

ROLE AND OPERATION OF THE PESTICIDE ANALYTICAL SERVICE
AT THE
CHEMICAL CONTROL RESEARCH INSTITUTE

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FOREWORD

In recent years, large-scale applications of organic chemicals for control of forest insect pests have been the subject of considerable concern regarding their total impact on the environment. Nevertheless synthetic pesticides play an important role in protecting Canada's forest resources, and it is anticipated that their use will continue to serve this purpose, for the foreseeable future. The apparent trend in chemical control will be toward more effective uses of the nonpersistent pesticides that are currently available and the development of new chemicals that have different modes of action and are more selective for any particular target species. Research with new pesticides and the necessary re-evaluation of certain other pesticides currently in use will require extensive studies of their possible impact in the forest environment.

In order to develop a greater understanding of the fate of pesticides, while utilizing their direct benefits, the Chemical Control Research Institute of the Canadian Forestry Service has recently extended its research program to develop an analytical capacity for the detection, identification and quantification of pesticide residues in the environment with special reference to residues resulting from forest spray operations.

This report outlines the resources, the expertise and the facilities available at the Analytical Service Unit at CCRI and its accomplishments since its inception.

James J. Fettes
Director, CCRI.

INTRODUCTION

In recent years various organochlorine, organophosphorus and carbamate insecticides have been used experimentally and operationally for controlling insect pests in Canadian forests. The environmental significance, fate and persistence of these chemicals in various substrates such as soil, water, air, flora and fauna, are not yet fully explored. The Analytical Service Unit began operating in January 1972, as an extension of the existing research facilities at the Chemical Control Research Institute, primarily to analyse and study the distribution of pesticide residues in various components of the forest environment and to assist in evaluating the impact of these residues on the ecosystem. The functions of the Unit are principally fourfold:

1. to provide necessary analytical service, in consultation and cooperation with research personnel of the Forestry Service by analysing pesticide residues in various components of the forest environment.
2. to carry out methodology research for the analysis of currently used and experimental pesticides* and their breakdown products.
3. to define procedures for collecting, processing and storing of samples from sprayed areas and to provide information on the properties of pesticide residues and their methods of analysis, and

* Common names of pesticides are used throughout the text. Chemical and/or trade names are given in Appendix 1.

4. to collaborate in mission-oriented research programs undertaken by the research personnel of the Forestry Service and other cooperating agencies.

So far much of the Unit's work was concerned with residue determinations and methodology developments for organochlorine and organophosphorus insecticides present in various substrates of the forest environment. This report describes the facilities available, the analytical methods in use and progress made by the Analytical Service Unit at this Institute during the calendar year, 1972.

LABORATORY FACILITIES

1. Laboratory Space

The pesticide analysis facilities at the Institute comprise a suite of chemical laboratories for mechanical treatment of field samples, solvent extraction, cleanup and for the conducting of other wet chemical experimentation for pesticide detection; balance room, instrument rooms, cold room for storing field samples, store and solvent rooms for keeping chemicals and solvents used in the analytical work. Due to increasing demand currently on the Service Unit, laboratory space is at a premium which limits further expansion, productivity and in particular its expanded usefulness to the research personnel.

2. Equipment

The analytical laboratory is equipped to detect and estimate quantitatively trace amounts (10^{-9} g) of various organochlorine and

organophosphorus pesticides and their metabolites present in forest environments resulting from spray operations. Certain analyses require the use of GLC (Fig 1), TLC (Fig 2), IR (Fig 3), UV (Fig 4) and radiochemical techniques (Fig 5 and 6) for detection, quantitation and characterization of residues. Gas chromatography and spectrophotometry play important roles in the identification of minute quantities of the breakdown products that are isolated. Radioisotope-labelled insecticides are detected after exposure by scintillation counting or chromatogram scanning. Paper and thin-layer chromatography assist in the purification and characterization of pesticide residues. In coping with a backlog, samples are freeze-dried (Fig 7) and stored at 0°C in tightly-capped glass jars for further analysis. Water samples are generally analysed immediately on arrival. Table 1 lists essential analytical instruments available at the Laboratory. Trade, company and model names of the instruments are included only for the benefit of readers and do not in any way imply their endorsement over other instruments available on the market.

The Service Unit is stocked with necessary chemicals, solvents, chromatographic accessories, several types of grinders for homogenizing samples and all kinds of glassware needed for macro and micro analysis.

3. Personnel

The Service Laboratory began to function in early January 1972 as the main responsibility of the author, who, with Dr. W. N. Yule,

TABLE 1

ANALYTICAL INSTRUMENTS IN THE LABORATORY

Equipment	Use
Radiochromatogram scanner (Packard 7200) with recording ratemeter	Radioactive uptake studies and breakdown tracer with TLC
Spectrophotometer IR (Beckman IR 20A)	Analysis of pesticide residues and formulations identification
Gas chromatograph (Varian 610D)	Identification of pesticide residues, and co-extractive background, formulation quantitation (EC*, FID)
Gas chromatograph (HP 810)	Quantitation of pesticide residues and identification (EC*, FPD)
Gas chromatograph (HP 5750)	" "
Gas chromatograph (HP 7610A)	" "
Liquid scintillation counter (Ansitron II Picker Nuclear)	Counting radioactivity. Distribution and metabolism tracer
TLC Equipment (Desaga)	Pesticide identification, metabolism studies
Spectrophotometer UV/visible (Beckman Acta CIII)	Analysis of pesticide residues, formulations and spray deposits identification

