponifying the oil and observing the temperature (the Evers turbidity temperature) at which the saturated fatty acids of higher molecular weight crystallise out of solution under standard conditions. A similar procedure has been applied to other vegetable oil, and is then generally known as the Bellier test, the original work having been carried out by Bellier in 1899[4].

The minimum temperature of the onset of turbidity in the Evers test for the pure oil has been the subject of disagreement in recent years, as illustrated by the fact that it was given as 37°C in the 1973 B.P., 36°C in the 1978 B.P. Addendum, and "about 36°C" in the 1980 B.P. Also, a more general criticism, that all empirical identification tests such as the Evers test should be abandoned altogether in favour of a determination of the fatty acid profile, has been made[5,6].

Internationally agreed ranges of values for the fatty acid composition of ten oils and fats have been published[6] although some disagreement remains on the level of the minor component, linolenic acid, in arachis oil[7]. These ranges are rather wide. In terms of the medians of each range, and ignoring those acids which are below 1·0 per cent., a typical composition of arachis oil would be:

Fatty acid	*Per cent.*
Oleic acid, $C_{18:1}$	50
Linoleic acid, $C_{18:2}$	28
Palmitic acid, C_{16}	10·6
Stearic acid, C_{18}	3·8
Behenic acid, C_{22}	3·1
Arachidic acid, C_{20}	1·7
Lignoceric acid, C_{24}	
Eicosenoic acid, $C_{20:1}$	1·2

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The Bellier turbidity test was originally intended to determine the extent of adulteration of oils, such as olive oil, with arachis oil, and indeed is still used for this purpose[8,9]. However, in 1936 Evers[3] stated that the test ". . . forms an excellent guide to the purity of arachis oil". The test solution contains the neutralised saponified oil, acetic acid, acetate ion, alcohol and water. As the mixture cools from a temperature at which all the components are in solution, solid acids begin to precipitate when the concentration of one or more of the acids exceeds its equilibrium solubility at that temperature. The consistently high temperature at which this precipitation occurs for arachis oil is due to the almost uniquely high amounts of the three long chain saturated acids, arachidic, behenic and lignoceric acids, which are present in arachis oil. However, as will be shown below, the observed Evers turbidity temperature is also a function of the amounts of the other constituent fatty acids present in an oil.

Various parameters affecting the results of the Evers test have been studied.

Apparatus

Varian Aerograph (1200) Gas Chromatograph, with flame ionisation detector. Column: 5 ft by 0·25 inch i.d. glass column packed with 3 per cent. w/w OV-1 on acid Chromosorb W.

Carrier gas: Nitrogen, flow rate 20 ml/min.

Reagents and Materials

Reagents for the identification (Evers) test specified in the B.P. monograph on Arachis oil[1], as follows:

Ethanolic potassium hydroxide, 1·5 M

Acetic acid, 33 per cent. w/w (6 M after 1980).

Ethanol, 70 per cent. v/v (made up from Industrial Methylated Spirit).

The above reagents were assayed after preparation and during the study.

Other Materials

Palmitic (Hexadecanoic) acid (C_{16}); 99 per cent. (ex Sigma).

Stearic (Octadecanoic) acid (C_{18}); 99 per cent. (ex Sigma).

Oleic (cis-*9-Octadecenoic*) *acid* ($C_{18:1}$); 99 per cent. (ex Sigma).

Linoleic (all cis, *12-Octadecadienoic*) *acid* ($C_{18:2}$); 99 per cent. (ex Sigma).

*Arachidic (*n-Eicosanoic) acid (C_{20}); 99 per cent. (ex Aldrich).

*Behenic (*n-*Decosanoic) acid* (C_{22}); ex B.D.H. and recrystallised from 95 per cent. ethanol to a 99 per cent. purity.

*Lignoceric (*n-*Tetracosanoic) acid* (C_{24}); 99 per cent. (ex Sigma).

The purity of the fatty acids was checked by GLC.

Storage and Treatment of Samples

Samples of arachis oil and groundnuts were obtained and stored at −18°C. Oil was cold extracted by stirring the finely chopped groundnuts for one hour with petroleum spirit (40–60°). After extraction the solvent was evaporated at room temperature in a fume cupboard.

Methods

(a) SAPONIFICATION

One gram of an oil was saponified by boiling over a small flame in a 100 ml flask under reflux with 5·0 ml of 1·5 M ethanolic potassium hydroxide for 5 min as directed in the B.P. After cooling, 1·5 ml of 6·0 M acetic acid and 50 ml of 70 per cent. alcohol were added and the test solution warmed to dissolve the fatty acids.

(b) TURBIDITY TEMPERATURE

The test solution was stirred magnetically while cooling in air, the 100 ml flask being clamped 4 cm above the magnetic stirrer plate to avoid the effect of heat generated in the unit. The flask was viewed with side illumination against a dark background. A faint opalescence was first seen and within 0·2°C the turbidity was unmistakable. The lower figure thus obtained was recorded.

(c) FATTY ACID SEPARATION

The mixed fatty acids responsible for the Evers turbidity with a given sample were collected at various temperatures on a glass fibre filter (Whatman GFC cut to fit a filter stick) and washed with 70 per cent. V/V alcohol at the same temperature. The glass fibre filter was then placed in the esterification mixture. After the acids had been esterified the esters were partitioned into petroleum spirit (40–60°) and chromatographed.

(d) ESTERIFICATION AND CHROMATOGRAPHY

Methyl esters of the fatty acids were prepared by the sulphuric acid-methanol procedure of the AOAC[10], which was considered to be intrinsically safer and more convenient than the AOAC BF_3 procedure[11]. The identity of the fatty acid methyl esters was established by reference to predetermined Kovats retention indices. It has previously been shown that the relative response of an FID to methyl esters of fatty acids from caprylic to arachidic is close to unity[12]. This was confirmed for arachidic, behenic, and lignoceric acids, therefore, methyl ester peak areas relative to the total peak areas were determined to obtain the composition of the mixture of acids.

