FOREWORD

by the

President of the Association of Public Analysts.

This Journal is intended to fill a gap that has become increasingly apparent during the last decade. Analysts, consultants, chemists in the Food & Drug industries and others particularly interested in various aspects of the work of the public analyst, including agriculture and water, have told us that they miss the essentially practical interchange of ideas and information that was at one time possible, and how useful it would be to them if it were known what public analysts are thinking and how they are proposing to tackle the various problems that come their way.

The Association has done much since its inception in 1953 to foster a spirit of co-operation, negotiation and mutual understanding between those responsible for making the laws relating to food and drugs, those responsible for their enforcement and those who have to tread warily to avoid committing an offence. It has also helped in ensuring that public analysts speak with one voice on all subjects of major importance whilst retaining the individual approach which we regard as essential.

The co-operation between public analysts throughout the country has been encouraged by the distribution of the confidential Bulletin of the Association, circulated monthly to all members and, during the last year, of the separate Monthly Report which, not being confidential, has been read also by assistants in public analysts' laboratories. This Monthly Report, under the editorship of Dr. E.C. Wood, has formed the basis of this new quarterly Journal, which is available on subscription to all who are interested in and affected by our work, and particularly to our brother chemists and analysts in this country and, we hope, abroad.

The Journal will include analytical and technical contributions, correspondence, general items of interest, legal notes and reports of cases of special importance and, from time to time, the Official Views of the Association. Its pages are open to subscribers and others who wish to contribute and, indeed, the success of the Journal will depend on the degree to which these contributions are forthcoming. The conception of the Journal is that it should be a medium for the exchange of ideas, technical information, compositional data and analytical notes and methods of mutual interest to subscribers. This first issue is one-sided in that it contains only contributions from public analysts. A cordial invitation is extended to readers to redress this unbalance by the time the next issue is due.

D. D. MOIR.

SPECIAL ANNOUNCEMENTS

FOOD STANDARDS : OFFICIAL VIEWS OF THE ASSOCIATION

A General Meeting of the Association was held on the 11th October, 1962 on the subject of "Food Standards and Codes of Practice". The object of the meeting was to consider and, if thought desirable, to adopt certain Resolutions that had previously been circulated relating to minimum standards of composition for certain foods for which no legal standards exist. Each Resolution began with the words "This General Meeting resolves that the following be published as the official view of the Association - " and it was the intention that each Resolution adopted at the meeting be publicised by a statement in the Monthly Report of the Association and in other journals if necessary. Any Public Analyst asked for an opinion on these points could then not only put forward the standard contained in the Resolution but could also say that it was the official view of the Association and could if necessary prove this in Court by production of the relevant issue of the Monthly Report. Certain Resolutions were adopted, after discussion at the meeting, all unanimously or nearly so, and these are set out below.

1. Sausages and Sausage Meat

   (a) Meat Content Foods described as Pork Sausage(s) or as Pork Sausage Meat should have a meat content of not less than 65 per cent. Foods described as Beef Sausage(s) or as Beef Sausage Meat, or as Sausage(s) or as Sausage Meat should have a meat content of not less than 50 per cent.

   (b) Fat The lean fat-free meat content must not be less than half the required minimum meat content, that is, not less than $32\frac{1}{2}$ per cent or 25 per cent as the case may be.

2. Luncheon Meat

   Products sold under any description which includes the words "Luncheon Meat" or implies that the product is being sold as luncheon meat should have a meat content of not less than 80 per cent. 'Meat Content' means the percentage by weight of all meat including meat fat, calculated as raw meat, in the cooked luncheon meat.

   'Meat' includes pork, bacon, ham, beef, mutton and veal, but does not include offals defined as "prohibited offals" by the Offals in Meat Products Order, 1953, namely, brains, feet, fries, gut (including chitterlings), manifolds, paunches, udders, sweetbreads, tripe, melts or lites, spinal chord, uteri, pigs' maws, and calves' vells.

(Note - an agreement in exactly the above words was made in 1959 between the Food Manufacturers' Federation Inc. and the Association of Public Analysts and has been operative since the 1st October, 1959).

3. Flavoured Milk

Foods described as Flavoured Milk should contain not less than 85 per cent of Full-Cream Milk (complying with the Food and Drugs Act, 1955) and not more than 15 per cent of any of the following or of a mixture of them - sugar, liquid glucose, fruit juices and fruit acids, added flavouring, added colouring. The additions to be incorporated in no more water than is reasonably necessary to ensure their solution. Any label to bear a declaration of ingredients and a statement of the minimum volume of the contents.

4. Marzipan, Almond Paste

Foods described as Marzipan or Almond Paste, other than those sold as sugar confectionery, should contain at least 25 per cent of ground almonds ($23\frac{1}{2}$% dry substance) and of the rest not less than 75% should be sugar solids.

5. Candied Peel, Cut Peel

Foods described as Candied Peel or Cut Peel should contain at least 64 per cent of soluble solids as determined by refractometer at 20° C.

(Note - an agreement to this effect was made between the F.M.F. and the A.P.A. at the end of 1957. As regards the method of determination, see below).