* Detectors

- EC - electron capture
- FID - flame ionisation
- FPD - flame photometric

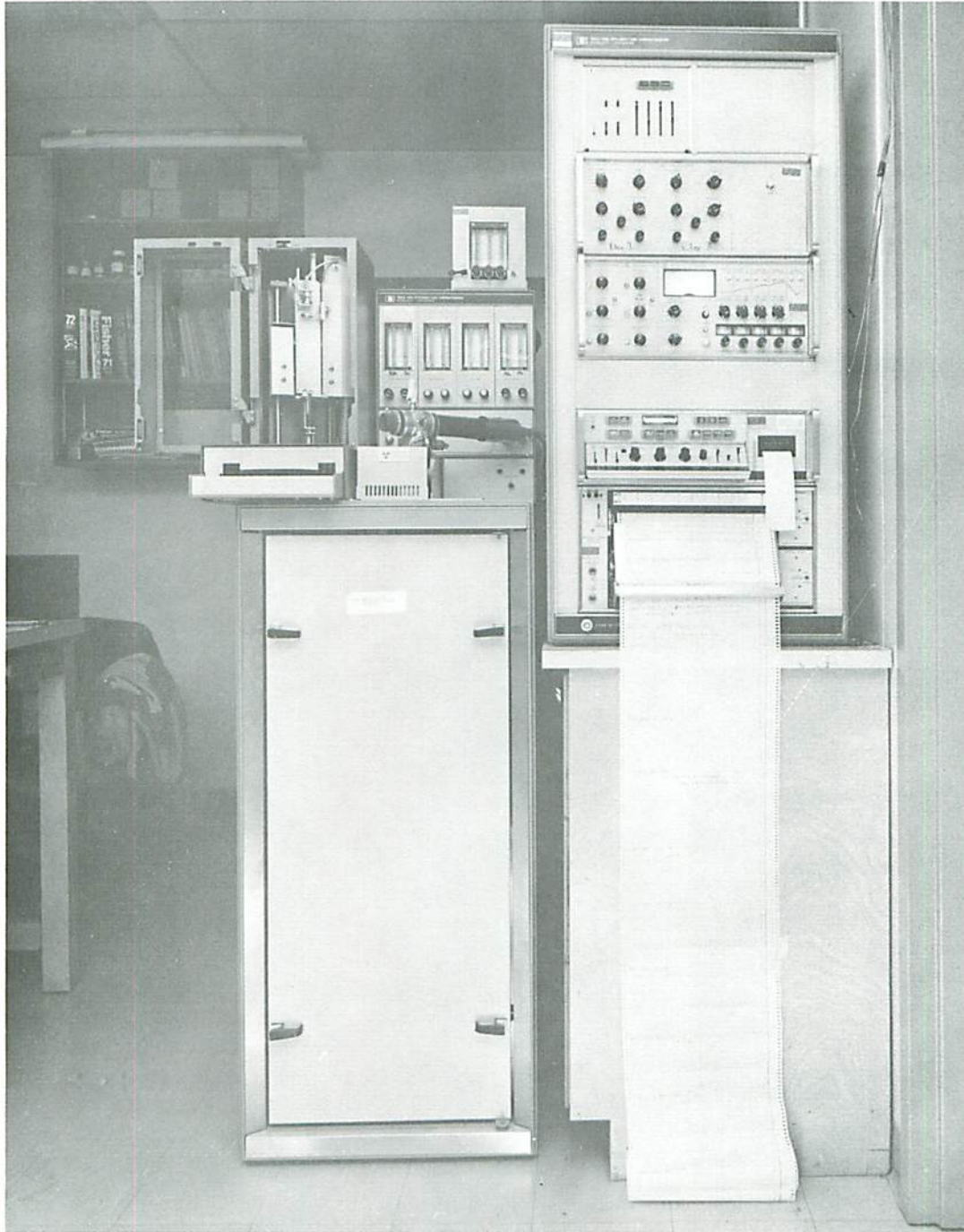


Fig 1

Gas chromatograph for the quantitative analysis of pesticides and their breakdown products.

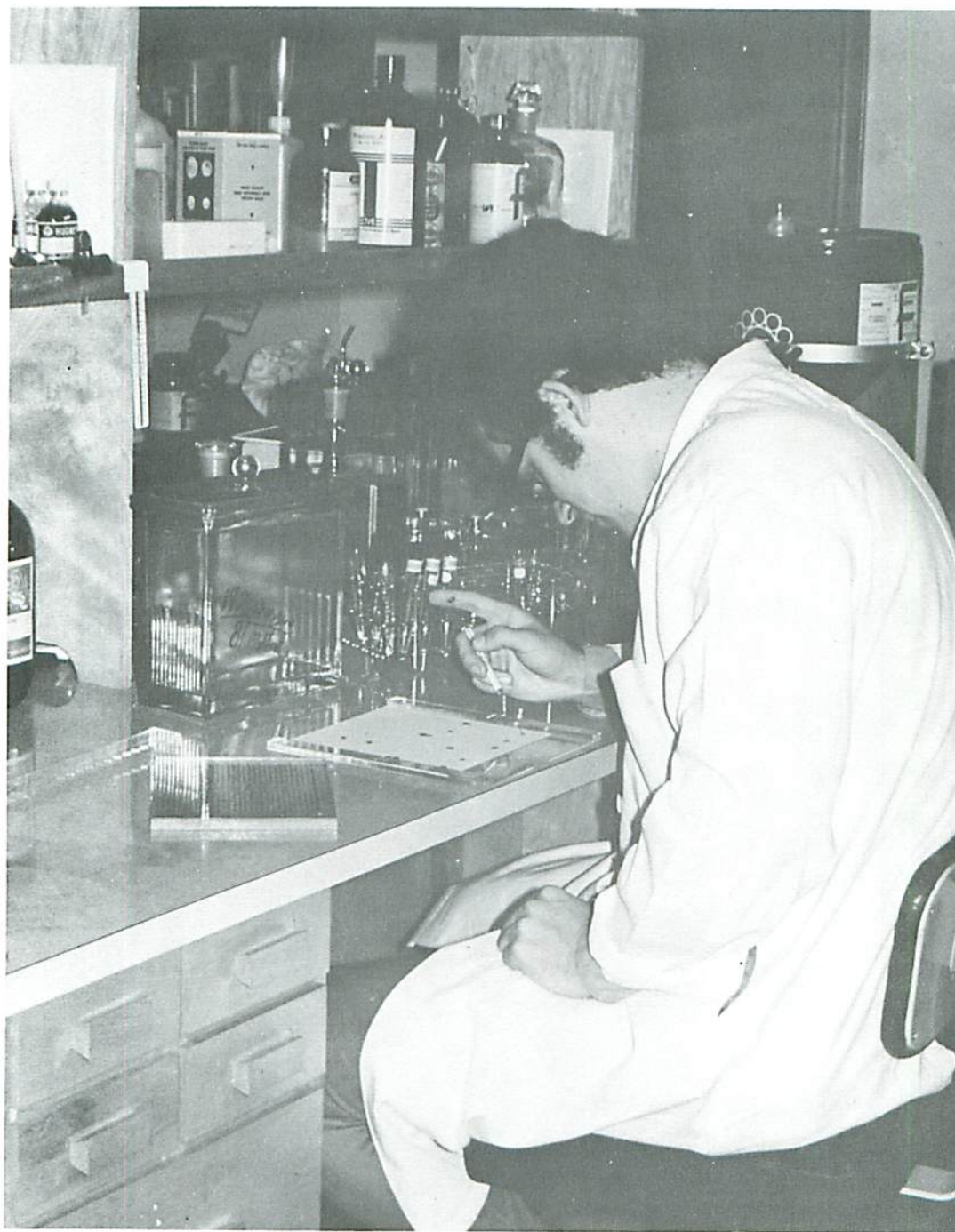


Fig 2 Thin-layer chromatography for determining pesticide residues.

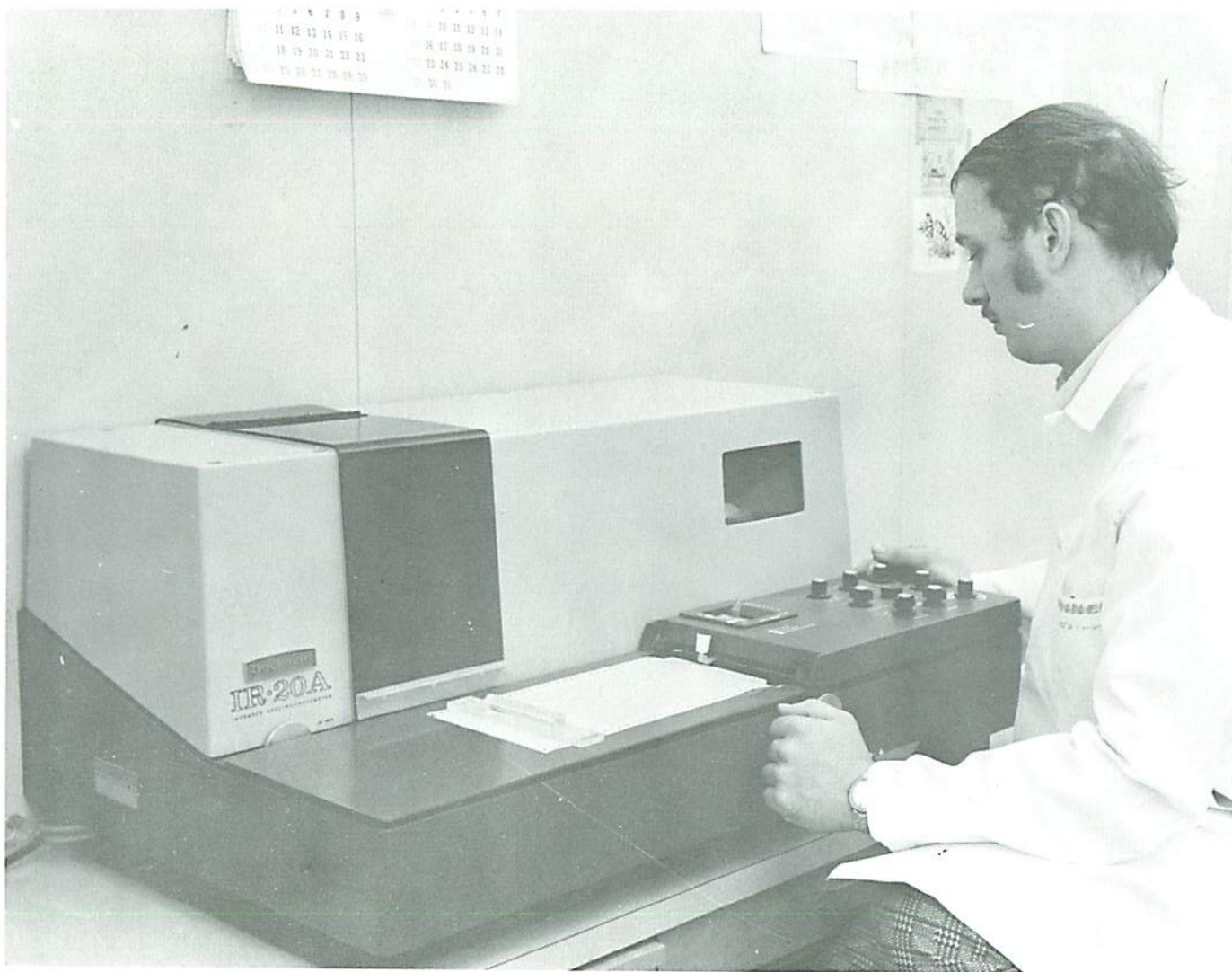


Fig 3 Infrared spectrophotometer for the identification of pesticide residues.