(e) INVESTIGATION OF THE EFFECTS OF ADDED ACIDS

The relative influences on the Evers turbidity temperature of the acids which comprise the Evers precipitate were investigated as follows: One gram of an arachis oil was taken and the Evers turbidity temperature was obtained in the usual B.P. manner. Incremental additions of an acid were made to the B.P. test solution, the mixture was warmed to redissolve the acids and the successive new turbidity-temperatures were noted after each such addition. The acids investigated were palmitic acid, stearic acid, arachidic acid, behenic acid and lignoceric acid. Cerotic acid was not investigated since, although it occurs in the Evers precipitate at levels similar to those of palmitic acid (Table I), its concentration in the oil itself is considerably lower than that of palmitic acid[6].

The effect of varying the concentration of acetic acid was also studied. A number of test solutions were prepared using the same oil but with different concentrations of acetic acid, and with all the other reagents at their normal values.

Results and Discussion

A typical set of results for the fatty acids comprising the precipitate responsible for the Evers turbidity is shown in Table I. These data show that all the saturated acids known to be present[13] in arachis oil made some contribution to the observed turbidity and that the major components were behenic, lignoceric and arachidic acids in that order (See Table I).

TABLE I

FATTY ACIDS RESPONSIBLE FOR THE TURBIDITY IN THE EVERS TEST (COLLECTED AT 35°C)

Fatty acid	Composition *per cent.*
Palmitic acid C_{16}	1·3
Stearic acid C_{18}	2·3
Arachidic acid C_{20}	7·9
Behenic acid C_{22}	49·1
Lignoceric acid C_{24}	37·5
Cerotic acid C_{26}	1·9

The composition of the precipitates obtained at various temperatures at which they were filtered off are shown in Table II. The results obtained for the composition of the precipitate at various temperatures are compatible with the known mutual solubility effect of one fatty acid on another.

The repeatability of the Evers test was examined by making ten determinations of the turbidity temperature on the same arachis oil over a period of six weeks using fresh sets of reagents each time. The results are summarised in Table III. The standard deviation of the observed Evers turbidity temperature was found to be 0·3°C.

The effects on the Evers turbidity temperature of small incremental additions of the fatty acids of chain length equal to and greater than C_{16} present in arachis oil are shown in Figure 1.

It did not prove possible to use the same arachis oil for each acid investigated, and thus the turbidity-temperature at zero acid addition differs from curve to curve in Figure 1. This however is considered to enhance the generality of the results.

Repeated heating and cooling of the test solutions was not found to affect materially the observed turbidity temperatures. Table IV shows the relative influence of the added acids. Figure 2 shows the turbidity temperature as a function of the concentration of acetic acid used in the test.

Bellier[4] (1899) used 70 per cent. v/v alcohol containing 1 per cent. v/v hydrochloric acid. Ibarra[14] examined the effect of added hydrochloric acid and found that the turbidity temperature increased with the concentration of

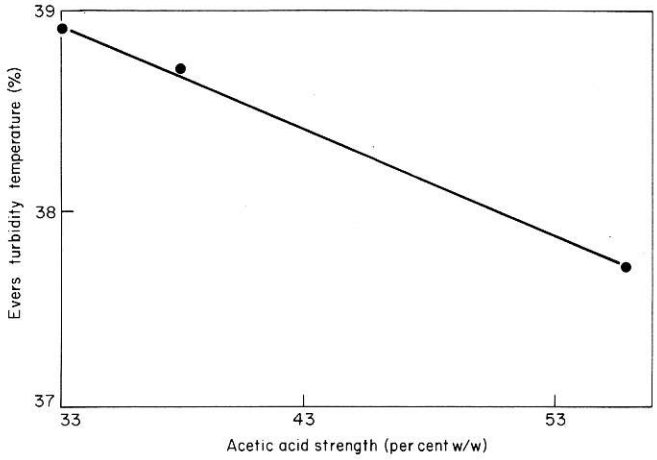

Fig. 1. Influence of added fatty acids on the observed Evers turbidity temperature. ●, Arachidic acid; ○, linoleic acid; ■, oleic acid; □, palmitic acid; ▲, stearic acid; △, behenic acid; ▽, lignoceric acid.

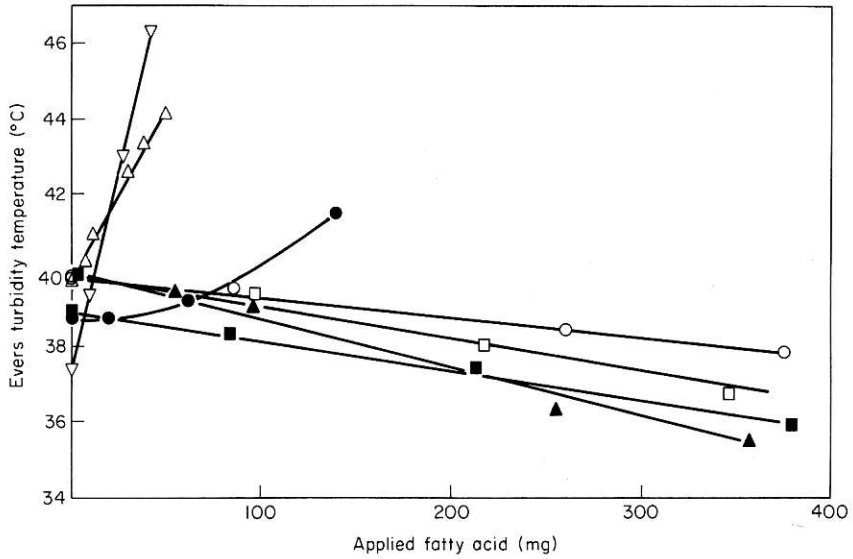

Fig. 2. Influence of strength of acetic acid used on the observed Evers turbidity temperature.

hydrochloric acid in the test solution. In contrast to the effect of hydrochloric acid, the results presented here (Figure 2) show that increasing the strength of acetic acid used in the test decreases the turbidity temperature. It is interesting to note that in the 1980 B.P. the strength of acetic acid specified is 6 M, which is slightly stronger than the 33 per cent. w/w acid (5·6 M) specified in previous editions. However, this change in concentration will, from the data obtained, cause a decrease of only 0·2°C in the observed turbidity temperature (Figure 2).