ANALYTICAL AND TECHNICAL CONTRIBUTIONS

(A Note from Council)

It will be remembered that in 1958 agreement was reached between the Association of Public Analysts and the appropriate section of the Food Manufacturers' Federation that Candied Peel and Cut Mixed Peel should contain not less than 64 per cent of soluble solids as determined by refractometer at 20° C. The agreement has worked well; but recently some queries have arisen as regards the correct way to take the reading. This point was not mentioned in the agreement. It is always possible to cut a thin slice with a razor and place it directly between the prisms, but some analysts squeeze out a drop of syrup by pressure and take a reading on this. D.G. Forbes has examined 17 samples by both methods. Agreement between them was in general good; the slice method gave the higher

reading on 11 samples and a slightly lower reading on 3 samples; while on the other 3 the readings were the same or practically so.

The Standards Committee, having considered these results, have recommended - and Council have approved the recommendations - that the slice method should be used in all cases.

DETECTION OF INSECTICIDE RESIDUES IN FRUIT AND VEGETABLES

by P.S. Hall

We had in the Presidential Address last year a full account of the problem raised by the use of the new Organic Pesticides. The object of this meeting is to take the discussion further with an account of some preliminary investigations which we have carried out in this field. We intend this afternoon to confine the discussion to insecticides alone, since we feel these constitute the main hazard to the consumer.

The common types at present in use can be broadly classified into chlorinated hydro-carbons which have found considerable use as seed dressings. This application has, however, been now much curtailed following the widespread deaths to wild-life which were traced to their use: and the organo-phosphorus pesticides, the earlier types of which were based on derivatives of pyro-phosphoric acid such as schradan, but are now mainly based on ortho-phosphoric acid derivatives. The detection and determination of these latter compounds is complicated by the series of toxic metabolites which they form in the plant, these metabolites being sometimes more effective than the parent compound.

Faced with the problem of testing for such a variety of chemicals, the cry of the Public Analyst will be - 'Show me a simple Sorting Test'. Clearly one simple chemical test for such a varied assortment of compounds is not possible, but a biological test might well provide the answer. Quite a lot of work has already been published[1] on the use of biological methods in pesticide residue analysis. Most of this work, however, has been concerned with the determination of the amount of a known pesticide. The Public Analyst is more concerned in the first place with the development of a simple Sorting Test to determine whether his sample contains pesticide residues at an objectionable level, and is not in the happy position of knowing the identity of the pesticides concerned.

The published work, however, does indicate that the choice of organism, bearing in mind ease of breeding and susceptibility, lies between Drosophila melanogaster, Aedes

aegypti and Daphnia with Drosophila being the first choice on both counts. Unfortunately this species is not ideal, since it appears that it shows low susceptibility towards some insecticides. It might be advisable, therefore, to use a second organism. We have experience of Drosophila only, so I will confine my remarks to a description of our methods using this organism.

Breeding of Drosophila

The flies are bred in jars containing a suitable medium in the bottom. The dual purpose 'Kilner' two pound jar is very satisfactory for this purpose. This type has a metal lid and a hole about one inch in diameter is cut in the lid and closed with a cotton-wool plug.

Many different formulae have been proposed for the breeding medium. A typical one is shown below:-

Agar	25 g.
Maize Meal	500 g.
Sugar	250 g.
Yeast (Dried, Brewers') .	31 g.
Nipagin M in alcohol	20 ml of 10%
Water	2.4 litres

The water is heated and when it is nearly boiling, the agar (soaked in a little cold water) is added. The mixture of maize meal, sugar and yeast is then added slowly, stirring to remove lumps. The mixture is allowed to boil for ten minutes. The preservative solution is then added and thoroughly stirred.

To prepare the culture jars the liquid medium is poured into a jar to a depth of approximately one inch and allowed to cool. It is advisable to pour the medium into a warm jar to prevent condensation of steam on the inside of the jar.

A much simpler medium, especially when breeding is carried out on only a small scale, is mashed banana. Plain mashed banana is frequently found to be rather too wet. This can easily be overcome by incorporating a sufficiency of maize meal. The mixture is preserved with 0.1% methyl para hydroxy benzoate.

The cultures are kept at a temperature of 25° C, and at this temperature the development time from egg to adult is about 9 - 10 days., i.e., egg... 1 day; larva... 5 - 6 days; pupa... 2 - 3 days.

It is advisable to sub-culture, i.e. transfer all the adult flies into a fresh clean jar every 2 - 3 weeks.

Handling

Some workers find it necessary to anaesthetise the flies for handling purposes, but this practice is open to much criticism and we find it quite easy by means of a funnel inverted over the hole in the top of the jar to transfer and even count flies passing into the test jar.

Detection of Insecticide Residues using Drosophila

It is possible merely to expose the flies to a pulp of the sample under examination. This method, however, is much less sensitive than exposure to the dried residue obtained from a preliminary solvent extraction. If the first method is used, it is necessary to incorporate a little preservative in the pulp to prevent mould formation.

Extraction Procedure

The recent paper by Laws and Webley[2] indicates that the use of dichlormethane as an extraction solvent may be almost universally applicable to the extraction of pesticide residues. This solvent is therefore used and the procedure is as follows. A representative sample of the fruit or vegetable is prepared by slicing or grating, and after well mixing, a 50 g. portion of this is transferred to a macerator. 100 ml. of dichlormethane is added and the whole macerated for about 15 minutes. The mixture is filtered through a Buchner funnel and the solid on the filter pad is washed with 50 ml. of dichlormethane. The filtrate is transferred to a separating funnel and the organic layer is run off, the aqueous layer being rejected. The dichlormethane solution is reduced in bulk by heating on a hot plate in a current of air and is then transferred to a 100 ml. volumetric flask and made up to the mark.