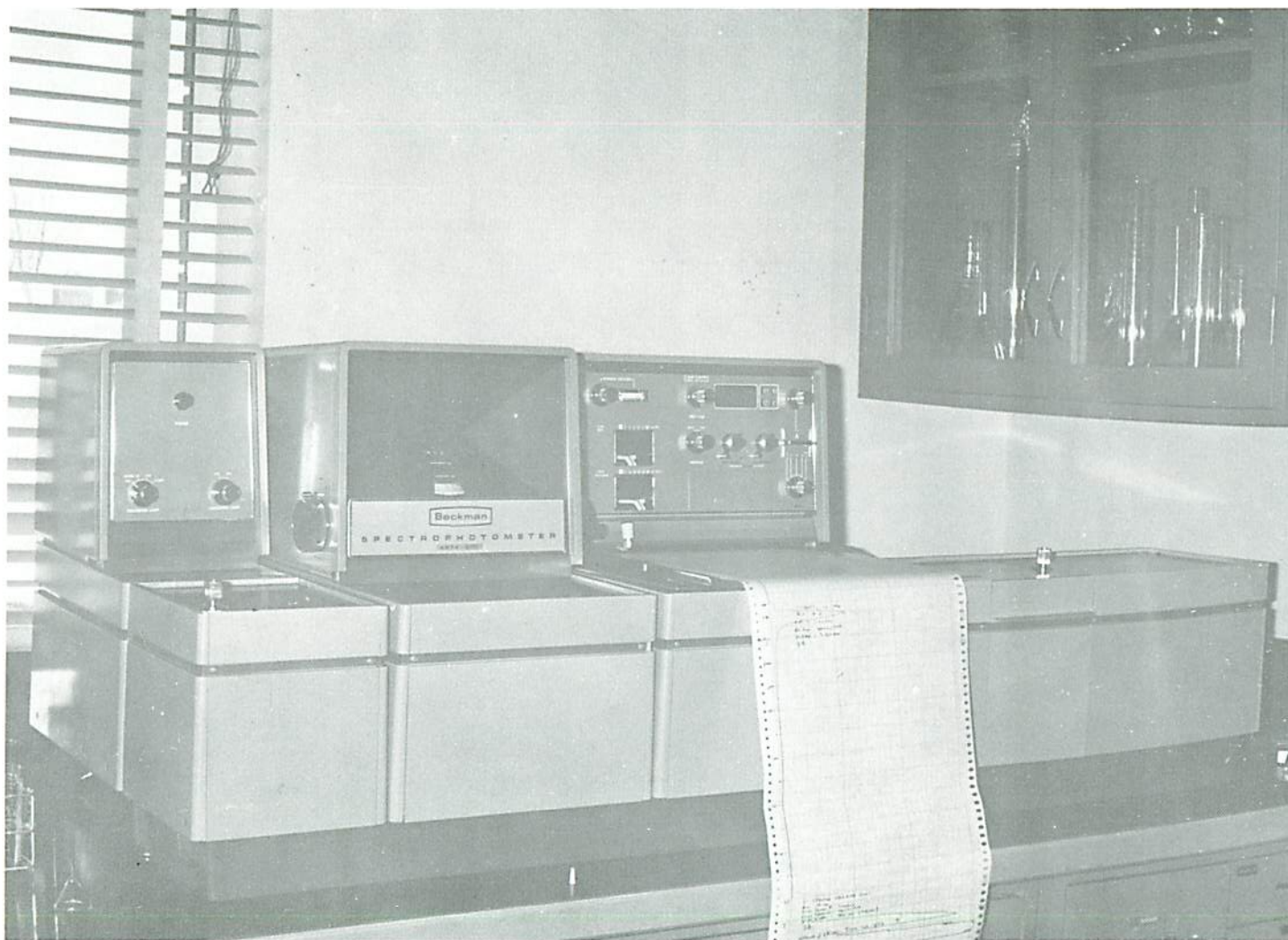


Fig 4 Ultraviolet spectrophotometer for the analysis of pesticide residues, formulations and spray deposits.

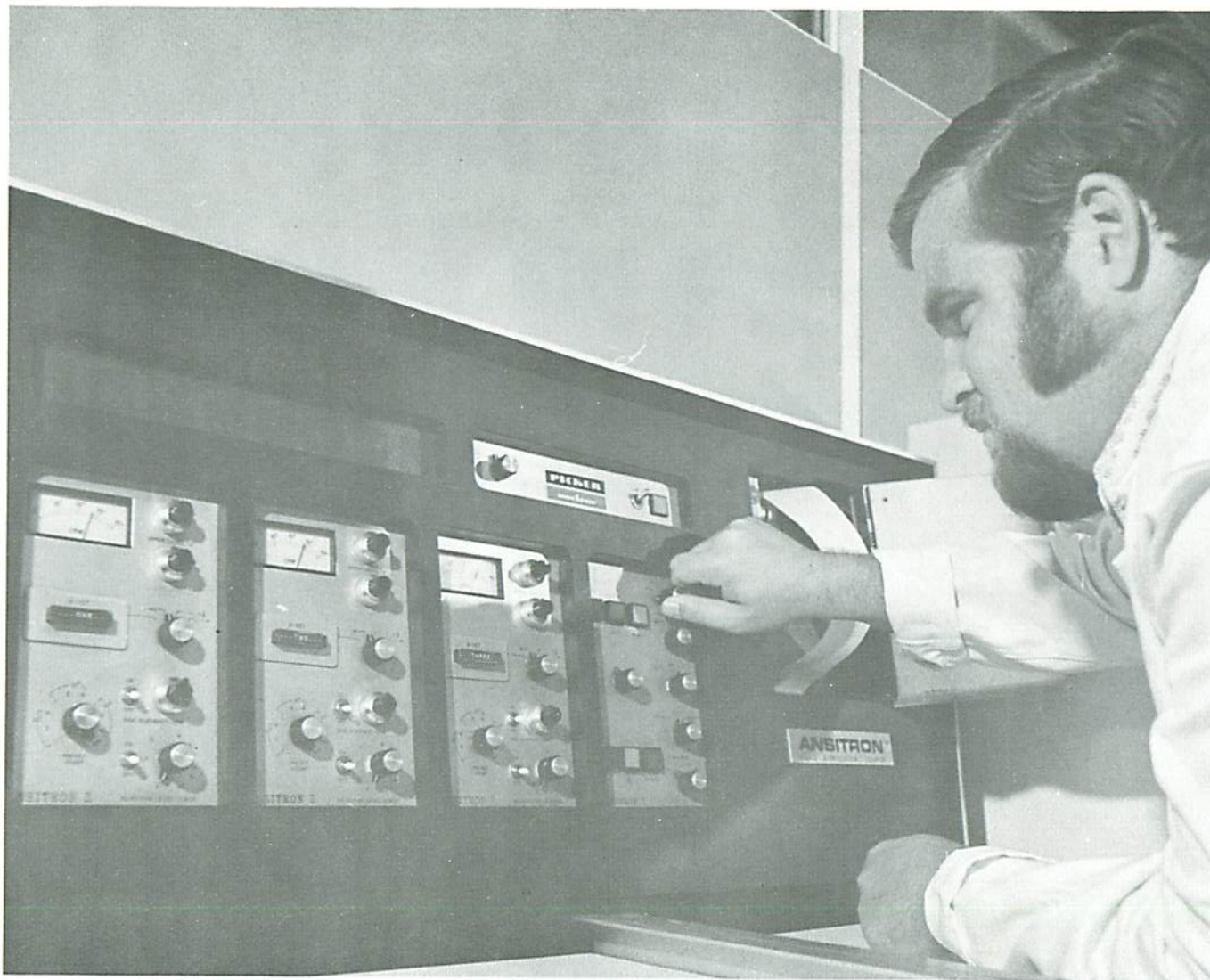


Fig 5

Scintillation counter for the detection of radioisotope-labelled insecticide residues.