A survey of the Evers turbidity temperature of a number of samples of arachis oil was carried out. The results are summarised in Table V, and include one

TABLE II

COMPOSITION, PER CENT. W/W, OF THE PRECIPITATE RESPONSIBLE FOR THE TURBIDITY IN THE EVERS TEST AS A FUNCTION OF TEMPERATURE

Fatty acid	Temperature °C 37/38	35/36	29/30
Palmitic acid	0·9	0·8	1·3
Stearic acid	1·3	1·9	2·4
Arachidic acid	9·8	6·8	18·5
Behenic acid	36·7	39·0	37·1
Lignoceric acid	44·8	45·1	39·8
Cerotic acid	6·5	6·4	0·9

TABLE III

REPEATABILITY OF THE EVERS TEST (10 DETERMINATIONS)

Weight of oil taken *g*	Evers turbidity temperature, °C *range*	*mean*	*s.d.*
1·0035–0·9954	38·9–39·7	39·27	0·3

TABLE IV

RELATIVE EFFECTS OF ADDED FATTY ACIDS ON THE EVERS-TURBIDITY-TEMPERATURE

Fatty acid	$\Delta t/mg$, m°C*	Per cent. compositional change†
Palmitic acid	−9	93
Stearic acid	−13	250
Oleic acid	−8	18
Linoleic acid	−5	69
Arachidic acid	+20	36
Behenic acid	+86	56
Lignoceric acid	+214	29

* Magnitude and direction of the change in the Evers turbidity temperature when the cited acids were added to the test solution.

† Estimate of the percentage compositional change, in terms of a shift away from the median fatty acid composition of the oil which would result in a change in the Evers turbidity temperature of 1°C.

sample which was not arachis oil as defined earlier. The effect on the turbidity temperature of a sample of oil of adding any one fatty acid will depend on its original content of that fatty acid; for example, adding 10 per cent. of palmitic acid when the oil contains 6–15 per cent. of that acid will have a different effect from adding 10 per cent. of stearic acid, of which the oil contains only 1·3–6·5 per cent. This explains why Table IV includes an estimate, for each fatty acid, of the percentage change that would be required in the total amount of that acid to produce a 1°C change in the turbidity temperature of an oil having the median composition given in the third paragraph of this paper. The Evers test can

TABLE V

SURVEY OF EVERS TURBIDITY TEMPERATURE, t_t, FOR ARACHIS OIL

Year of test	No. of samples	Origin	t_t
1979	8	Obtained as oils	39·7, 39·1, 38·7, 38·5, 37·5, 40·8, 39·6, 37·3
1979	7	Extracted from groundnuts	
		Origin unknown	39·5, 39·9, 40·0
		Argentina	37·6
		Gambia	38·6
		Senegal	38·7, 39·8
1980	1	R. E. Worthington	51·1*
1982	2	Extracted from groundnuts	
		Local purchase	37·9, 38·9
1983	3	Extracted from groundnuts	
		Local purchase	40·0, 41·5
		Hong Kong purchase	39·5
1985	1	Obtained as oil	37·3

* This was not arachis oil as defined but was extracted from wild peanuts, *Arachis villosulicarpa*, and is not a commercially available genotype. It is unusual in containing relatively large amounts of behenic and lignoceric acids, 14·0 and 5·9 per cent. respectively[15].

therefore be considered to be sensitive to abnormally low levels of arachidic, behenic or lignoceric acids, or to an abnormally high level of stearic acid.

From Table IV and Figure 1 it is clear that varying the amounts of stearic, oleic, palmitic and linoleic acids in a test portion of the oil will produce very similar results, since the temperature shift per milligram of acid added is in the same direction and approximately the same magnitude for each acid. Palmitic, oleic and linoleic acids are major components of the fatty acids of arachis oil[6] and the individual amounts of each would be expected to be, to a large extent, interdependant. It follows then that variations, from one sample of arachis oil to the next, in the concentrations of palmitic, oleic and linoleic acids will have relatively little effect on the Evers turbidity temperature.

A reduction of the turbidity temperature, to below the usual range would therefore normally be expected only to result from a fall in the levels of the three higher saturated acids namely arachidic, behenic and lignoceric. Authentic arachis oils of unusual composition have been reported, in that for a few samples, arachidic and lignoceric acids have been found to constitute less than one per cent. of the total fatty acids[13,16,17]. However, no report has been found in the literature of abnormally low values of behenic acid, which would affect the turbidity temperature to a considerable extent.

Despite variations in the basic technique, the Evers turbidity temperature of arachis oil has remained consistently in the upper portion of the range 35–40°C for 70 years (Table VI). The test is rapid, provided the reagents are to hand, simple to perform and interpret, and must be seen as being complementary to information obtained from the GLC fatty acid profile of the oil. It has a useful quality control function, even if it does not alone provide a positive identification due to the possibility of authentic samples of arachis oil of unusual fatty acid profile. This limitation, however, applies equally to identity or purity

specifications written solely in terms of the fatty acid composition. Worthington[7], discussing fatty acid profile criteria has shown that setting a limit of "less than 1·0 per cent." rather than "less than 0·1 per cent." for linolenic acid in arachis oil allows undetectable adulteration to the extent of 10 per cent. or greater with oils of appropriate fatty acid profile. Obviously the Evers test cannot work to the same limits as GLC techniques but it is interesting to note that even GLC based fatty acid profiles may not resolve the issue.

