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"Problems of Pesticide Residue Analysis in Food"

Pesticide residue analysis is a relatively young science. Begining with insensitive, time consuming, tedious and uncertain methodologies of the 50's, it has now grown into a highly sophisticated science. ~~[illegible]~~ All these advances have taken place mostly during the last decade – the "After Carson" days when general Public became increasingly concerned about contamination of food stuff by poisonous pesticide chemicals. In this all round advance no subject concerned with pesticides have been neglected.

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It has benefitted the analytical technology, led to intensive studies on the toxicological and ecological effects of pesticides; led to lesser use of persistant first generation pesticides, search for safer pesticides have been intensified, hopes for alternate third generation methods of Pest control have increased.

design of better chemicals to day poised for a break through.

To day pesticidal chemicals ~~are~~ used in the field of agriculture and public health cover hundreds of established organic compounds. But the ones which are most frequently used and contribute maximally to the residue problem in food are few ~~and~~ ~~can be~~ These can be categorised into

(i) Chlorinated hydrocarbons DDT, BHC, endrin
toxaphene, endosulfan, aldrin
2,4-D etc PCNB

(ii) Organophosphorous compds: malathion, ethyl parathion
DDVP, Phorate, metasystox, fenitrothione etc.

Tech problem extraction of metabolites

(iii) Carbamates : Carbaryl, Carbofuran, dithiocarbamates
(iv) Fumigants : Phosphine, methylbromide, EDB, CCl_4

non volatile (v) Herbicides : Atrazine, Simazine, paraquat etc.

The situation for the Residue Chemist is further complicated by the fact that these chemicals can be used in admixtures at the simultaneously or at different times giving rise to multiresidue problems

→ As Residue chemists all of you know that there are several ~~steps in the analysis~~ prerequisite steps in this analysis. ~~two of which~~ These are (a) Sampling and sample storage (b) sample preparation (c) extraction (d) clean up and lastly (e) analysis. It appears to me that the major effort of the residue chemist in the past decade has been on the last steps. For years he has striven to devise more sensitive and specific methods of analysis which has taken him from microgram, through nanogram to picogram level. Limits of detection of pesticides by various analytical techniques have been raised during the past 20 years ~~from~~ by a factor of more than 250 million. (from 25 µg to 0.1 pg)

25 µg Arsenic

Emphasis on the Emphasis on the

Improvements in instrumentation is still continuing. Newer and more sophisticated ones are being offered for the Residue Chemist. This continued Search for smaller & smaller — for the hypothetical Zero has created some unbalance. The residue chemist tends to forget that other steps are equally important. For him a gas chromatograph has become a symbol of accuracy irrespective of how he has collected the sample and how he has followed other steps. This is misleading, by increasing the absolute sensitivity of the final method of analysis the over all detectability of residues in food is not necessarily improved. Other links in the chain are equally important.

From the point of view of the first step — extraction — food materials can be classified into three types — Predominantly wet (vegetable, leafy parts) — Predominantly dry (Cereals, dried plant matter etc) — Predominantly fatty (oils & Fats, oily nuts etc)

A Attempts a general clean up.

For the first two types extraction procedure and solvents are well defined — generally a mixture of immiscible (hexane, $CHCl_3$, etc.) and miscible phase (acetone, ethanol, isopropyl alcohol) is used. It works satisfactorily in most cases. Extraction with a miscible phase like acetone or iprOH — dilution and reextraction back to hexane also works fine in most cases. All these

however is not satisfactory for some metabolites forming conjugates with food matter on which they are sorbed. The existence of such conjugates with have been known for sometime and have been isolated only after hydrolysis with acids. So far no direct solvent extraction method have come into general use. After stripping with usual solvents another extraction with 70% aqueous acetone or isopropyl alcohol should take out such conjugates.

It is with fatty matter that a special problem is encountered. Acetonitrile has been the solvent of choice for many years now but it is very costly and entirely imported. Clearly more work is needed in this area to find out the efficacy of other polar solvents like DMF, Dimethyl acetamide as extractants. (1)

~~It is also well to realise that residues due to post harvest application of are easier to ex~~

The second step of "clean up" also offers a lot of scope for development and innovation. It is probably the most exacting step in residue analysis. Extraction brings out co-extractives of diverse nature varying from substrate to substrate and depending often on its physical nature. These may cause

tailing of chromatographic eluates, clogging of columns and syringes, fouling and overloading of detectors, over loading of tlc plates etc. Although many GLC instruments makers advertise - "No clean up required - inject directly" - in the interest of long life of columns and detectors clean up is necessary.