Fig 6 Chromatogram scanner for the estimation of isotopically labelled pesticides and their metabolites.

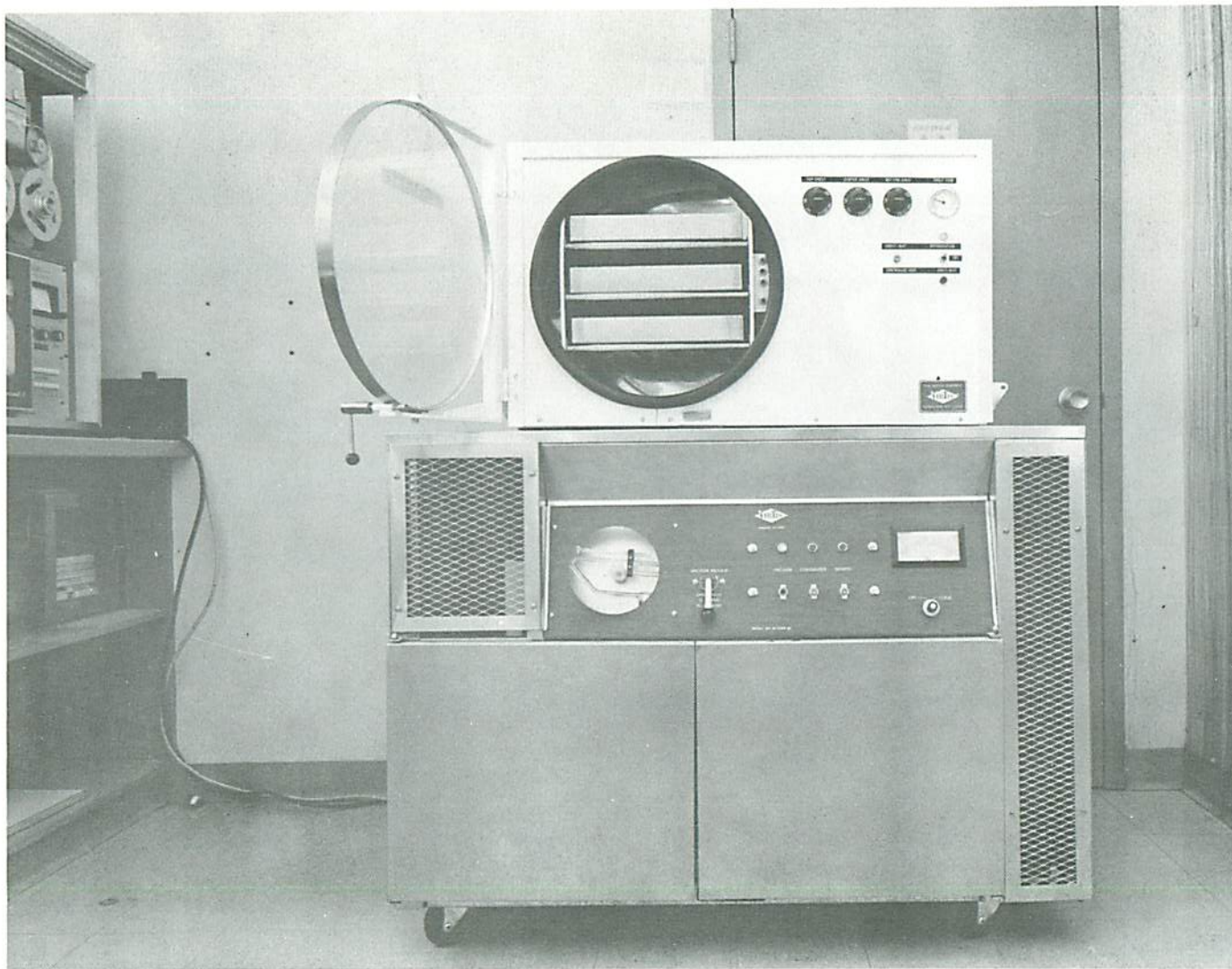


Fig 7 Freeze-dryer for the preservation of plant and animal tissues for future analyses by drying them at ca -15°C .

supervises the planning and organization of work, methodology research development, and the analytical work. One senior technician is responsible for supervising the practical work ordering supplies, and scheduling samples for analysis. Equipment operation is handled by an instrument technologist who is trained in the use of gas chromatographic procedures and optical techniques. The routine bench work is carried out by two trained technicians. To complete the work on schedule, seasonal help, especially undergraduate students with training in analytical chemistry, is sought in summer during the field season when the workload is high. All personnel in the Service Unit are made familiar with health and safety hazards associated with pesticides and other hazardous chemicals and various types of equipment being used in the laboratory.

METHODS OF ANALYSIS

During the past year, the Analytical Service Laboratory has carried out 16 different types of analysis on a total of 942 samples collected from forest environments and submitted by different researchers within the Forestry Service for residue assessment. The following is a breakdown of the various types of analysis performed in the laboratory.

DDT residues (<u>o</u> , <u>p</u> -DDT; <u>p</u> , <u>p</u> '-DDT and DDE), pH and moisture content of soils ...	18
Fenitrothion content, temperature and pH of natural waters ...	190
Methoxychlor residues (<u>o</u> , <u>p</u> -MC; <u>p</u> , <u>p</u> '-MC and MCE) and moisture content of white pine leaders ...	81
Fenitrothion content in honeybees, honey, beeswax and pollens ...	66
Fenitrothion residues (fenitrothion and its oxon) and moisture content of foliage ...	99
Moisture, pH and fenitrothion residues in soil ...	58
DDT residues (<u>o</u> , <u>p</u> -DDT; <u>p</u> , <u>p</u> '-DDT and DDE) in the brain tissues of small mammals ...	362
Fenitrothion in aerial spray deposits ...	60
Fenitrothion content of commercial spray formulations ...	2
Fenitrothion in air samples ...	5
Acidity and alkalinity (expressed as CaCO ₃) of water used for preparing spray mixture ...	1

Experimental designs and methodologies for organochlorine (DDT and methoxychlor) and organophosphorus (fenitrothion and Gardona) insecticides and their metabolites from such diverse samples as air,

water, animal and plant tissues, soil, sediments, honeybees, honey, beeswax and pollen grains have been either developed or available techniques were used as such with or without modifications. Methodologies varied in complexity depending on the types of samples under investigation and the nature of interfering materials and other coextractives present. Various steps that are currently used in the total residue analysis from a forest spray operation are given in the flow chart (Fig 8) and the details are outlined below for the benefit of other analytical chemists. Complete description of all the analytical methods developed are reported elsewhere (Sundaram 1972a, b, and c) or being processed for publication.