TABLE VI

TURBIDITY TEMPERATURES OF ARACHIS OIL 1898–1985

Year	Turbidity temperature °C	Remarks	Ref.
1898	35–38	The separated higher saturated fatty acids in 100 ml 90 per cent. alcohol	18, 19
1912	40·0–40·8	Adler's results, confirmed by Evers	2
1914	—	B.P. (1914) Evers test not included in its modern form	
1930	*c.* 40	A French standard method of the time, similar to that of Evers	20
1932	≥39	B.P. 1932	
1936	39–40	Evers, 13 samples	3
1943	40	Methods similar to that of Evers	21
1943	39·5	Methods similar to that of Evers	22
1945	34·5–38·0	29 samples. AOAC investigation of the Evers (1936) method	23
1948	39·0–42·5	Evers method, filtration of final solution and use of methyl red indicator to ensure that it was acidic	24
1948	≥39	B.P. 1948	
1952	39·7–42·5	Essentially Evers method	26
1953	36·5–40·5	Excess HCl (3–12 drops) added	14
1954	39–41	Bellier/Adler method	25
1958	≥39	B.P. 1958. However the quantity of oil tested was increased from 1·0 ml (0·91 g) to 1·0 g	
1973	above 37	1973 B.P.	
1978	above 36	B.P. Addendum 1978	
1979	above 37	The Pharmaceutical Codex, 11th Ed.	
1980	about 36	B.P. 1980	
1980	39–40	20 samples (India)	9
1979–1985	37·3–41·5	Present work, 21 samples	

Omission of the Evers test from the B.P. monograph on arachis oil would not, therefore, be justified. However, a *specific* lower limit for the Evers turbidity temperature should be reintroduced. The lowest Evers turbidity temperature found in the present survey was 37·3°C (Table VI). Allowing for the slight change in strength of the acetic acid specified in the B.P. since that particular determination was made (causing a decrease of 0·2°C—See above and Figure 2), and subtracting three times the standard deviation of the method, a figure of 36·2°C is obtained. Therefore, it is suggested that a specific lower limit of "not less than 36°C" be reintroduced in the Evers test in the B.P. monograph on arachis oil. It should be noted in passing that none of the oils tested would have had difficulty in satisfying the requirement of the 1973 B.P. of "not less than 37°C".

Conclusion

A rationale has been given for the narrower range of values for the Evers turbidity temperature of arachis oil, supporting the view that the test should not be dropped from the B.P. monograph. The description of the test should be expanded, however, to include reference to stirring the test solution and the importance of viewing with side illumination against a dark background as an aid to reproduceability. A specific lower limit for the Evers turbidity temperature should be reintroduced, and the results of the present survey, together with the historical data presented, support a limit of "not less than 36°C".

The authors record, with gratitude, the influence of the late R. Sinar at whose suggestion this work was undertaken, and also thank the following for providing samples of arachis oil or groundnuts: M. Ranson, Ranson & Son Ltd; S. A. Wood, Bush Boake Allen Ltd; N. Nix, The Boots Company Ltd; R. E. Worthington, University of Georgia.

References

1. "The British Pharmacopoeia", vol. 1, HMSO, London, 1980, p. 38.
2. Evers, N., *Analyst*, 1912, **37**, 487–492.
3. Evers, N., *Analyst*, 1937, **62**, 96–100.
4. Bellier, J., *Ann. Chim. Anal.*, 1899, **4**, 4.
5. O'Connor, R. T., and Herb, S. F., *J. Amer. Oil Chem. Soc.*, 1970, **47**, 186A, 195A, 197A.
6. Spencer, F. G., Herb, S. F., and Gorminsky, P. J., *J. Amer. Oil Chem. Soc.*, 1976, **53**, 94–96.
7. Worthington, R. E., *J. Amer. Oil Chem. Soc.*, 1977, **54**, 167–169.
8. "The British Pharmacopoeia", vol. 2, HMSO, London, 1980, p. A107.
9. Chakravorty, K. L., *J. Assoc. Publ. Analysts*, 1980, **18**, 137–140.
10. "Official Methods of Analysis", 10th Ed. Association of Official Agricultural Chemists, Washington DC, 1965, 26.052.
11. "Official Methods of Analysis", 11th Ed. Association of Official Agricultural Chemists, Washington, D.C. 1970, 28.058.
12. Ettre, L. S., and Kabot, F. J., *J. Chromatogr.*, 1963, **11**, 114–116.
13. Iverson, J. L., Firestone, D., and Horwitz, W., *J. Assoc. Off. Agric. Chem.*, 1963, **46**, 718–725.
14. Ibarra, A., *An. Ass. Brasil. Quim.*, 1953, **12**, 151–155.
15. Worthington, R. E., and Hammons, R. O., *J. Amer. Oil Chem. Soc.*, 1977, **54**, 105A–108A.
16. French, R. V., *J. Amer. Oil Chem. Soc.*, 1962, **39**, 176.
17. Worthington, R. E., and Holley, K. T., *J. Amer. Oil Chem. Soc.*, 1967, **44**, 515.
18. Tortelli, M., and Ruggeri, R., *Zeit. Angw. Chem.*, 1898, 850–853.
19. Tortelli, M., and Ruggeri, R., *Chem. Zeit.*, 1898, **22**, 600–603.
20. Marcille, R., *14me Congr. Ind. Paris 1934, Chem. Abs.*, **29**, 6449.
21. Da Silveira, L., *Industria y Quim.*, 1942, **4**, 70–73.
22. De Mingo, M., and Romero, L. M., *Ion* 1943, **3**, 329–335.
23. Riggs, T. H., and Kirsten, G., *J. Assoc. Off. Agric. Chem.*, 1945, **28**, 293–295.
24. Brunetti, S. E., *Rev. Sanided y Asistencia Social (Venezuela)*, 1948, **13**, 486–494.
25. Leclercq, L., and Fagard, J., *J. Pharm. Belg.*, 1954, **9**, 325–330.
26. Amato, C., and Wohlers, de A. E., *Engenharia e. Quim.*, 1952, **4**, 7–13.