Clean-up Procedures

Several reports suggest that certain vegetable extracts may show toxicity to the test organism. With the crops with which we have worked we have no experience of this occurring. It is undoubtedly true, however, that the sensitivity of the test may be seriously affected not only by the weight of residue in the test jar, but also by its character. Thus the sensitivity of the test using one crop may be quite different from that using another. It is therefore necessary to carry out parallel control tests using an extract of the type of crop under examination, but which is known not to have been treated with the pesticide.

The Public Analyst is faced with two difficulties:-

(1) that of obtaining a specimen of crop known not to have been treated

and (2) with the fact that he will be dealing with a large number of different crops.

The first difficulty can be solved fairly simply by exposing an extract of the proposed control crop to the test organism for a prolonged time, say 3 - 4 days. If no significant deaths occur in this time, it is reasonable to suppose that the crop is satisfactorily free and may be used as a control. The second difficulty has to be accepted.

When searching for low levels of pesticide below about 1 p.p.m., the presence of the residue may so reduce the sensitivity of the test that a clean-up of the extract becomes necessary.

Tew and Sillibourne[3] working with cyclodiene insecticides, pass the extract through a column of magnesium oxide - celite. It is unlikely that this procedure is universally applicable but Laws and Webley[2] describe a clean-up procedure which appears to be applicable to most of the common insecticides.

Method of Test

The test jar consists of a 4 oz. wide-neck bakelite capped jar. A hole $\frac{1}{2}$" in diameter is cut in the bakelite cap and plugged with cotton wool. A small aliquot of the extract solution is pipetted into a jar, which is then rotated, causing the extract to evaporate on the walls of the jar, it being so manipulated as to achieve an even film of extract over the wall. The jar is left on its side for 10 - 15 minutes to ensure that all traces of solvent have evaporated, and the top is then screwed in place. About 20 flies are introduced into the jar by means of a funnel as previously described, and the hole in the cap is then plugged with cotton-wool which has been saturated in 10 per cent. solution of honey and squeezed out.

The jar is examined at intervals of 10 minutes for the first hour, and thereafter at hourly intervals for the next 5 hours, and then at convenient intervals up to 3 days.

The jar is maintained at about 25° C throughout the test. It is our experience that if no more than 1 or 2 deaths occur in this time the level of pesticide residues is satisfactory.

It is the usual practice to count "knock down" rather than death. It is quite easy to differentiate flies resting on the side of the jar from those which have been knocked down merely by rotating the jar. Flies which have succumbed, or are quite dead, will roll around inside the jar and can be quite easily counted. Some of those suffering the effects of the poison will show typical spasms of the limbs on being disturbed.

At the end of the test all flies are killed to enable the total number in the jar to be counted.

It is unwise to recover survivors of a test since they

may eventually breed a strain which has acquired tolerance.

Typical results on four common insecticides showing the time for 50 per cent mortality are shown in the following table:-

Insecticide	µg. per jar	L T 50
S 1752	2	25 mins.
	1	50 mins.
Demeton Methyl	2	23 hrs.
D.D.T.	25	120 mins.
	250	85 mins.
Dieldrin	1	200 mins.

L D 50 figures for 24 hours exposure for two insecticides to which Drosophila are least sensitive, namely D.D.T. and Demeton Methyl are shown below:-

Insecticide	L D 50
D.D. T	2 µg.
Demeton Methyl	2 µg.

These figures are of the same order as thos quoted by Tew and Sillibourne[3].

A positive reaction indicates the presence of some insecticidally-active material, and further examination by chemical methods becomes desirable. Such further examination falls naturally into two parts:-

(1) Examination for organo-phosphorus insecticides.

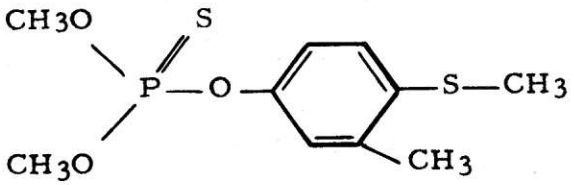
(2) Examination for organo-chlorine insecticides.

Examination for organo-phosphorus insecticides

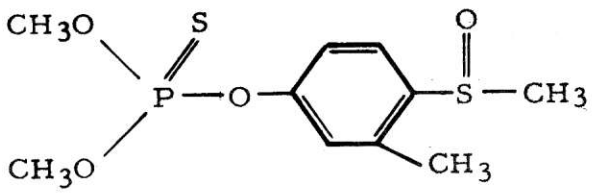
Laws and Webley[2] in their recent paper describe a general method for insecticide residues. This consists of an initial extraction with dichlormethane, which is then removed by evaporation, the residue being partitioned between Light Petroleum and aqueous methanol. The petroleum-soluble fraction is purified by chromatography on alumina and the eluate is examined for organic phosphorus. The active residues in aqueous methanol are extracted into chloroform and this extract is purified by chromatography on active charcoal, and also examined for organic phosphorus.

As a basis for a general examination of organo-phosphorus insecticides, this procedure has much to recommend it, but in certain cases an attempt to classify the unknown insecticide in the form given by Laws and Webley[2] in their paper may be misleading. The reason for this is that organophosphorus pesticides break down in the plant to a series of biologically-active metabolites. These metabolites do not

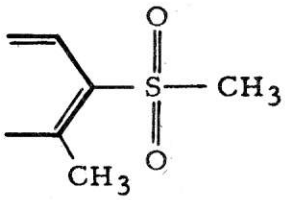
always behave physically and chemically as the parent compounds, e.g., the compound S 1752, which has the composition:-



is shown by Laws and Webley[2] as appearing in the petroleum-soluble fraction in their procedure. Such compounds in vivo are known to metabolise within 24 hours mainly to a sulphoxide:-



and also the sulphone:-



and the oxygen analogues of these compounds.