Procedures Used in Pesticide Residue Analysis

Field sampling:

For obtaining reliable and meaningful residue data in forest spray operations, it is essential that proper attention be given to sample collection, subsampling, sizing and handling of the samples to be analysed. The chemists' residue data may be precisely determined but may not be accurate because of inadequate field sampling. In some of the present residue analysis studies, representative samples were collected (Fig 9 and 10) from properly designed experiments (establishment of sizeable plots, availability of replicated and control samples, uniform coverage of pesticide etc). The gross samples collected were reduced to subsamples by mixing and quartering and stored in suitable containers (for liquids glass jars

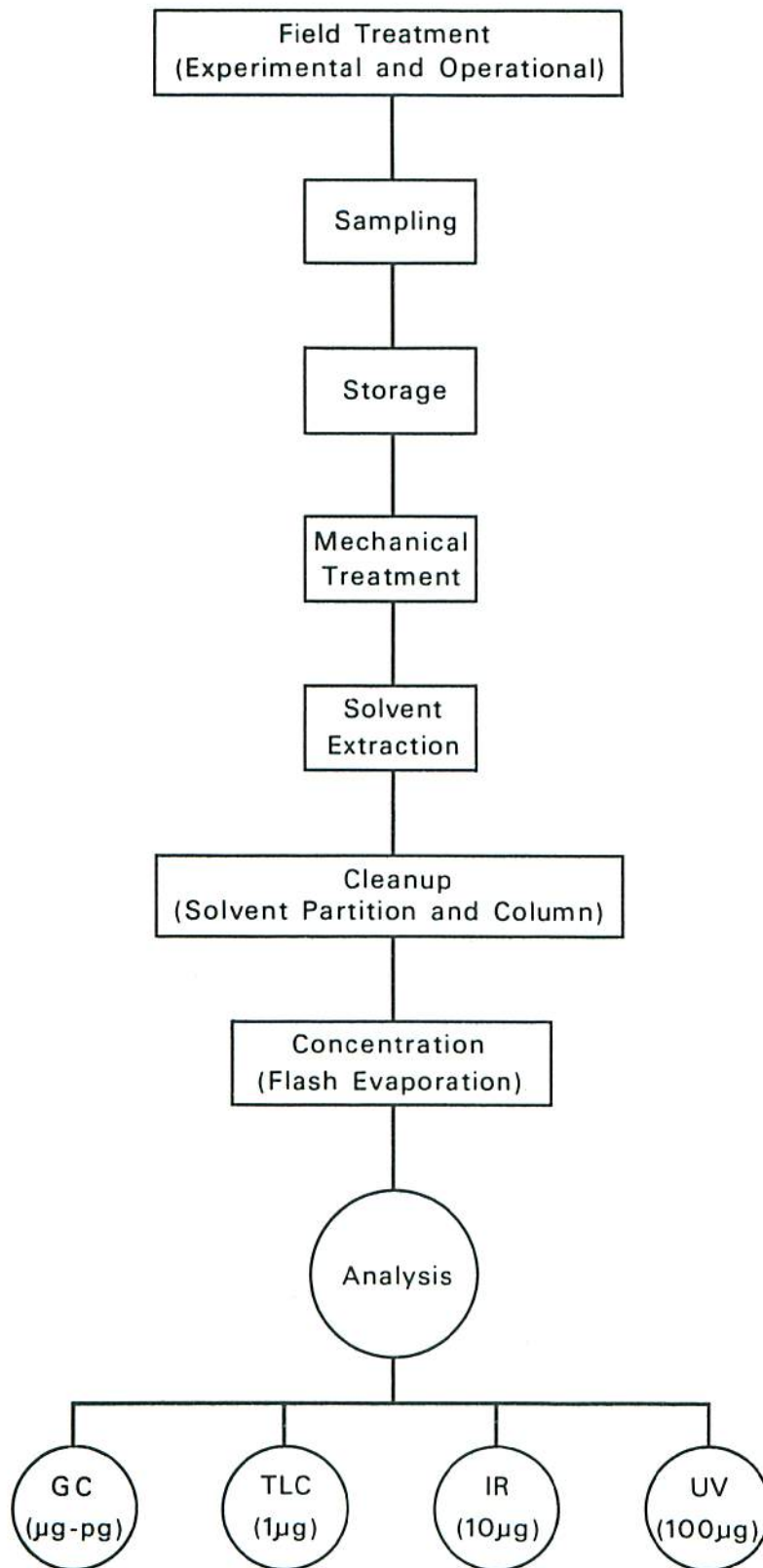


Fig. 8 Flow chart showing the various steps involved in pesticide residue analysis. Values in parenthesis show the minimum concentration limit that could be detected.



Fig 9 Field sampling of foliage for residue determination.

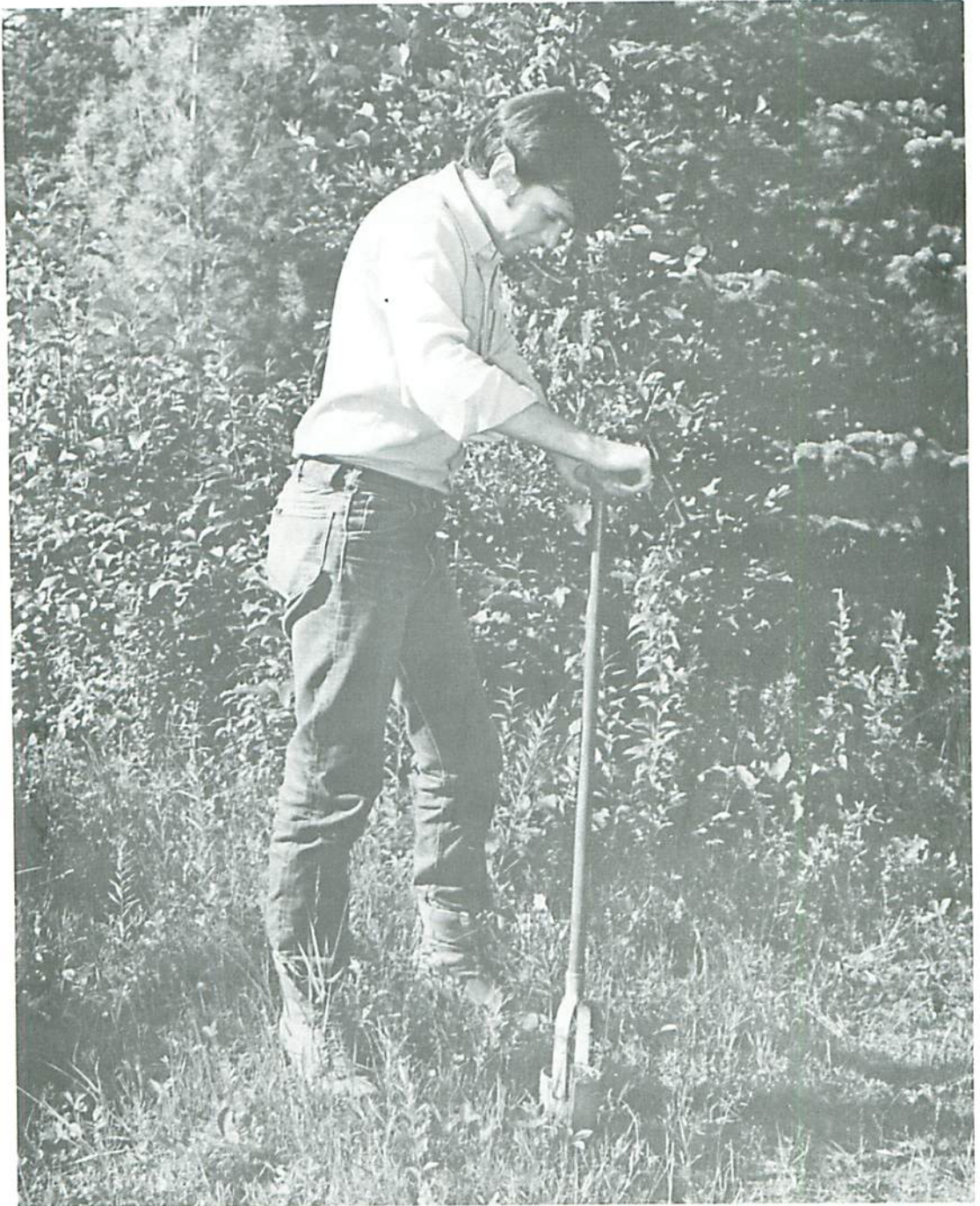


Fig 10 Sampling of forest soil for pesticide residue analysis.

with aluminum foil covers and screw caps and plastic bags for foliage, soil etc), stored below 0°C in rigid insulated containers and transported immediately to the chemical laboratory for analysis.

Mechanical treatment:

The subsamples (animal and plant materials) were comminuted either by using hand clippers or "Hobart" choppers, composited and mixed well (Fig 11). This produced an intimate mixture of substrate material from which the pesticide residues were extracted with high efficiency.