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Determination of Hydroxyproline in Meat Products: Collaborative Trial

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Twelve Public Analysts' Laboratories participated in a collaborative study of the British Standards Institution method for the Determination of L(−) hydroxyproline content of meat products. Repeatability and reproducibility have been calculated at three levels of hydroxyproline content.

The British Standards Institution (BSI) method for determination of hydroxyproline in meat and meat products[1] is widely used by Public Analysts to estimate collagenous connective tissue. The method specifies a precision requirement, viz: "The difference between the two calculated values obtained simultaneously or in rapid succession from the duplicate test portions by the same analyst shall not exceed 5 per cent. of the arithmetic mean value". There is however no record that the precision requirement was established from collaborative testing of the method.

Jonas and Wood[2] reported the results of a collaborative trial of the method involving 26 Public Analysts' Laboratories. Of the four samples analysed in their trial, only one had a hydroxyproline content within the range normally encountered in raw meats and meat products, i.e. 0·2 to 1 per cent. That particular sample contained a mean of 0·43 per cent. of hydroxyproline which gave rise on analysis to a calculated repeatability value of 30 per cent. (i.e. repeatability expressed as a percentage of the mean hydroxyproline content of 0·43 per cent). This conclusion served to indicate that the trial repeatability was much poorer than the internal repeatability achieved by Public Analysts within their own laboratories.

To re-evaluate the precision achievable using the method, the Analytical Quality Assurance Sub-Committee of the Association of Public Analysts decided to organise its own collaborative trial using the guidelines established by the Sub-Committee[3].

Samples and Organisation of Trial

The trial coordinators selected three proprietary pet foodstuffs to represent meat products in the trial at the hydroxyproline levels of interest viz:

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Sample	Manufacturer's stated ingredients	Approximate hydroxyproline content *per cent.*
A	Cereals, vegetable protein extracts, meat and animal derivatives, fish and fish derivatives, minerals, oils and fats, yeast, antioxidants, preservatives	0·1
B	Cereals, vegetable protein extracts, meat and animal derivatives, fats and oils, sugars, marrowbone, stabilisers, minerals, vitamins, preservatives, antioxidants, colourants	0·4
C	Animal protein concentrates, vegetable protein concentrates, cereals, beef, animal fat, minerals, colours, antioxidant	1·0

Each sample was ground and homogenised and subject to repeated analysis in the coordinating laboratory to verify homogeneity before distribution to the collaborating laboratories. The samples were despatched simultaneously by Data Post so that analysis by the twelve collaborators could be commenced on the same day. Analysts were asked to test each sample in duplicate and report analyses in the format laid down by the BSI method. In addition copies of calibration graphs were requested. The results are given in Table I.

TABLE I

HYDROXYPROLINE IN MEAT PRODUCTS: INDIVIDUAL RESULTS OF A COLLABORATIVE TRIAL

Laboratory	Sample A *per cent.*	Sample B *per cent.*	Sample C *per cent.*
1	0·14, 0·14	0·42, 0·44	1·00, 1·00
2	0·13, 0·13	0·43, 0·41	1·01, 1·03
3	0·13, 0·13	0·44, 0·44	1·03, 1·01
4	0·12, 0·12	0·42, 0·42	1·06, 1·02
5	0·13, 0·13	0·44, 0·44	1·01, 1·01
6	0·14, 0·14	0·43, 0·43	1·00, 0·97
7	0·14, 0·13	0·44, 0·42	1·01, 1·01
8	0·13, 0·13	0·43, 0·41	0·97, 0·97
9	0·13, 0·13	0·40, 0·42	1·00, 0·96
10	0·18, 0·16*	0·57, 0·56*	0·98, 1·03
11	0·13, 0·13	0·37, 0·40	0·94, 0·94
12	0·11, 0·11	0·40, 0·39	0·95, 0·93

* Rejected from data analysis (see text).

Statistical Analysis of Results

Analysis of the data in Table I was carried out using the procedures given in British Standard 5497—Precision of test methods[4]. For Sample A the results

from Laboratory 10 were classified as outliers by Dixon's test and Cochran's test and were rejected. The results from Laboratory 12 were classified as stragglers by Dixon's test but not noted by Cochran's test. These results were not rejected. For Sample B the results from Laboratory 10 were classified as outliers by Dixon's test and rejected. For Sample C no results were noted by Dixon's nor Cochran's tests.

Inspection of the calibration graphs supplied by participants indicated that Laboratory 10 had used calibration figures which were approximately 80 per cent. of those used by other laboratories. This offered an explanation of that laboratory's outlying data. It should also be noted that the BS test method requires that results are reported to two places of decimals. Sample A was found to have a mean hydroxyproline level of 0·13 per cent. Five per cent. of this mean is 0·0065 per cent. and for more precise estimation of repeatability at this level analytical data would have to be reported to four places of decimals.

The calculated values for repeatability and reproducibility are shown in Table II.

TABLE II

HYDROXYPROLINE IN MEAT PRODUCTS: MEAN RESULTS, REPEATABILITIES, AND REPRODUCIBILITIES IN COLLABORATIVE TRIAL

	Sample A	Sample B	Sample C
Mean hydroxyproline content (per cent.)	0·13	0·42	0·99
Repeatability			
Absolute	0·01	0·04	0·06
Expressed as a percentage of the mean	8	7	5
Reproducibility			
Absolute	0·02	0·06	0·10
Expressed as a percentage of the mean*	15	12	10

* Calculated from raw data.

TABLE III

EQUIVALENT CONNECTIVE TISSUE CONTENT OF MEAT PRODUCTS: MEAN RESULTS OF COLLABORATIVE TRIAL

	Sample A	Sample B	Sample C
Mean hydroxyproline content (per cent.)	0·13	0·42	0·99
Equivalent connective tissue content (per cent.)*	4·8	15·5	36·6
Repeatability expressed as equivalent connective tissue content (per cent.)	0·4	1·1	1·9
Reproducibility expressed as equivalent connective tissue content (per cent.)	0·7	1·9	3·7

* Wet fat free connective tissue = Hydroxyproline × 37.