We have shown that these compounds appear in the aqueous methanol phase and an examination therefore of a fruit treated with S 1752 would show the presence of water-soluble organo-phosphorus compounds and very little petroleum-soluble compounds. In fact, if 28 p.p.m. of S 1752 and 22 p.p.m. of metabolites are present on strawberries, only 2 p.p.m. of S 1752 remain 5 days later, but 37 p.p.m. of metabolites are present; after 12 days the amounts are 1 and 12 p.p.m. respectively.

It is worth mentioning here that the standardisation of the adsorbents used in chromatography is most important. The alumina should be acid-washed, not the slightly alkaline material usually supplied. We find it very useful to use the Brockmann Grading[4] of alumina, which is very simply carried out. A Grade 5 alumina appears to give satisfactory results, and the method given by Laws and Webley[2] for the preparation of the active charcoal should be carefully adhered to.

Once again when residues of the order of less than 1 p.p.m. are expected, control samples are necessary since although the clean-up procedure described removes much of the naturally occurring phosphorus compounds, small traces still remain and an allowance must be made for their presence.

Identification of Organo-Phosphorus Insecticides

A number of papers[5, 6] have been published claiming that it is possible to identify and also to estimate organo-phosphorus insecticides by paper chromatography. Information derived from such tests it seems must always be of doubtful value because of the numbers of metabolites usually produced by these compounds. Several of these insecticides do, of course, give colour reactions which, though not always specific, may give some guide to their identity.

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THE DETERMINATION OF CHLORINATED PESTICIDE RESIDUES

by J.A. Potter

My colleague has described biological sorting tests for pesticide residues and the determination of organo-phosphorus compounds. I propose to deal with the residues of chlorinated pesticides.

In the event of a positive result being obtained in the Drosophila test we adopt the following procedure:-

(1) Extraction of the pesticide from the sample.
(2) Determination of the total organic chlorine, using the micro-Stepanow Method, and mercuric oxycyanide titration, and, if a significant quantity of organic chlorine is found:-
(3) Identification and semi quantitative determination of the pesticide using paper chromatography.

In most methods the pesticide is stripped from the sample by a solvent e.g. benzene, cyclohexane, petroleum ether and acetone, dichlormethane, etc. Very often in extracting large amounts of plant and animal tissue one obtains a solution containing pigments, oils and waxes the presence of which may well interfere with the analysis. The analyst then has recourse to a clean-up procedure which generally consists of adsorption chromatography. Unfortunately, however, no

single adsorbant exists that is suitable for all chlorinated pesticides. For instance, magnesium trisilicate can be used successfully for D.D.T., Lindane, Chlordane, Heptachlor and Heptachlor Epoxide while Aldrin is only eluted partially and Dieldrin and Endrin not at all. Another technique, in use at the Government Laboratory to clean up D.D.T., Lindane, Chlordane, Heptachlor and Heptachlor Epoxide involves celite and fuming sulphuric acid. The method employed by the Pesticide Sub Committee of the A.M.C. of the S.A.C.[1] uses activated alumina. This adsorbent works well with D.D.T. residues but is useless if applied indiscriminately.

An alternative clean-up procedure however consists of separation of the pesticide from the sample by steam distillation.

Gunther and Blinn in their book "Analysis of Insecticides and Acaricides" published in 1955, observed that "Aldrin, Chlordane, Heptachlor and Lindane are readily steam volatile" and Davidow and Sabatino in a paper published nine years ago[2] recommended steam distillation as a preliminary in a biological screening test (they actually used goldfish) for the detection of D.D.T., Lindane, Heptachlor, Dieldrin, Chlordane, Toxaphene and Aldrin. They found that all these pesticides could be recovered by steam distillation from a wide range of products including fruit, milk and feeding stuffs. Thus there is nothing novel in the idea, although it seems to have been strangely neglected. The simplicity of the technique is an advantage in laboratories where large numbers of food samples have to be examined for pesticide residues.

The procedure we have used consists of steam distillation, solvent extraction of the distillate, and identification and estimation by paper chromatography. It should be noted that steam distillation can be satisfactorily employed with most foods except those with a very high fat content.

Davidow and Sabatino used 100 g. of sample in a 500 ml flask and found that virtually all the pesticide came over in the first 200 ml. We actually use 50 to 100 g. of sample in a 1 litre flask and distil over about 500 ml. It is most important to use an all-glass steam distillation apparatus. The distillate is then extracted three times with 100 ml of ether, the ether extract washed twice with glass distilled water and the ether evaporated off carefully. It is convenient to use a Kuderna-Danish[3] evaporative concentrator, fitted with a 50 ml flask.

The whole of the extract or an aliquot can be taken for the micro-Stepanow determination. We broadly follow the procedure given in the report of the Pesticides Sub Committee[1] which is briefly as follows:-

To the residue in the flask add 4 ml of isopropanol and 0.3 g. of sodium, boil under reflux for half an hour on a micro-burner, then add 2 ml of 50 per cent isopropanol. When all the sodium is dissolved, discontinue heating and rinse the condenser with 5 ml of distilled water. It is essential, of course, to run a blank at the same time. Cool the flask, (this is where we prefer to follow Appendix 2 in the 1957 Method), acidify to methyl red with 3 N sulphuric acid and extract with ether. Run off the aqueous layer into the original flask, make just alkaline and boil down to about 10 ml. Transfer to a 25 ml conical flask, make just acid with 0.01 N H_2SO_4, boil to expel CO_2, cool, add 2 ml of neutralised 2% mercuric oxycyanide and titrate with standard sulphuric acid to the original tint. Recognition of the end-point is more readily achieved by comparison with a reference flask containing boiled distilled water plus methyl red. We found the end-point very difficult using standard sulphuric acid weaker than N/250. Blanks vary but are usually not more than 60 micrograms.

Some years ago we attempted to use a colorimetric method instead of the oxycyanide titration. We tried to adapt the method of Swain[4] for determination of traces of chloride in boiler waters using mercuric thiocyanate. After an encouraging start we ran into difficulties and could not get consistent results. However, Sergeant and Thompson claim to have successfully modified the Swain method. We have not tried their method[5] but if it is satisfactory it has obvious advantages over the titration method.

Now we come to the identification of pesticides by paper chromatography. L. C. Mitchell has published a long series of papers on the paper chromatography of pesticides in the J.A.O.A.C. and in his 1958 publication[6] will be found R F values for 114 pesticide chemicals of all types. Other workers, mainly in the United States have published papers based on Mitchell's technique[7, 8, 9, 10, 11] which utilises reverse phase paper chromatography, with two solvent systems, aqueous and non-aqueous. We have had no experience of the latter but understand from the Government Laboratory that it shows no advantages over the aqueous system. The general principle consists of development by upward displacement of the pesticides spotted on paper impregnated with liquid paraffin or soya bean oil followed by some form of treatment to render the spots visible. The treatment is aimed at dechlorinating the pesticides, treating with silver nitrate, and exposing to sunlight, or ultra-violet light. In the earlier papers a variety of chromogenic agents was employed e. g. spraying with methanolic potassium hydroxide in silver nitrate or spraying with silver nitrate, formaldehyde, potassium hydroxide and finally with concentrated nitric acid and 30% hydrogen peroxide. Mitchell, however, has

introduced a single chromogenic reagent solution which is more effective as well as being more pleasant to use. Moreover, spraying seems to have gone out of fashion, and it is generally agreed that dipping is more satisfactory. Furthermore, the need for stocking a large selection of spraying bottles is avoided.

The procedure we have adopted is as follows:-

We use Whatman No.1 paper 15 cm wide which can be bought in 100 metre rolls. We cut 10" strips, rule a starting line with pencil 1" from the end, and spot on between 5 and 10 micro-litres of an ether solution of the residue isolated by steam distillation. R F values vary somewhat between runs and so it is important to run standards alongside the samples before drawing any conclusions as to the pesticide present. When the samples and standards have been applied, the paper is then subjected to a quick dip in a 10% solution of liquid paraffin in ether. After two or three minutes drying in air the paper is suspended by clips in a chromatographic tank and eluted with 70% aqueous acetone. After development, which takes 4 to 5 hours, the paper is removed and dried thoroughly with a hair dryer. It is advisable to hold the paper at the top only, since the chromogenic reagent readily reveals fingerprints. When the paper is dry it is immersed in the chromogenic solution, the composition of which is as follows:-

Silver nitrate	3.4	g.
Water	130	ml.
2-phenoxy-ethanol	20	ml.
Ethanol	50	ml.

If necessary, add more ethanol to obtain a clear solution. This solution should be kept in the dark. It keeps for several weeks and tends to darken though it still remains effective. Some people recommend the addition of a drop of 30% hydrogen peroxide.

The paper is dipped in the chromogenic solution just as quickly and evenly as it was dipped in the liquid paraffin-ether solution. We use rectangular polythene developing dishes for the purpose. The chromatogram is then dried and subjected to ultra-violet light until the spots develop. It is essential to use low wave length ultra-violet light (2537 A°) such as is obtained from a Phillips TUV 15-watt germicidal strip lamp. The paper is irradiated at a distance of about 10 to 15 cm from the tube, which should be well screened to prevent exposure to the eyes and skin. After an interval of ten minutes or so the paper is turned over and irradiated on the reverse side. By 20 to 30 minutes the spots should have fully developed. Most workers recommend that the papers be washed before use, either simply with water, or with silver nitrate solution, followed by ammonia and finally by water. We have tried using washed papers but we

decided that the results obtained were not worth the additional bother. In any case the inorganic chloride on the paper migrates with the solvent front and since none of the pesticides examined has an R F approaching 1, the resulting brown mass at the top of the paper does not obscure anything of value. Specimen R F values are as follows:-

Aldrin	0.15
Heptachlor	0.19
D.D.T.	0.24
Dieldrin	0.32
Heptachlor Epoxide	0.35
Lindane	0.48

I should mention that sometimes steam-volatile fatty acids distilled over from the sample may interfere on the chromatogram. We encountered this difficulty on steam distilling some samples of feeding stuffs, actually poultry mashes with a high free fatty acid content. The developed chromatogram showed very bad streaking of the sample spots. However, we found the difficulty could be overcome by a rapid wash of the ether solution with 1% NaOH, which removed the acids. We found that this treatment did not affect the recovery of 50 ug of added D.D.T., Lindane, dieldrin, aldrin, and heptachlor.