Solvent extraction:

Aliquots of the homogeneous mixture were weighed, macerated or blended with a suitable polar and/or nonpolar solvent or solvent mixtures for a standard time using a high-speed blender such as the Waring Blender or the Omni-Mixer and the liquid phase filtered using a fritted glass funnel containing a column of anhydrous sodium sulphate to absorb the water from the extract. The blending techniques used with a combination of polar and nonpolar solvents resulted in both high extraction efficiency and consistent results. The efficiency of extraction procedures were evaluated using spiked specimens.

Cleanup procedures:

It was necessary to remove from the sample by several cleanup steps, most of the contaminants that were extracted along with the pesticide, otherwise impurities seriously interfered with subsequent



Fig 11

Mechanical treatment of foliage samples prior to solvent extraction.

identification and quantification and yielded misleading results. Partitioning of pesticide-containing extracts between two immiscible liquid phases (Fig 14) was used for separating pesticides from extraneous or interfering materials such as fats, waxes and other organic co-extractives. Trace contaminants, affecting sensitive GC detectors (Fig 12 and 13), if still present, were removed by column adsorption chromatographic technique (Fig 15) using Florisil, silicic acid, Celite, activated charcoal, etc., as retentive materials. Since many metabolites of a pesticide may be present in a sample extract, selective elutions, using different solvents and/or solvent mixtures, were necessary for estimating all spray residues from various substrates.

Concentration:

The eluates collected from chromatographic columns were concentrated in a flash evaporator at reduced pressure and room temperature to minimize possible vaporization, isomerization and other physicochemical transformations. The concentrates were dissolved in nonpolar organic solvents, transferred quantitatively to a centrifuge tube and diluted further to a known volume.

Quantitation:

Gas chromatography played an important role in the quantitative determination of the pesticides and their breakdown products. The specificity of the GC procedures is determined by the column packing, operating parameters and the detector systems [electron capture detec-

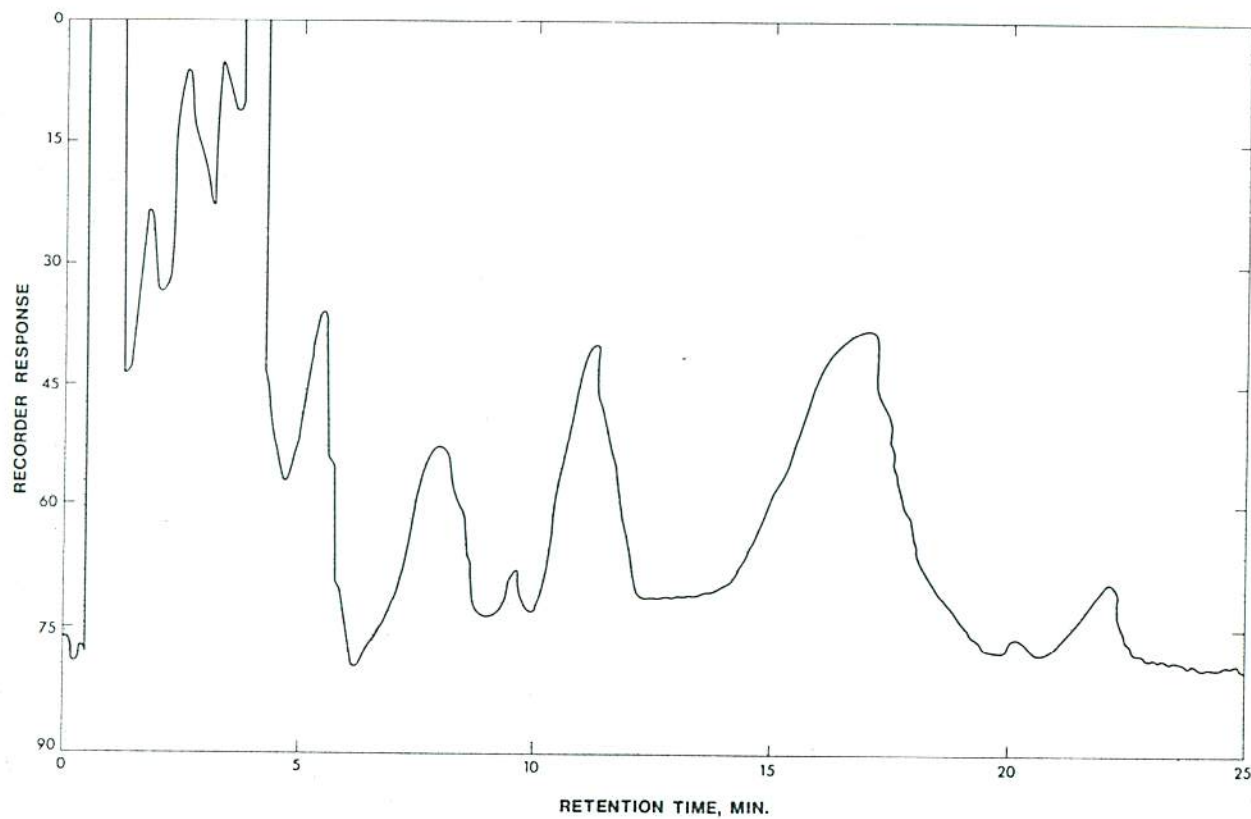


Fig 12

Chromatogram of hexane extracts of animal tissues before the solvent partition and column cleanup. The sensitivity of EC detectors makes the presence of trace contaminants significant.

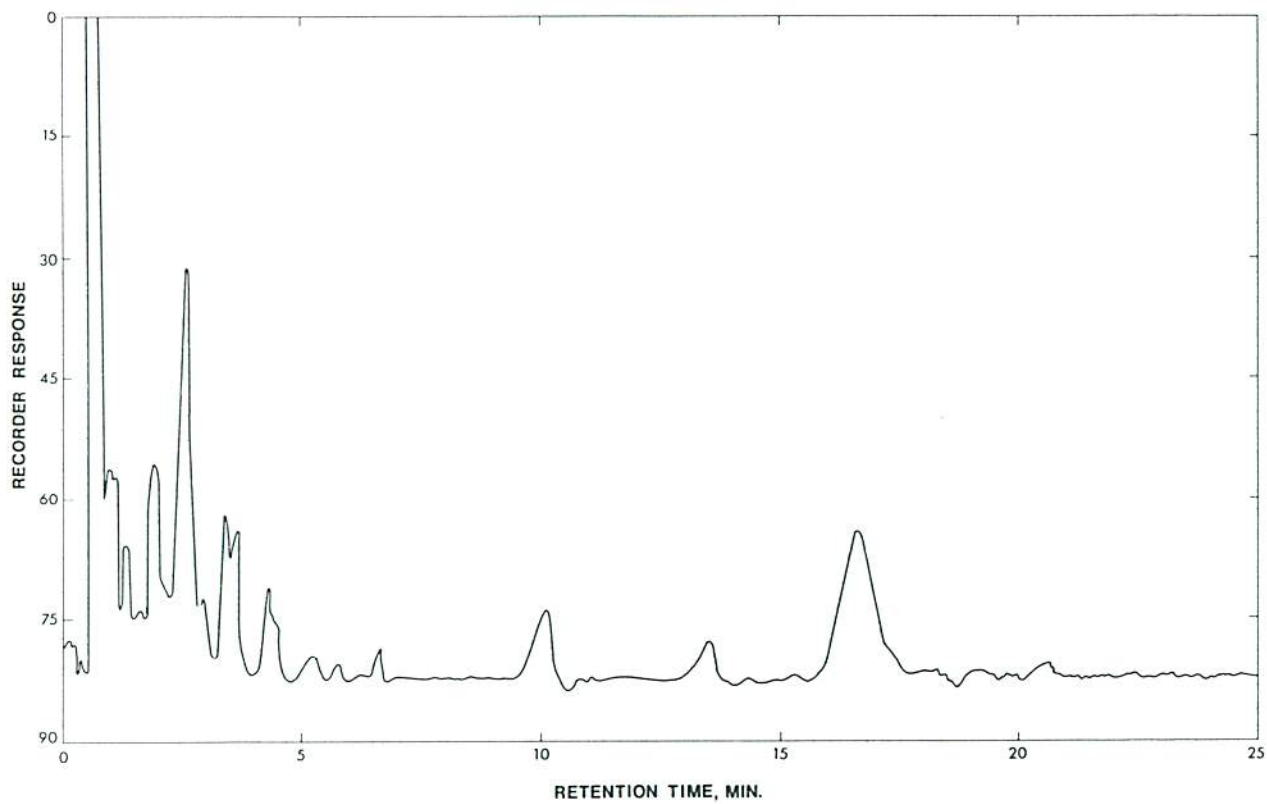


Fig 13

Chromatogram of hexane extracts of animal tissues after the solvent partition and Florisil column cleanup.