Discussion

The B.S. test method specifies a repeatability requirement equivalent to 5 per cent. of the mean of two test results. Apparently this is arbitary and without any mathematical basis, and as such may not always be suitable. For the purpose of

enforcement analysis by Public Analysts the results obtained in the present trial demonstrate that the method can produce data of adequate precision. This may be more obvious if the results are translated into connective tissue content by using a conversion factor; Table III is based on the use of a conversion factor of 37 as we have previously proposed[5].

References

1. "British Standard Methods of Test for Meat and Meat Products. BS 4401, Part 11, Determination of L(−)- hydroxyproline content". B.S.I., London, 1979.
2. Jonas, A. D., and Wood, R., *J. Assoc. Publ. Analysts*, 1983, **21**, 113–121.
3. Lord, D. W., *Anal. Proc.*, 1984, **21**, 394–397.
4. "Precision of Test Methods". BS 5497, Part 1, B.S.I., London, 1979.
5. Lord, D. W., and Swan, K. J., *J. Assoc. Publ. Analysts*, 1984, **22**, 131–140.

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Collaborative Trials: Their Use and Abuse

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The design and operation of Collaborative Trials are discussed, with special reference to their value to Public Analysts and to the responsibilities of both the organisers and the participating laboratories.

Many Public Analysts have recently been involved in several[1,2,3,4,5,6] collaborative trials (referred to hereafter as Trials) of analytical methods for the determination of a component or components of foodstuffs and the like. Some Public Analysts have questioned the value of these Trials, requiring as they do the expenditure of much time by assistants whose primary function is the analysis of samples submitted officially by the Local Authority; this criticism can be answered only by consideration of the cost-effectiveness of these Trials. It may therefore be of value to discuss Trials in general from first principles, including the reasons for performing them; their value to analysts and in particular to Public Analysts; and the conditions to be observed by both organisers and participants if the results are to be of maximum benefit. It is assumed in what follows that the reader is acquainted with the fundamental principles of statistics and the meaning of such terms as "accuracy", "precision", and "standard deviation".

The Rationale of Collaborative Trials

Suppose that a new law requires a minimum content of a certain component C in a foodstuff F. Public Analysts now have the duty of determining the proportion of C in any sample of F officially submitted to them and of reporting the result. Suppose also that a method for doing this has already been published but until the new law came into force had received little attention. The actions that probably would (and certainly should) be taken by a Public Analyst are as follows.

1. The method is tested on a random sample of F, (a) to see if it "works", (i.e. it reveals no obvious practical snags); (b) to ascertain whether it can be completed in a reasonable time; and (c) to ensure that it gives a well-defined quantitative result that can be satisfactorily replicated. In other words, the method must pass tests for practicability and precision.

2. Unless the sample of F tested has a known C content (often this is not so in practice), he cannot tell whether the method is also accurate; the Analyst must then make "recovery tests" after addition of known amounts of pure C to the sample.

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3. Even if the method passes this test for accuracy also, this is true only for the one laboratory; two or more operators may have analysed the sample with satisfactory agreement between their results, but it is still possible that all the results were affected by a constant error, such as that due to an incorrectly standardised titrant.*

4. The Analyst then requests other Public Analysts to analyse sub-samples of the same sample by the same method and compare their results with his. Whether two or twenty-two laboratories participate, a Trial has been made.

The Value of Collaborative Trials

All these steps are essential stages in the evaluation of a method intended for use by the Public Analyst if he is going to inform his Local Authority that he is now ready to receive samples of F taken officially by the Sampling Officer and to report their content of C. For this implies that in certain cases (see below) he is prepared to issue an official certificate stating that a sample is deficient in C content to an extent that he knows is likely to result in the Local Authority taking legal proceedings against the vendor. The defence may then produce a certificate from another Public Analyst who has analysed the vendor's counterpart of the same sample and has found a higher C content than did the first Public Analyst. If the difference between the two is large enough to cause the Magistrates to dismiss the case, this will mean at the very least substantial damage to the confidence of the Local Authority in their Public Analyst. It is a major virtue of a Trial that each participating Public Analyst can feel confident that he will not find himself "out on a limb" if he gives official certificates of non-genuineness relating to samples of foodstuff F analysed by the tested method.

But this is only a qualitative approach. Whenever the law prescribes a minimum value for the content of a component in a food, the Public Analyst has to fix for himself a certain figure, less than the legal minimum, to divide the deficient samples on which he gives only an informal report from those so deficient as to justify a formal adverse certificate and consequent legal proceedings. If he chooses too low a value for this partitioning (let us call it the Criterion), too many manufacturers of inferior food will go unpunished; if it is too high, he will risk, as explained above, the possibility that a prosecution might fail. He needs quantitative data to assist him in solving his dilemma.

Use of Statistical Analysis

These data are obtained by the statistical examination of the numerical results of the Trial. A well-known example of historic interest arises from the determination of extraneous water in milk from its freezing-point depression. After what has been in effect a long series of Trials, the accuracy and precision of this measurement under carefully standardised conditions are known closely

* The author has recently become aware of a Trial in which the results of the participating laboratories agreed well with each other except that those from one were all about 20 per cent. different from the rest. This was traced to an inaccurate calibration graph; the error was unsuspected by the laboratory in question until the results from the Trial as a whole showed that an investigation was necessary.

enough to enable the risk of error in certifying non-genuineness to be stated within close limits; for example, the B.S.I. recommendation* that 0·525 m°C. should be taken as the smallest f.p.d. for a genuine herd milk is based on the calculation that the risk that a genuine milk will have a smaller f.p.d. is only 0·1 per cent.