As little as one ug of the pesticide can be readily detected but between 5 and 10 ug is the desirable range for matching the intensity of the spots with the standards. The chromatograms keep for a few weeks if left in the dark and their life may be extended by washing with 1% thiosulphate solution followed by water.

I have quoted R F values for only six chlorinated hydrocarbons, those probably most often encountered in residue analysis. There remain of course many other possible substances, some of which have been investigated by Evans of the Government Laboratory[11], e.g., pp'DDE, pp'TDE, Endrin, Methoxychlor, Endosulfan A, Endosulfan B, and Endosulfan Alcohol.

It is unfortunate, but hardly surprising, that the one solvent used, 70% acetone, fails to separate all 13 pesticides, (including metabolites), that have so far been mentioned. Evans quotes three pairs with coincident R F values;

Dehydrochlorinated pp'TDE and heptachlor
Endosulfan A and endrin
Endosulfan B and gamma - BHC.

Obviously new solvent systems will have to be found to separate these substances.

Another point of interest emerges from Evans' work; endosulfan alcohol migrates with the solvent front, a fact which would seem to make the use of washed paper advisable.

I have tried to describe a procedure for the determination of pesticide residues that would be useful for small laboratories. The attractive technique of gas chromatography using the electron-capture detector is, however, an obvious tool for those who possess the necessary equipment. This application has been developed by E.S. Goodwin et al[12].

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THE DIFFERENTIATION OF CANNED PROCESSED AND CANNED GARDEN PEAS

by A. Houlbrooke

Part IV of the Labelling of Food Order, 1953, provides that Canned Processed Peas must be labelled as such and that Canned Peas described as "Fresh", "Garden" or "Green" must not consist of Processed Peas. As is usual in such Orders and Regulations there is a careful avoidance of the admittedly tricky problem of precise definition. Nevertheless, the Public Analyst is left to tackle the problem of deciding, as he must, how to differentiate between these two classes of peas.

In this laboratory this particular duty became inescapable in 1957 when it was asked to determine whether a certain sample of "Canned Peas" consisted of processed or garden peas. It was decided to seek advice from the Director of the Fruit and Vegetable Canning and Quick Freezing Research Association at Chipping Campden. Very useful information was most willingly provided by the Director, Mr. W.B. Adam, and this is gratefully acknowledged.

The difference between processed and garden peas lies in the degree of maturity. Processed peas are reconstituted dried peas and are, therefore, at their final stage of maturity while most garden peas are canned in a reasonably immature state. With increasing maturity both the Alcohol-Insoluble Solids and the Total Solids increase. Up to 1957 Chipping Campden had found that the A.I.S. of canned garden peas of the wrinkled variety ranged from 11% to 16% though with a possible maximum of 20%. The rounded variety of peas gave an appreciably higher figure which was as much as 25%. The A.I.S. of canned processed peas was found to be 26% to 30%. The Total Solids are higher than the Alcohol-Insoluble Solids, the difference being about 10% at the recommended canning stage for green peas but falling to 4% to 6% when the peas are substandard as regards maturity. The T.S. of wrinkled varieties rarely exceed 26% for canned garden peas, whereas the normal values for canned processed peas may rise to 28% to 34% or even higher.

Since receiving this information from Chipping Campden in 1957, 66 samples of Canned Peas have been submitted to this Laboratory under the Food & Drugs Act 1955. A.I.S. and T.S. have been determined on all samples with the following results:-

		A.I.S.	T.S.
Garden Peas (42 Samples)			
	Average	14.8%	19.8%
	Range	10.1% to 19.7%	13.2% to 24.6%
Processed Peas (24 Samples)			
	Average	25.1%	28.8%
	Range	21.8% to 27.0%	26.1% to 30.6%

These figures agree reasonably well with those obtained previously at Chipping Campden. It is of interest to note that, in the comparatively small number of samples examined, there is no overlap of figures for the two groups. It is possible that the garden peas examined consisted almost entirely of the wrinkled seed types and that, had a significant proportion of rounded seed types been examined there would have been some overlap in the A.I.S. and T.S. figures. The U.S. Food and Drug Administrative Regulations relating to Canned Peas do not appear to

differentiate between "Garden" and "Processed" but do fix maximum figures for A.I.S. of 23.5% for smooth skin varieties and 21.0% for wrinkled varieties.

The methods used for determining A.I.S. and T.S. were kindly supplied by Mr. Adam and are given below. The method for A.I.S. determination is substantially the same as that laid down in the U.S. Regulations and quoted in "A.O.A.C. Methods of Analysis" as well as in Jacobs' "The Chemical Analysis of Foods and Food Products".

Preparation of Sample

Drain the liquid from the peas on a suitable sieve. Wash in water and drain again. Wash and drain finally for two minutes. Pass the peas twice through a mincer and place immediately in a screw-capped jar.

Total Solids

Weigh duplicate samples of about 10 g into flat-bottomed glass dishes provided with rods. Spread the weighed sample into as thin a layer as possible to facilitate drying. Dry in steam oven at 96° - 98° overnight (or to constant weight). Duplicate analyses should agree within 0.25%.

Alcohol-Insoluble Solids

Weigh 10 g into a small beaker. Transfer with 150 ml of 80% alcohol to a 250 ml flask and boil under reflux for 30 minutes. Filter with suction, using a Buchner funnel, through a 9 cm No.1 Whatman filter paper which has been dried at 98°-100° C and weighed after standing for 30 minutes in a desiccator. Wash with 80% alcohol until the washings are colourless. Dry filter paper and residue at 98° - 100° C for 2 hours; stand for 30 minutes in a desiccator and weigh.