~~Although~~ The specific clean up procedure will vary enormously depending on "How much of what is to be cleaned up from how much of what", But the emphasis these days is on the development of universal type of clean up techniques. Various clay mixtures in combination with active charcoal have been used, single step extraction and clean up, on which some of my colleagues have spoken, have also have been developed and many new innovations done.

~~The techniques range from (a) physical methods like chromatography, Codistillation, partition, precipitation etc. (b) Chemical methods like dehydration, dehydrohalogenation, oxidation, hydrolysis etc.~~

→ The major problem encountered is again clean up from extracted fats and waxes. Partition between acetonitrile and hexane or, use of florisil column or hydrolytic removal of fats is the usual technique again but makes the process tedious. Extraction with acetone - concentration and chilling to remove solid fats and waxes - dilution and extraction back into hexane is sometimes a better method.

Analytical methods which do not require very thorough clean up are GLC and Mass spectrometric. The later has not been fully utilised in residue analysis but I think wherever budget permitting the future lies in this mode of instrumentation.

Regarding the last step, that is actual analysis, I do not have to say much before you experts. But there I only want to make a few observation regarding future development in analytical trends in this field. All of you know that- the begining with colorimetry (limit of detection 10^{-5}) the instruments and techniques that can be employed for actual quantitative analysis of trace amts of organic chemicals have undergone so much improvements and sophistication that today search for "needle in a haystack" is not at all difficult. Limit of detection using spl. GLC with EC detector has been raised to 10^{-12} gm (pg) levels. Analysis can be done rapidly and automatically. But all this increases cost and makes setting up of a residue laboratory in all areas in a country such as ours difficult. Maintainance of sophisticated equipment like GLC is possible only in a few centres.

Unless easier methods like TLC is fully developed there is not much hope of seeing ~~real~~ adequate residue studies in a large country like ours.

(2) Combination of different technique such GLC coupled to colorimetry; U-V or IR are other areas where future development is bound to take place specially where absolute identification of the compounds involved is necessary.

Two other areas of instrumentation when fully developed might solve some of the problems faced by the residue chemist. These are ① use of mass spectrometer — where the vapour of a sample produced in high vacuum at about 200-400°C is subjected to electron bombardment and the masses of the +ve ions produced are determined by a combination of electric and magnetic fields. Thus every compound gives a characteristic member of fragment which can ~~identified~~ be located to identify the compound. The method has all the advantages you want (a) sensitive 10^{-6} g (b) multi residues can be easily analysed (c) clean up is not required. In fact paper chrom. spots can be utilised with paper — so also TLC bands with silica gel — without separation. Properly developed This will be a great help — but Cost again is a deterent factor.

The second one is High Speed Liq. Chrom. where a thin column of conventional materials is utilised under constant flow system. Resolution is like GLC but it is generally applicable for non volatile compds. Most metabolites and conjugates and are polar and non volatile

HSLC
HPLC

and can not be subjected to gas liquid Chromatographic techniques. It is in this area that High Speed liquid Chromatography will be most useful. The acetone extracts with only minor clean up can be utilised to give a full picture of non-volatile metabolites. Thermally labile substances which can not be subjected to glc techniques can be used here with great ease. High speed liquid Chromatography will become more widely applicable in near future. But its cost again is the deterent factor.

There are certain other general problems. I shall mention one or two. For example since the results of individual analysis are depended on methods of sampling and methods of actual analysis there is great need for adopting "agreed methods or referee methods all over the country". This has been advocated by Dr. Bammi and others at ISI several times. But this kills the spirit of innovation and experimentation and researchers tend to become technicians. Again established Referee methods tend to become quickly outdated. WHO also agrees that it is impractical to attempt to specify a "Referee method of analysis" — It may be better to say "Referees procedure" and for routine use it is better to use the term "Regulatory method of analysis.

(3) WHO

Secondly it is well known that residues of common chemicals can be vastly reduced by good cooking procedures. Residues of EDB on wheat for example comes down for 50 p.p.m to 8 ppm after aeration

During bread making it goes down to 0·5 ppm
When food is cooked in open vessels, as is done in 90% of Indian household - I am sure many of the contaminants will go down to a low level but there is not much data on what these good cooking procedures do to residues and why these values should not be utilised for calculating ADI values and tolerance limits. (4)

The residue chemist by and large in our country has remained content with only with monitoring. Their primary job is to develop good plant protection schedules — so that residues on crops can be predicted and that is being done only in a few solitary places. It is time to think a little beyond monitoring — to think of such problems as of decontamination by suitable processing; to think of traces of more toxic products in technical grade materials — of methods like TLC coupled with high speed colorimetry which can be utilised by larger number of smaller laboratories and finally time to think of education and extention so that people do not misuse pesticides.

Safer pesticides through newer formulations