Fig 14

Solvent partition of pesticide residues between two immiscible liquids.

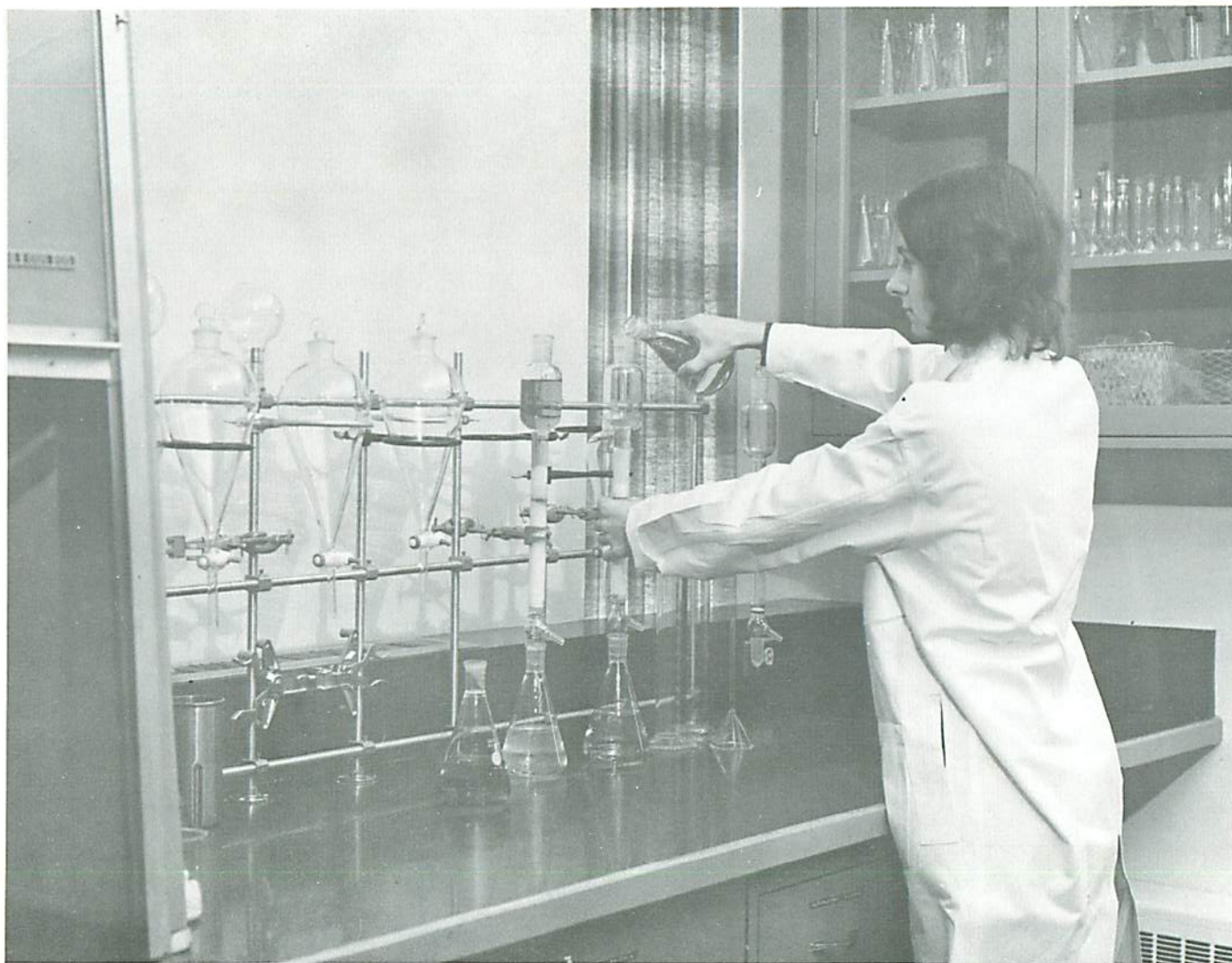


Fig 15

Cleanup of pesticide residues by column chromatography.

tor for organochlorines and flame photometric emission detector using P(526 m μ) and S(394 m μ) filters for thio-organophosphorus compounds]. The operating conditions of the GC equipment used for the quantitative estimation of various organochlorine and organophosphorus pesticides are fully discussed by Yule and Duffy (1971) and Sundaram (1972a, b, and c). Thin layer chromatographic and spectroscopic (UV and IR) methods provided valuable corroborative evidences leading eventually to positive identification of pesticide residues.

The various analytical methods developed or utilized at this Service Laboratory for the estimation of pesticide residues are not described in detail but the sequence of steps are outlined in Table 2.

OPERATION OF THE SERVICE LABORATORY

Usually the service function development of the Analytical Laboratory paralleled the Institute's research program. In order to set work guide lines and work program, researchers who needed chemistry service in their projects were asked to discuss their requirements with the scientists in charge of the Analytical Chemistry Section well in advance of the start of the field season. Discussion of proposals for collaborative work are normally made at the project planning stage, and again before the field sampling stage was undertaken. Also during the preliminary discussions, agreements were reached on the purpose of analysis, mode of analyses required, types and numbers

TABLE 2

DETERMINATIVE PROCEDURES FOR PESTICIDE RESIDUES

Sample	Determination	Mechanical Treatment	Solvent Extraction	Cleanup	Reference
Soil	<u>p,p'</u> -DDT, <u>o,p'</u> -DDT and DDE	50 g composite, sieved (mesh 8), moist soil	2 x 100 ml 2:1(V/V) hexane : acetone mixture. Sorvall	Solvent partition (300 ml hexane and 600 ml water) followed by column cleanup using preconditioned Florisil and eluted with 2 x 100 ml 15% (V) benzene in hexane	Yule (1973)
Brain Tissues of Small Mammals	<u>p,p'</u> -DDT, <u>o,p'</u> -DDT and DDE	Individual samples of mass <u>ca</u> 0.3 g, stored in absolute ethanol	20 ml hexane + 2 g anhy. Na ₂ SO ₄ . Sorvall	DMF - hexane partition; DMF + Na ₂ SO ₄ (aq) - hexane separation. Hexane layer passed through activated Florisil column and eluted with 70 ml of hexane	Sundaram (1972a)
White Pine Leaders	Methoxychlor (<u>o,p'</u> , <u>p,p'</u> and MCE)	10 g composite sample in finely divided form	Macerated overnight with 200 ml 1:1(V/V) CH ₂ Cl ₂ :CH ₃ OH	Solvent partition (<u>n</u> -hexane - acetonitrile) followed by Florisil column cleanup and eluted with 450 ml 1:4 CH ₂ Cl ₂ : <u>n</u> -hexane (V/V)	Sundaram (1972c)
Natural Waters*	Fenitrothion	Decantation of supernatant liquid	Progressive extraction of aliquots with hexane		

TABLE 2 (Cont'd)