ANALYTICAL DATA FOR GRAPEFRUIT JUICE

by J.G. Sherratt and R. Sinar

In the last issue of the Monthly Report, J.H. Hamence gave analytical data for Israeli grapefruit juice. We have examined juice extracted in the laboratory from whole fruit from various countries. The samples marked with an asterisk in the following table were Israeli grapefruit flown in and sent on to us by the importers immediately they arrived in this country.

Members may find the figures for sodium in the juice have some interest, since the concentrations of this element seem in general to be lower than is found in most natural waters.

Potassium, Sodium and Chlorine in Grapefruit Juice

(Milligrammes per Litre)

Country of Origin	Potassium as K	Sodium as Na	Chlorides as Cl
Trinidad 5 samples	740 - 1200 mean 1052	6.5 - 11 mean 8.5	24.8 - 74.5 mean 39.7
Cyprus 4 samples	1320 - 1540 mean 1445	6.5 - 12.5 mean 9.5	30.7 - 49.7 mean 36.3
S. Africa 6 samples	1175 - 1600 mean 1358	3.5 - 8.5 mean 6.0	39.0, 28.4 (2 only)
Cape 1 sample	1420	5	32.0
Israel 12 samples	1180 - 1880 mean 1406	5.0 - 23.5 mean 11.4	25.0 - 142.0 mean 61.6 (9 samples)
*12 samples	1180 - 1680 mean 1418	5.5 - 12.0 mean 7.7	-

* Analysis of mixed juice of strained samples:-

Ash	0.32% w/v
Alkalinity per 100 ml.	35.0 ml N/10 HC 1

(Editor's Note. The data from Hamence referred to by Sherratt were contained in a note as follows.)

Several members have been concerned at the analytical characteristics of grapefruit juice from Israel during 1961/62, and there has been correspondence between the A.P.A. and the Israeli Government. Figures for phosphoric acid, potash, etc. have been very low compared with those for grapefruit juice from other sources. This season's grapefruit have now become available in Israel, and J.H. Hamence arranged for six fruits to be flown to his laboratory to minimise any possible drying out. They were divided into two samples of three each. The juice was squeezed out and submitted to the usual analysis, with the following results:-

	Sample A per cent	Sample B per cent
Percentage of Juice in Fruit	42.0	43.8
Total Solids in Juice	9.16	8.64
Invert Sugar	3.89	3.70
Cane Sugar	2.87	2.71
Total Sugars	6.76	6.41
Acidity (as Citric Acid)	1.74	1.71
Mineral Matter (Ash)	0.33	0.33
including -		
Phosphoric Acid (P_2O_5)	0.032	0.032
Potash (K_2O)	0.172	0.172

THE DETERMINATION OF THE ALMOND CONTENT OF MARZIPAN

by H. E. Monk

A difference of opinion on a recent sample of marzipan has led to some reconsideration of the problem of determining its almond content. It was certified as 22 per cent in this sample and a higher figure was given in a report produced by the makers. This said inter alia that the minimum oil content quoted in the literature was 41.6 per cent : that this figure is exceptional and normal minimum figures are higher than this, the various authorities quoting 44, 47.1 and 48.9 per cent. The suggested criterion was 55 per cent oil for air-dried almonds with a moisture content of about 6 per cent. The maximum figure recorded in the literature was stated to be about 60 per cent. For protein, the minimum figures quoted have been between 16.6 and 18.7 per cent and the suggested protein figure to be used for calculation is 21.0 per cent.

For about the last ten years it has been my practice to multiply the oil content of marzipan by 1.7 and the protein content by 4.35. By this means one usually obtains two closely agreeing figures and the average of the two has been the percentage of almond content certified.

The statement produced on behalf of the firm led to a search of literature available here and also examination of our own records:-

COMPOSITION OF ALMONDS

Source	Ref.	per cent Oil	per cent Protein
Jacobs	1	54.1	18.6
Geigy	2	54.1	18.6
Wooster and Blanck	3	54.1	18.6
Sherman	4	54.1	18.6
McCance and Widdowson	5	53.5	20.5
Bridges	6	53.5	20.5
Winton : Highest	7	60.0	25.3
Lowest		48.9	16.6
Average		54.9	21.0

It is obvious that the first four are derived from the same source, which according to Jacobs is Charlotte Chatfield and George Adams, U.S. Dept. Agric. Circ. 549 (1940). It also seems likely that Bridges is quoting McCance and Widdowson's figures. The figures given by Winton are for Californian sweet almonds and it is stated that for bitter almonds the average protein is about ten per cent more and the average oil about ten per cent less than in sweet almonds.

Our own figures are at considerable variance with the foregoing. During the last five years we have had 173 samples of ground almonds as sold in Kent and the figures are as follows:-

	per cent Oil	per cent Protein
Highest	66.7	27.1
Lowest	50.2	20.0
Average	58.5	23.2

With regard to the suggested oil criterion of 55 per cent we have in fact had only one sample with less than this amount and it was probably not genuine. Of our 173 samples, 122 had an oil content within the range of 57 - 60 per cent a much smaller degree of variability than is found in many natural products. So far as the suggested figure of 21 per cent for the protein criterion is concerned, only four of our samples had less than this and of these two were probably not genuine. We had 126 samples with protein in the range of 22 - 24 per cent. The average moisture content of eleven of these samples was 4.4 per cent.

This note is published in the hope of inducing colleagues to bring forward figures from their experience. It is possible

that ground almonds as commonly sold in this country may contain a not very large proportion of bitter almonds. If Winton's statement is correct, while this might account for the difference in protein it would not explain why our average oil figure is higher than those given in "the literature". It does not seem possible that the ground almonds used by the manufacturers of marzipan should be different from those on retail sale.

There is one word of caution to be added; the above figures are of course not applicable when de-bittered bitter almonds have been used. Specimens of such marzipan are occasionally encountered; generally they are of continental make. We did succeed in obtaining in one instance a sample of the de-bittered almonds from which the marzipan had been made; it gave the following results:-

Moisture 4.5, oil 52.5, protein 31.1, ash 3.45 per cent, iodine value of oil 97.7, Bieber test negative.

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ITEMS OF INTEREST

New Types of Sterilised Milk D.R.A. Davies (Jersey) has sent some interesting information about two new types of sterilised milk. The first is produced by a high-temperature-short-time process which appears to bear the same relation to ordinary sterilisation as H.T.S.T. pasteurisation bears to the old 'holder' process. The milk is first pre-heated to about 60°C in a heat exchanger and then homogenised. A second heat exchanger raises the temperature to about 80°C and the milk is then de-aerated in a vacuum tank; the firm making the equipment regard this as important in preventing the build-up of deposits on the plates in the sterilising section, which comes next and in which the milk is raised to 135° - 140°C for 2 to

4 seconds only. The milk is immediately cooled to 70° - 75° C for filling into sterilised containers under aseptic conditions. (This of course differs from the process for ordinary sterilisation, which is conducted in the bottle). It is claimed that whereas ordinary sterilised milk acquires a 'boiled' taste and brownish colour, the 'flash-sterilised' milk is not altered from fresh milk in appearance or taste.

An even more novel type of 'bacteria-free' milk, as it is called by the producers, is produced by first pre-heating the milk to 80° C and then injecting superheated steam, which raises the temperature in a fraction of a second to 150° C. It is held at this temperature for 2.4 seconds and is then transferred to a vacuum chamber where it is 'Expanded' and the temperature reduced to 80° C the steam being recovered and re-used. "Fully automatic controls ensure that the milk retains its specific weight and that its composition remains unchanged", claim the inventors of the process, which they refer to as 'Uperising'; it is of Swiss origin. A Danish firm has evolved a similar plant in which pre-heated milk is sprayed into superheated steam; the product is called 'Palarizet' milk. All these sterilised milks are being filled into 'Tetra-Pak' containers, which many members may have seen; they consist of tetrahedra of paper, coated inside with plastic and outside with wax. The coated paper as delivered to the dairy in rolls is almost free from bacteria, but to ensure absolute sterility the strip is treated with hydrogen peroxide in a thin layer before it is shaped into a continuous tube. It is then heated from inside with air at 200° C which decomposes the peroxide and leaves the tube dry and sterile. The milk is fed in to the tube which is sealed and cut off at intervals to give the final pack.

'Uperised' milk in Tetrapaks has been on sale in Switzerland since December 1960, and has proved acceptable to the consumers, who in large scale tests are said not to have noticed the difference from ordinary pasteurised milk. The milk will 'keep' for at least a month. In Denmark, the product is still in the experimental stage, so Davies is informed. Steam injection is claimed to have less effect on the taste than contact with hot plates, but any P.A. would wonder about the possible addition of water to the milk. Davies had submitted to him samples of Palarized Milk from Denmark, in Tetra Pak containers. He says "Although it was nearly a month old, it had only a very mild, slightly 'nutty' taste. I fear that I was initially sceptical about the whole idea, and rather disposed to judge wholly on the final product. I was surprised when I obtained figures which left me no option but to report the milk as genuine despite the possible addition of water as steam." Analysis of two samples gave the following results.

	Sample A	Sample B
Total Solids (weighed)	13.12%	13.09%
Fat (Gerber)	3.98%	4.00%
Solids Not Fat	9.14%	9.09%
Freezing-Point Depression (Hortvet)	0.536° C	0.534° C

The Hortvet thermometer was standardised with A.R. sucrose. The milk did not conform to the turbidity test for sterilised milk.

Davies says that "costing calculations indicate that the carton will cost a little over 1d. as compared to the present returnable bottle which is stated to be about ½d. However, savings in the frequency of deliveries should make up the difference." It is understood that these types of milk and of pack may soon be introduced into this country.

Polenske Flasks, Availability of The flasks to be used in the determination of butter-fat content by the standard Reichert-Polenske-Kirschner method (see for example B.S.S. 769) have a capacity of 310 ± 10 ml. Some time ago, the leading manufacturers of British glassware such as Pyrex and Hysil decided to 'rationalise' their standard sizes of flasks (and beakers) and the 300 ml size disappeared, there being no size between 250 and 400 ml. One or two small manufacturers were still prepared to make flasks to the B.S.S. dimensions but only if users were willing to take half-a-gross at one time, which ordinary users were not. E.C. Wood asked the B.S.I. to take some action in this matter and was supported by the S.A.C.

He has now been informed by the B.S.I. that these flasks are obtainable from Messrs. Griffin and George Ltd. against their catalogue reference S. 33-762-05. The B.S.I. have been assured that an adequate stock is held.