In the general case in which a properly organised and performed Trial has been statistically analysed, two figures which between them summarise the principal characteristics of the data will emerge—the repeatability r and the reproducibility R. By definition[8], these terms mean that if duplicate analyses of the same sample are made by one operator in any one of the participating laboratories, on 19 out of 20 occasions the difference between the results will not be greater than r, whereas if the two analyses are made in different laboratories the difference will not be greater than R. In probability language, the risk of a greater difference will be 5 per cent. If a higher degree of confidence is required, the corresponding figures for a 1 per cent. risk are 1·4 times r or R; for a 0·1 per cent. risk the factor is 1·7.

Armed with this knowledge, the Public Analyst can work out for himself how to fix his Criterion. For example, an actual Trial[4] involved 18 laboratories that determined total fat in cocoa powder. The results from three laboratories were excluded on statistical grounds; those from the remaining 15 gave a mean of 15·95 per cent. and the values of r and R were respectively 0·55 and 1·18 per cent. The quantities r and R, being estimates of the error within and between the laboratories, are themselves subject to error, and using the knowledge that 15 laboratories were involved their confidence limits can be calculated; for 95 per cent. probability these are 0·4 to 0·9 for r and 0·9 to 1·6 for R. Nevertheless, useful deductions can be made from the mean values given above.

The law requires a minimum of 20 per cent. of total fat in the dry matter of cocoa. From r, the Analyst knows that in his own laboratory, 19 out of 20 duplicate analyses should agree within 0·55 per cent., and that any larger difference needs further investigation, the next obvious step being to have further analyses made. From R he knows similarly that single analyses in his and another Public Analyst's laboratory will not differ, in 95 per cent. of comparisons, by much more than 1·18 per cent. and in 99 per cent. by much more than $1{\cdot}4 \times 1{\cdot}18 = 1{\cdot}65$ per cent. If both laboratories make duplicate analyses, the means will differ only in 1 per cent. of cases by more than $1{\cdot}65/\sqrt{2} = 1{\cdot}16$ per cent. (when a sample is analysed n times in each laboratory, R should be divided by $\sqrt{n}$). Bearing in mind the "95 per cent. range" of R as given above, this is of course an approximation; for practical purposes one can say that if duplicate analyses were made in any two of the participating laboratories (and therefore presumably in any other two laboratories comparable in the accuracy and precision of their results) the difference between their respective means will be significantly more than 1 per cent. in only one in a hundred such cases.

Moreover, in about half these unusual occurrences it will not be our Public Analyst's but the other laboratory whose mean result will be the lower, so that the risk of being confronted by a higher result therefrom is only one in two

* See reference 7 for a discussion of this matter and of the discrepancy between British and American recommendations.

hundred. Again, no Public Analyst is likely to issue an offical adverse certificate without having the evidence of at least *three* analyses, so if he adopts a Criterion of, for example, 18 per cent., he will know that no other reputable laboratory is at all likely to report more than 19 per cent. This means that even if a Magistrates' Court decided to accept the evidence of the defence's analyst, a significant deficiency of fat would have been proved. It may be that the average Public Analyst would consider a Criterion of 18 per cent. to be over-cautious and would favour 18·5 per cent. The exact figure to be adopted is a matter for personal judgment; the choice, however, is not a mere guess, as it would be if no Trial had been made, but is based logically on quantitative evidence.

Increasing Cost-effectiveness by "Screening"

It is unfortunately true that to increase the reliability of a result, more work must be done; that all work costs money; and that all Public Analysts have costs in the forefront of their minds. It is logical to devise a routine whereby the less suspicious a sample, the less work is done on it. For some types of determination, all samples can be analysed once and the obviously genuine samples excused duplication. A reasonable routine in the "fat in cocoa powder" case might be—all samples analysed once; all under 20·0 per cent. analysed again (preferably by another operator); all then giving a *mean* result of 19·5 per cent. or less given further examination. In other types of test (e.g. heavy metals) a relatively imprecise but speedy method may be available for separating the obvious "sheep" from the possible "goats", the latter being examined further. All such procedures will much improve the cost-effectiveness of the laboratory.

Responsibilities of the Organisers of Trials

If the object of a Trial is to give participants an opportunity to test their expertise against their peers, then the more that join in the better. But if the object is to test the reliability of a method, then the need for cost-effectiveness requires some restriction on the number of participating laboratories. Two Trials each involving 10 laboratories would enable two methods to be tested with no more expenditure of analytical man-hours than one Trial involving 20, with very little loss of statistical information.

Homogeneity of the test sample before it is divided into sub-samples is vital, as are precautions against changes taking place in transit. It is implied in the design that the "between sub-samples" variance is nil, and any departure could seriously affect the "between laboratories" variance and consequently the value of R. Two Trials were recently performed by the same laboratories on different samples of the same food; the improvement in the agreement between laboratories was such as to suggest lack of homogeneity in the sample used in the first Trial.

It is also assumed that the "between methods" variance is nil, and every care must be taken to ensure that this assumption is valid. The method to be used must be specified in every detail, even when it is believed that variations in time, temperature, etc. are not of importance. Failure to realise this has ruined many a Trial in the past. Vagueness must be eschewed. Even the size of the vessel

when following an instruction like "boil vigorously for ten minutes" may be relevant, and what does "vigorously" mean?

Finally (under this sub-heading) the details of the method to be used should, whenever practicable (and sometimes it is not), be circulated with the invitations to participate, so that the laboratory chief can consider carefully what he is being required to do before he accepts the invitation. There may be something in the method—use of a particular instrument, for instance—that would prevent some laboratories from taking part. To know this at once is much better, for the organisers as well as for the laboratory concerned, than having to withdraw after having originally accepted the invitation.

Responsibilities of the Participating Laboratories

The head of an invited laboratory must give very careful consideration to the implications; acceptance is emphatically not a matter to be treated casually. Conscientious collaboration requires adherence to certain rules.

1. The method prescribed must be closely examined to ensure that it is one with which at least some of his analysts are familiar and also that the specification contains no requirement outside the scope of the laboratory. For example, the instruction "ash at 430 ± 10°C "would require the use of a thermostatically controlled furnace, and the specification must be adhered to rigidly or not at all.

2. The analyses must be made only by skilled and experienced operators. The results will have to stand comparison with those from the other laboratories; this is a challenge to be accepted or declined, not "fudged".

3. Entry to the Trial must imply the firm intention to carry out the whole of the work specified. Obviously, causes such as illness may make this impossible, and statisticians have methods for compensating for a few missing values; but to drop out completely halfway through the Trial means that the laboratory has completely wasted its own time and in part that of the organisers.

4. All analytical results must be reported to the organisers on completion of the work, without deletion or amendment on the grounds that "it doesn't look right". If more than one operator is taking part they must not be allowed to consult or compare results with each other.

5. "Blanks" must be carried through in full, the method being adhered to rigidly except that there is no sample. This is more important than it may seem; the results can sometimes be enlightening (see later).

All this costs more than using the Trial as an exercise for trainees; but if the job cannot be done properly, it should not be undertaken.

The Uses of Collaborative Trials

Trials have three main uses. First, each provides a searching test of the analytical method employed, and this is often the primary reason for arranging it, the organisers wanting the information before making an official recommendation or incorporating it into legislation. Secondly, it can give the Public Analyst useful help in fulfilling his duties, as we have seen. Thirdly, it enables

each participating laboratory to test its quality against its peers, and this deserves further comment.

The published report of a Trial should, and usually does, contain a table giving all the individual results from every laboratory taking part and a statistical analysis thereof. This begins by noting where it has been found necessary to exclude some results because they were found by a mathematical test to be "outliers", i.e. not consistent with the rest of the data. Occasionally (rarely, but it does happen[4]) all the results from one laboratory are excluded because they show divergence as a whole—usually, because they are all too high or too low. The remaining results are then used to calculate means, r, R, and any other relevant quantities.

The report thus provides all who read it with a picture not only of the value of the method used and the mean results from each sample but also of the expertise of the analysts. Any exclusion of results by the statistician lowers the prestige of the laboratories as a whole. If half or more of the data from one participant are excluded,—much worse, if they all are—this is a cloud the shadow of which falls on all the others. Every laboratory finding that some of its results have been discarded should take this as a reason for internal enquiry and as a warning—"could, and certainly should, do better".

Another Type of Trial

Sometimes a number of laboratories are asked to make certain determinations by whatever method each normally uses for that kind of analysis. One such Trial[9] asked for the determination of lead and cadmium in six samples of foods. Six Government laboratories, 22 Public Analysts, nine other British laboratories and one West German laboratory took part. The results showed a disturbingly wide range of figures. Taking as an example lead in cabbage, although the results from the six Government laboratories ranged from 0·23 to 0·41 mg/kg. dry weight, with a mean of 0·31, the range of the results as a whole was 0·08 to 0·68, and nine of them were 0·44 to 0·68—unacceptably high if the "official" data are accepted as regards accuracy and precision.

The authors of the paper state that the results taken together show "striking differences between the performances of the laboratories and there is considerable room for improvement". No information is given about the methods used by the laboratories, and the wide spread of the results could be accounted for by:

(a) the use by some laboratories of a method lacking accuracy and/or precision;
(b) variations in analytical skill between laboratories;
(c) unsatisfactory precautions against adventitious contamination by, e.g., dust or incomplete cleaning of vessels;
(d) insufficient attention to the necessity of careful determinination of the "blank".

The last two are well worth consideration; when one is determining a quantity of the order of 0·5 part per million, the extraneous presence of no more than 0·1 p.p.m. represents a 20 per cent. error. Be that as it may, there is an obviously

urgent need for inter-method comparative experiments leading to the establishment of a method of choice accepted by all Public Analysts (and, one would hope, by the Government and other laboratories as well). One wonders how many of the participants in the reported trial had ever previously checked their results in this field of analysis by comparison with any other laboratory on the same sample.

Conclusion

If properly organised and performed, Trials are a valuable source of external Quality Assurance—a subject much discussed these days[10] as an addition to whatever internal procedures are in use, for they supply objective information that is unobtainable any other way. Just as every laboratory manager must spend money on insurance against such risks as fire and theft, so insurance against the risk of issuing reports based on faulty analyses is not merely desirable but could on occasion even prove crucial.

The author showed the first draft of this paper to J. Markland and learned that the latter had addressed a meeting of the A.P.A. on this subject when he was Public Analyst for Derbyshire. The author is indebted to him for helpful comments and criticisms.

References

1. Reynolds, S. L., Thorpe, S. A., and Wood, R., *J. Assoc. Publ. Analysts*, 1983,**21**, 47.
2. Jones, D. A., and Wood, R., *J. Assoc. Publ. Analysts*, 1983, **21**, 113.
3. Crimes, A. A., Hitchcock, C. H. S., and Wood, R., *J. Assoc. Publ. Analysts*, 1984, **22**, 59.
4. Judd, H. J., Percival, M. D., and Wood, R., *J. Assoc. Publ. Analysts*, 1984, **22**, 81.
5. Cummings, J. H., Englyst, H. N., and Wood, R., *J. Assoc. Publ. Analysts*, 1985, **23**, 1.
6. Lord, D. W., and Swan, K. J., *J. Assoc. Publ. Analysts*, 1985, **23**, 71.
7. McIntosh, L. J., *J. Assoc. Publ. Analysts*, 1983, **21**, 77.
8. British Standards Institution, "Precision of Test Methods", B.S. 5497, Part 1. London, 1979.
9. Sherlock, J. C., Evans, W. H., Hislop, J., Kay, L. J., Law, R., McWeeny, D. J., Smart, G. A., Topping, G., and Wood, R., *Chem. in Brit.*, 1985, **21**, 1019.
10. Lord, D. W., *Analyt. Proc.*, 1984, **21**, 394.