Sample	Determination	Mechanical Treatment	Solvent Extraction	Cleanup	Reference
Foliage (mixed spruces)	Fenitrothion, fenitro- oxon and 4-nitrocresol	100 g composite sample machine chopped (Hobart) and sieved	Homogenized with 400 ml of ethyl acetate for 8 min using Sorvall	Filtrate flash evaporated to dryness, dissolved in acetonitrile, partitioned twice with hexane, the polar phase passed through a charcoal column and eluted with benzene-ethyl acetate mixture. Fenitrothion, its oxon and nitrocresol were separated by passing through a silica column and eluting selectively with benzene and acetone.	Yule and Duffy (1972)
Soil	Fenitrothion, fenitro- oxon and 4-nitrocresol	100 g composite, sieved (mesh 8), moist soil	Sorvall with 400 ml of ethyl acetate for 8 min	Same as in foliage	See previous reference
Aerial spray deposits collected in petri dish*	Fenitrothion	-	Dissolved the residue in suitable aliquots (5 or 10 ml) of hexane and analysed by GC		
Fenitrothion EC	Fenitrothion	-	As above		
Air*	Aerosol and gaseous airborne fenitro- thion absorbed in DMF present in air sampler	-	-	Flash evaporated to dryness, dissolved the residue in suitable aliquot of hexane and analysed by GC	

TABLE 2 (Cont'd)

Sample	Determination	Mechanical Treatment	Solvent Extraction	Cleanup	Reference
Honeybees*	Fenitrothion	-	3-5 g bees + 10 g Na ₂ SO ₄ + 50 ml CH ₃ CN, sorvall for 5 min.	Solvent partition (CH ₃ CN - hexane) followed by charcoal-Celite and silica columns cleanup for acetonitrile layer. Eluted with benzene/ethyl acetate	
Pollens*	Fenitrothion	-	5-7 g of pollens + 10 g Na ₂ SO ₄ + 50 ml CH ₃ CN, sorvall for 5 min	Same as in honey-bees	
Beeswax*	Fenitrothion	-	10 g of wax + 10 g Na ₂ SO ₄ + 100 ml of hexane, sorvall for 7 min	Flash evaporated and solvent partitioned (CH ₃ CN - hexane). CH ₃ CN layer subjected to column cleanups as above and eluted with benzene/ethyl acetate	
Honey*	Fenitrothion	-	10 g of honey + 500 ml water + 50 ml hexane, sorvall for 10 min.	Hexane layer dried with Na ₂ SO ₄ , filtered, flash evaporated to 0.5 ml and analysed by GC	

TABLE 2 (Cont'd)

Sample	Determination	Mechanical Treatment	Solvent Extraction	Cleanup	Reference
Soil and foliage	Moisture	Composited and homogenized samples (10 g)	-	Oven drying method (105°C)	A.O.A.C. (1955)
Water and soil	pH	-	-	Measured by using IL pH Meter model 175 with glass pH indicating electrodes containing Ag/AgCl half-cells	Atkinson <i>et al</i> (1958)
Water	Alkalinity (expressed as CaCO ₃)			Aliquots of water (200 ml) titrated with standard HCl (0.05M) using methyl red as indicator	Dye (1958), Thomas and Lynch (1960)

* Sundaram, unpublished

of samples to be chosen, previous spray history of plots, field sampling, packing, storing and transportation etc. This facilitated planning of the work. Based on residue assessment requirements, a tentative analytical program was worked out for a particular research project, depending on the overall facilities available and the work load at the Laboratory; otherwise appropriate modifications were introduced.

As a preliminary step, literature surveys were made to ascertain what analytical methods were available for a specific purpose, and if required, methodology was either developed or the existing ones were modified and then standardized by performing trial experiments using spiked samples.

After completion of the practical work, the results were processed, spot checks were made if necessary, and a copy of the completed results was sent to the scientist concerned or if the program was a collaborative one, suitable interpretations and appraisals were given on the basis of residue analytical data obtained (Sundaram 1972a and b). The remnants of the analytical samples were stored for some time and then discarded, when all the determinations were completed and no further verifications were necessary.

CONTRIBUTIONS BY THE ANALYTICAL SERVICE

The Analytical Service is playing an important role in the various research programs of the Canadian Forestry Service by providing rapidly and on schedule, necessary residue determinations

and suitable interpretations. Guidance and advisory service on pesticide handling and formulations are also provided for the research personnel. During the past year, the Service Laboratory has carried out various types of analysis (see Table 2) of organochlorine and organophosphorus pesticides on a total of 942 samples obtained from such diverse substrates as animal tissues, insects, plants, water, soil and air. Residue determinations at ppm and ppb levels were made possible by the availability of analytical techniques, sensitive instruments, adequate facilities and trained personnel. Compared to private analytical service organizations, the overall cost involved in the analysis of a sample is nearly half.

Major research projects to which the Analytical Service has contributed, in addition to service work, are outlined below.

(1) Determination of DDT and its metabolites showed their persistence, but not to hazardous levels, in the tissues of small mammals (Sundaram 1972a and b) collected from DDT-contaminated forest areas, six years after the cessation of DDT spraying. The residue concentrations in the animal tissues were in proportion to soil DDT content (Yule et al 1972).

(2) Studies of methoxychlor treatment for the control of white pine weevil in plantations showed that the compound is effective in weevil eradication and ecologically safe, due to its normal degradation period in the field, with a half-life of 21 days (Sundaram 1972c).

(3) Studies of distribution, persistence and fate of fenitrothion in various samples of water, soil, foliage and insects showed its ready degradability (enzymatic and chemical) and low biological magnification compared to DDT and justifying it as a safer insecticide than DDT for controlling insect pests of Canadian forests.

(4) Analysis and critical evaluation of aerial spray deposits of fenitrothion collected on glass plates kept on the ground confirmed that in many spray operations only about 6% of the active ingredient reached the ground and that formulation development with the objective of improving recovery efficiency should play an integral part in the Institute's research programs.

RECOMMENDATIONS AND COMMENTS

Further development and full utilization of the Analytical Laboratory to meet current needs and problems are dependent in large measure on the following:

1. A rigorous planning of proposed research projects with collaborating researchers, to discuss early in the project, the purpose and scope of analysis, plot selection and sample collection, is of vital importance, if the end results are to be meaningful. In some cases, researchers may not be aware of the capabilities and limitations of the analytical techniques employed in

residue determinations. It must be emphasized that residue data can be precisely determined but may lead to inaccurate conclusions if there is inadequate communication by researchers.

2. Continuous research is necessary for development of sensitive analytical techniques by GC accompanied by confirmative determinations by other supplementary techniques, e.g. derivatization, TLC and spectrophotometric methods. Recent developments in quantitation and identification by combining GC and mass spectrometry can yield valuable and sensitive residue assessments and should be examined.
3. Attention and research should be directed to pesticide formulations and physical variations that affect their behaviour particularly their toxicity and persistence characteristics.
4. Methodology research and service function developments need an adequate and stable knowledgeable core of trained technical staff to maintain efficient functioning of the laboratory. Adequate space, facilities and material support are other essential requirements for this end.

It is hoped that in the near future many of these problems will be solved and the research and service functions of the Unit will *parallel* the Institute's expanding programs.

ACKNOWLEDGEMENTS

The successful operation and maintenance of the Analytical Service Laboratory required the full cooperation and assistance of all the technical staff of the Laboratory. The author is indebted to Gordon G. Smith, W. O'Brien, P. Davis and D. Bonnett for their technical assistance and cooperation which contributed greatly to the development of the Service Laboratory.

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APPENDIX I

Common, trade and chemical names of pesticides
mentioned in the text or tables

Common or trade name	Chemical name
DDT	Dichlorodiphenyltrichloromethane
<u>p,p'</u> -DDT	1,1,1-trichloro-2, 2-bis(<u>p</u> -chlorophenyl) ethane
<u>o,p'</u> -DDT	1,1,1-trichloro-2(<u>p</u> -chlorophenyl)2-(<u>o</u> -chlorophenyl) ethane
DDE	1,1-dichloro-2, 2-bis(<u>p</u> -chlorophenyl) ethene
Fenitrothion	0,0-dimethyl 0-(3 methyl-4-nitrophenyl) phosphorothioate
Fenitrothion	0,0-dimethyl 0-(3 methyl-4-nitrophenyl) phosphate
Methoxychlor	1,1,1-trichloro-2, 2-bis(<u>p</u> -methoxyphenyl) ethane
MCE (methoxychlor ethene)	1,1-dichloro-2, 2-bis(<u>p</u> -methoxyphenyl) ethene
Gardona [®]	2-chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate