Quality Assurance in Pesticide Sampling and Analysis

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Specifications for Quality Assurance program

1.1 Introduction

To achieve consistency in data sets and to assist in interpretation and comparison of results, at the beginning of this program, the Land and Water Resources R&D Corporation (LWRRDC) requested that common protocols be established for sampling, data production and reduction, validation and completeness, for use by the separate projects of the joint program. It was recognised that the methods of analysis vary slightly to suit local needs of each laboratory and project, but a common basis was necessary for assessment of data, to provide consistency and for valid comparisons to be possible. LWRRDC also requested that a Quality Assurance project (QA) be conducted as part of the Minimising the Riverine Environmental Impact of Pesticides R&D Program. Both the original recommendations for sampling and analysis and the results of the QA are described in this publication.

Most of the methods used by the research groups and laboratories involved in the program are based on recommended procedures of the US EPA or the Australian Standards Association; being validated. These should therefore produce high quality data.

1.2 Objectives

The quality assurance objectives involve measurements of data in terms of precision, accuracy, representativeness, completeness and ease of comparison.

The three main analytical laboratories involved in the program (Agricultural Chemistry, QDPI, Indooroopilly, Queensland; BCRI, NSW Agriculture, Rydalmere; NSW Department of Water Resources—now Department of Land and Water Conservation, Arncliffe) had NATA (National Association of Testing Authorities) accreditation and each was experienced in analysis of the full range of the pesticides whose behaviour is to be investigated in the program. Therefore, high standards of analysis were expected to be maintained. In cases where analyses were conducted elsewhere, careful reference to and use of procedures used in these laboratories was expected to be maintained.

In addition, to ensure consistency of data sets throughout the program, a small proportion (ca. 5%) of soil, water or other samples analysed in any laboratory were required to be confirmed by analyses of the same samples in at least one of the other laboratories participating in the program. Full details of the quality assurance program utilised in this program follow.

The Quality Assurance (QA) program proposed was designed to:

- Control and document the laboratory processes in general use in each of the participating laboratories. These are documented as appendices in this Manual.
- Through a program of exchanged samples from the various experimental sites, to conduct an analytical program to verify the reproducibility and general good quality of the data collected in each participating laboratory.
- The QA program was administered and assessed by the University of Sydney, a participant in the research of the joint program but one with an independent role, with relatively minor involvement in routine pesticide analysis. The results of the QA program were to be reported in a manner enabling the end-users of the results of the joint program to assess the data quality.

1.3 General principles

It was expected that normal operating procedures in each of the laboratories would provide mechanisms for identifying and correcting laboratory errors, as detailed elsewhere in this manual.

The laboratory QA programs were expected to control and document each step of the analytical process. For example, in each of the participating laboratories, it was expected that this would be accomplished by controlling and documenting:

- Laboratory capability—using established criteria for facilities, staff, equipment, and operational procedures to ensure that the laboratory has the ability to do quality work.
- Laboratory performance—by monitoring the day-to-day performance of the laboratory through the use of control samples to ensure consistency of quality performance.
- Matrix effects—by using matrix spikes, matrix duplicates, matrix spike duplicates, matrix surrogate recoveries, matrix standard additions, etc to assess the affect of the sample matrix on method performance and data quality.

As the main analytical laboratories involved in the joint program are NATA accredited (NSWDLWC, BCRI—NSW Agriculture, QDPI—Indooroopilly), this level of control was expected to be automatically applied. Other laboratory analyses in the program should ensure similar standards. In addition, an integral part of the program was the reporting of sufficient QC information to allow the joint program as a whole to form an assessment. Any reported information was to be supported by extensive documentation kept on file in the laboratory but available to those concerned upon request.

1.4 QA analytical program

Schedules for the interchange of analytical samples were circulated. These schedules catered for the following aspects:

- Analyses of samples were included for all aspects of the joint program, including soil, water runoff including sediments, drift and volatilisation, and foliage. In addition, all projects within the joint program were expected to participate by supplying samples of an appropriate nature for interchange (including rain simulator and ecotoxicology studies).
- An orderly shipment of samples from field sites was ensured by circulated spreadsheet schedules. QA samples were requested to be given priority for analysis, allowing corrections in procedures, indicated as necessary, to be made as soon as possible.
- An intensive period of sample interchange occurred during 1994 and 1995, and some sample interchange was to continue throughout the life of the joint program.

1.5 Criteria for acceptability

Based on comparisons of data from the various field sites obtained in the first year of the joint program, there did not appear to be major anomalies, although significant improvements were made in sampling and analytical procedures as a result of this experience. In particular, the effectiveness of methods for analysing runoff samples with a heavy sediment load were examined.

Given the fact that it is well known that repeated analyses of environmental samples for pesticides have standard errors often about one-third to one-half of the mean value, it was considered as undesirable to be too prescriptive about exact numerical criteria for assessment of QA. However, it is suggested that analyses of the same samples by different laboratories involved in the joint program should be expected to overlap in the same range of values in this variance range. Where multiple samples have been analysed (eg. of field soil cores), the variance of the data should fall in the same range for each of the different laboratories.

Standard statistical procedures for reproducibility were to be applied to the data, and the results expressed in a convenient graphical form.

1.6 Reporting of QA information

Most monitoring programs do not specify that any quality control (QC) data be reported with the analytical data. As a result, it is virtually impossible for the end-user to evaluate the quality of the data received. One program that is notable for the 'deliveries' required is the USEPA/CLP (Contract Lab Program). Deliveries specified by this program include all QC information plus all raw data generated during the analytical process. While some information above and beyond a table of analytical results is needed to evaluate data quality, it is debatable that the average user of environmental data quality, has the expertise to use and evaluate a data package which includes all of the raw data.

As a compromise between these 'all or nothing' approaches, a policy of preparing the following information for each data set in annual reports or in the Protocols Manual was recommended for the joint program:

- analytical results, reported with appropriate significant figures;
- reporting limits for each analyte (substance being analysed);
- method reference;
- results of laboratory control samples;
- results of reagent blanks;
- commentary on any anomalies encountered during the analysis; and
- recoveries of surrogates.

This information was expected to be extensively documented in material kept on file in the laboratory and available to the end-user upon request. This documentation should include:

- all raw data from environmental and QC samples;
- all calibration data associated with the samples;
- standard operating procedures which define each step of the analytical process (available at each laboratory—many of these are given in the appendices of the manual);
- descriptions of facilities, equipment and staff of the laboratory; and
- accreditations held by the laboratory.

The results of the QA program were to be used to monitor and improve analytical methods. In addition, a report of the QA program was to be made by the University of Sydney, to the annual workshop, and a final report at the end of the joint program.

Recommended protocols—sample collection and storage

As a result of consultation within the joint program, a set of recommendations were assembled. Their validity was assessed as part of the QA program. The recommendations, including modifications made during the program, are presented here

Each sample container should be clearly marked using permanent ink or laser-printed labels with the following information:

- date and time of collection;
- place of collection; and
- sample type and identification.

A chain of custody procedure would ensure the legitimacy of each sample. Logbooks and sample collection forms should contain information such as:

- site of sampling;
- date and time sampled;
- sample identification code;
- sample matrix (soil, water, composite);
- treatment such as preservation, if any;
- identity of sampler;
- method of transport;
- destination;
- specific analyses required (if applicable);
- date and time of arrival in laboratory; and
- name and signature of person taking custody.

The analytical laboratory should be advised (by fax) when samples are despatched, indicating number and type of samples and estimated arrival time.

2.1 Soil sampling

All analytical data for soil was to be reported on a moisture-free basis, using dry weight obtained in a forced-air oven on exposed soil sub-samples dried overnight at 60–105°C. Particularly with volatile pesticides such as endosulfan, care should be taken to prevent losses by using soil samples for extraction with minimum preparation.

Thorough mixing of soil, preferably at the time of sampling to ensure homogeneity for sub-sampling, was to be carried out on a stainless steel or glass tray using a clean or disposable spatula. Equipment can be cleaned by brushing, washed with detergent and tap water, rinsed in acetone or isopropanol, and rinsed three times with clean tap water and allowed to air dry or by use of a clean paper towel. Alternatively, soil samples could be mechanically mixed in the laboratory using equipment such as the Robot Coupe Processor R301 Ultra with stainless steel bowl.

2.1.1 Storage

Soil samples are best stored in airtight solvent-washed or brand-new glass jars, verified as pesticide-free, sealed with aluminium or Teflon foil liner, fitted with new plastic screw-caps (see Australian Standard 2031.1, 1986). Jars should be transported to the field with caps fitted, to minimise the possibility of contamination. Field samples should be cooled to 4°C or less as soon as possible for transportation and then held in a deep-freeze until it is possible to perform solvent extraction. The use of polythene bags for storage at ambient temperature, of material freshly sprayed with pesticides such as endosulfan, is not encouraged, unless samples can be extracted immediately.

2.1.2 Samples

Each soil sample should be selected from a bulked composite of at least 10 well mixed cores, to reduce the sample variance. Depth will depend on the purpose of sampling and the nature of each chemical. For estimating total soil burden of a chemical not subject to leaching (such as endosulfan), either 5 or 10 cm deep cores would be appropriate, or to cultivation depth. For greater sensitivity in detecting recently applied chemicals, 2.5 cm samples taken carefully with a calibrated trowel, would be preferable. Sampling depths must be specified. To assist in comparisons between sites, presentations of residue data as a total burden per unit area of cotton fields (g/ha) should be provided as an alternative to giving concentrations of residues in soil in mg/kg (ppm).

It could be appropriate on cotton fields to recognise at least three classes of cores, from the tops (T) of beds adjacent to cotton plants, from the edges (E) of irrigation furrows (more prone to erosion) and from the base of furrows (F), where eroded soil may accumulate.

2.1.3 Sampling design

Selection of soil cores on cotton fields should be based on a properly justified sampling design. While the nature of this design will depend on the factors to be examined, wherever possible an accepted sampling design suitable for statistical analysis should be used. Stratified designs favoured at the Narrabri and Warren sites, are described in Appendix 1. Based on the degree of uniformity indicated by statistical analyses of data from such designs, it was later considered that a zigzag transect of at least 200 metres, with random sampling of 10 cores for preparing a well-mixed composite sample, would be adequate. For each cotton field of ca. 50 ha, a minimum of five such composite samples was found to be required.

Sufficient samples should be taken to establish spatial variability in pesticide residues. Samples taken for such purposes should be clearly labelled, indicating location in the field, whether from top, the particular edge or bottom of furrow, and depth.

2.2 Water/sediment samples

Extraction was to be performed on water or turbid water to obtain total pesticide content, or after removal of particulate matter by filtration (GFA, 1.2 mm glass membranes) or centrifugation at 2,000 rpm in glass vessels, with separate extraction of water (soluble or colloidal fraction) and sediment where sediment loads are significant. The sediment load should be determined on all water samples, to establish the eroded fraction. The concentration of pesticide residues should be given as mg/L (ppb).

2.2.1 Storage

Water samples of 1 L should be stored in solvent-washed or brand-new (amber) glass bottles verified as uncontaminated, sealed with aluminium foil or Teflon, fitted with new plastic screw-caps (see Australian Standard 2031.1, 1986) and chilled immediately to less than 4°C in a refrigerator. Organic solvent (eg. dichloromethane) can be added immediately where convenient to limit volatilisation or hydrolysis, although care to prevent leakage is essential. Extraction of water samples with organic solvent should be made within 48 hours and immediately on receipt. Even so, it can be anticipated that samples containing endosulfan isomers will lose chemical by volatilisation if jars are not properly sealed, ideally with Teflon (Guerin and Kennedy, 1992), or by hydrolysis if the pH of the water is above 8. Freezing of samples for longer-term storage may also be desirable where facilities are adequate, provided bottles are only half-filled to prevent breakage and there are no other problems. The half-life of endosulfan by chemical hydrolysis to endosulfan diol in river water at pH 8.5 is claimed to be less than two days at ambient temperatures (Peterson and Batley, 1993), and it may be desirable to adjust pH to below 7 by addition of phosphate buffer (pH 6) or acetate buffer (pH 5.4) to 1–5mM, even for cold storage.

Although a number of pesticides were examined during the joint program, endosulfan was the main chemical examined particularly at the early stages of the research. For that reason, the QA program focussed on endosulfan since it was being analysed by each laboratory involved and it was considered that this chemical provided a convenient model for other pesticides.

2.3 Foliage

Methods are required to estimate the retention of pesticides on cotton plants, as well as to provide estimates of that washed off in rain and losses by others means such as volatilisation.

The extraction of plant or vegetable material may present special problems, related to the range of chemicals including pigments that occur in plant tissues. Tissues are normally homogenised to improve the efficiency of extraction using acetone-hexane or acetone-dichloromethane and clean-up is achieved on a silica column, such as Florisil. A decision whether results will be expressed as mg/kg or per unit area needs to be made, so that the surface area of a sample weight of leaves used for extraction can be estimated using a suitable planimeter.

2.3.1 Storage

Plant material may metabolise pesticides such as endosulfan rapidly, so it is important to extract leaves as soon as possible, or to freeze samples immediately before extraction later. Substantial quantities of endosulfan sulphate are formed in cotton leaves from endosulfan formulations within several days (Coleman and Kennedy, 1993).

2.4 Drift samples

Down-wind samples in drift studies (Centre for Pesticide Application Safety, C-PAS, University of Queensland, Gatton) were collected on mobile 10 m telescopic towers fitted with vertical copper wires, aluminium cylinders, nylon gauze, or pipe cleaners, or on filter papers of ca. 200 cm^2 attached by elastic bands to aluminium tables supported on wooden dowelling at crop height. Wires were cut into appropriate lengths, wound on aluminium rods and samples stored in glass containers (eg. McCartney bottles) sealed with aluminium foil. All collector media were stored by the same method and Nanograde acetone was added (5–10 mL) as soon as possible to prevent losses by volatilisation (of endosulfan), although immediate storage of samples held in the deep freeze was also effective.

2.5 Bottom sediments

Corers capable of sampling sediments under water were developed at the NSW EPA Centre For Environmental Toxicology (J. Chapman). An effective method of sampling sediments is by using a PVC tube fitted with a screw-cap seal following insertion of the tube in sediments under water (S. Kimber, University of Sydney).

Extraction of field samples

All solvents must be verified as contaminant-free prior to use, equivalent to Nanograde (contaminant-free solvent).

3.1 Dichloromethane extraction of water samples

At the Biological and Chemical Research Institute (BCRI), NSW Agriculture, water samples were extracted two or three times with dichloromethane, the extract concentrated and the sample cleaned up on an alumina column. The eluate, diluted in hexane, was gas chromatographed on a 30 m capillary column and quantitated by electron capture detection.

The method used at the NSW Department of Land and Water Conservation laboratory at Arncliffe (laboratory code WELOR101) is based on USEPA Method 3510, ‘Separatory Funnel Liquid-Liquid Extraction’, for the isolation and concentration of organic compounds from test water samples.

The sample, either as received or pH adjusted, was carefully extracted three times with dichloromethane (in a separating funnel). The extract, dried with anhydrous sodium sulphate, was then concentrated using a Kuderna-Danish flask fitted with a 10 mL graduated tube and a three ball Snyder Column. The solvent was exchanged to the specific solvent required for either the analysis or the clean-up stage.

Full details of the scope of use, reagents, apparatus, precautions and procedures are given in Appendix 2.

3.2 Extraction of soil for GLC

Extraction of pesticides from soil requires more polar solvents than hexane or dichloromethane alone. A mixed extracting solvent with added acetone, or the use of methanol as the primary extracting solvent, provides improved extraction of residues. The effectiveness of whichever solvent is used should be verified by spiking of soil with pesticide standards and by using surrogate analytes such as dibutylchlorandate or octachlorobiphenyl, to estimate extraction efficiency and subsequent handling of extracts in the laboratory.

A procedure, developed using acetone-dichloromethane, in the Department of Agricultural Chemistry and Soil Science, University of Sydney, for analysis of endosulfan residues including endosulfan sulphate, endosulfan ether, endosulfan hydroxy ether and endosulfan diol in cotton-growing soil (Kimber and Kennedy, in preparation), is fully described in Appendix 3. (Dichloromethane has the advantage of non-flammability, although its characteristics as a organochlorine should be respected). The procedure has been optimised for recovery of these compounds (better than 80% of recovery of each), while allowing the removal of several substances that would interfere with gas chromatographic analysis by electron capture detection of peaks, particularly when using packed columns but also with capillary columns.

3.3 Extraction of foliage

The extraction of foliage should be by blending with a mixture of dichloromethane and acetone (80:20) in an omnimixer or by soxhlet extraction. Extraction efficiencies should be established by spiking foliage samples at four different levels, one very close but slightly above the detention limit of the method with replication to establish the variation of the method.

The procedure employed at the BCRI, NSW Agriculture, is as follows. Place leaf sample into extraction thimble within an appropriate concentration of surrogate standard. Extract via soxhlet extraction using 25 mL of dichloromethane:methanol (80:20) for at least four hours. Concentrate to 5 mL and exchange the solvent to n-hexane. Transfer concentrated extract to 18 cm plastic (polythene) membranes and shake for 24 hours in 100 mL cyclohexane. Concentrate to 5 mL. Pass 2ml aliquot through 1g Al_2O_3 column and elute with 10 mL of 8% acetone in hexane solution. Submit for GC analysis at a final volume of 25 mL containing an appropriate concentration of internal standard.

Another method using acetone-hexane as a solvent (see Appendix 3) was extensively adapted from one developed by Chopra and Mahfong (1977) for extraction of endosulfan and metabolites from tobacco leaves. It has been employed for study of the rate of dissipation of endosulfan from cotton leaves (Coleman and Kennedy, 1993).

3.4 Cleanup

Batches of solid supports (eg. alumina, Florisil, etc.) should be pre-assessed for their ability to retain polar compounds and to allow elution of pesticide residues. The cleanup system used for removing coextractives from extracts should be calibrated by eluting mixtures of standards of interest through the cleanup columns and the elution pattern and volume recorded. Each time a new batch of alumina or silica gel is made the elution pattern and volume should be redetermined.

3.5 Extraction of soil for immunoassays (ELISA)

An immunoassay method for the detection of endosulfan in water and soil has been developed (Lee *et al.*, 1995; Lee *et al.*, 1997). Soil (10 g) is weighed moist into a glass jar, 25 mL of 90% methanol added and the sealed sample shaken and stored overnight. Endosulfan content is then estimated on 100 µL of the extract following a minimum dilution of 1/100 in water (Lee *et al.*, 1997). The dry weight of soil is measured following thorough air and oven drying of the sample on removal of the solvent. More details are found in Appendix 5.

Gas chromatographic analysis

4.1 Preface

All aspects of good laboratory practice are expected to be in force in analytical laboratories. All sample handling, extraction, cleanup, analysis, chromatography and confirmation should be referenced to the laboratory manual and standard operating procedures (SOPs). In reporting results, the precision and accuracy limits as defined in the standard operating procedure of a method should be maintained. Duplicate analyses with more than 15% coefficient of variation should be repeated for that sample. Data on precision and accuracy should be provided for each matrix by replicate analyses of spiked samples just above the detection limits of the method and at three other spike levels for establishing linearity of each analyte. All negative results should be reported as below these established detection limits rather than as not detected.

4.2 Gas chromatographs and chromatographic columns

Analytical instruments should be maintained as specified by manufacturers. Laboratory personnel responsible for performing such preventive maintenance should record this in log books available for inspection. No sample analysis can be conducted with instruments with not meeting performance specifications.

Each analysis should be confirmed on columns of different polarity, reporting both sets of results. Ideally, a split injection system with two columns fitted to separate detectors may be used to ensure verification of the identity of peaks.

It is anticipated that analysis will usually be performed on capillary columns, thus reducing problems of sample cleanup. However, a packed column providing a separation of all the endosulfan breakdown products was described by Guerin *et al.* (1992).

4.3 Standards

Calibration standards, like internal standards, should be prepared from certified standard material and kept no longer than six months with new preparations being evaluated against the old standard. All standard solution compositions, concentration and lot numbers should be recorded in the log books.

Every tenth field sample should be analysed in duplicate and each batch of ten should have a matrix blank, a reagent blank and one spike recovery at 0.1 mg/g of analytes of interest, such as a-endosulfan, b-endosulfan and endosulfan sulphate.

4.3.1 Matrix spike samples and blanks

Matrix spike and duplicate matrix samples shall also be analysed and results of these analysis submitted with each batch of 10 or 20 samples. Blanks should be analysed at the frequency of one in 20 samples. All the blanks should be fortified with surrogate compounds.

4.3.2 Internal standard and calibration standards

All analyses performed on GC/MS will require internal standards for analyte quantisation. Internal standards shall be prepared from certified reference standards. No solution should be kept longer than six months. All new preparations of the internal standard solution need to be verified against the old solution to ensure acceptability and records kept in the standard log book.

4.3.3 Surrogate and matrix spike standards

All surrogate and matrix spike standards should be prepared from certified reference standards and control charts for recoveries should be maintained. Information regarding analyte concentration, composition and lot numbers should be recorded for traceability purposes. No solution should be kept longer than three months. New preparations of surrogate and matrix spike solution should be analysed prior to use to ensure correct concentration levels and absence of any contaminants. The concentration of surrogates should be approximately 0.2ppm in solid matrices and 1 ppb in liquid.

4.3.4 Data analysis, validation and reporting

Preliminary data reduction performed in laboratories in this program should include analyte identification, contaminant or interference identification, elimination of false positives and manual quantisation of analyte concentration (eg. GC/MS) when peak splitting occurs. The separation of chromatographic peaks should be to the baseline and the minimum height of the reported peak should be at least 10% of the total chart deflection. Peak heights of standards and samples should not exceed 15%.

Analyses of blanks should demonstrate that reagents and glassware are clean and interference-free. Data from chromatograms must be collated and reviewed by a qualified chemist for each set of samples. Raw data relating to these samples should be examined for consistency with calculated results and spot checks on some of the calculations performed manually. Summary reports should be signed by the chemist and made available for inspection by the supervisor.

4.4 Calibration procedures

4.4.1 Instruments

All instruments used in the production of data for these projects should be calibrated according to procedures as prescribed in the SOPs of each method. For example, balances should be calibrated at least once according to manufacture's specifications and the calibration log book maintained for each balance. Class S weights should be used to verify electronic calibration.

4.4.2 GC/ECD

Gas liquid chromatograph should be calibrated at four levels of standard, one very close but above the noise level of the instrument and then at intervals of 10 times for each analyte. Retention time windows for each analyte should be established at base line separation. These retention times should be established before each batch of analysis. Instrument log books shall be maintained and will contain information regarding the usage and maintenance, problems and corrective actions. Prior to any sample analysis the GC operating condition must be maintained so that p,p'-DDT has a retention time of between 23–28 minutes on a DB_5 capillary column 30 metres long and of 0.32 mm internal diameter. Following this adjustment the retention time windows of each analyte must be determined. Calibrated GC operation mandates the analysis of various standards before sample analysis. The calibration will remain valid for 72 hours if the individual component pesticide standard meet acceptance criteria during this period. Recalibration will be mandatory after 72 hours.

4.4.3 GC/Mass Spectrometry

Prior to any standard, sample, or blank analysis the GC/MS system hardware must be tuned to meet the ion abundance criteria for the analysis of two reference standards such as decafluorotriphenylphosphine and p-bromo-fluorobenzene. Then a calibration derived from 20, 50, 80, 120 and 160 ng shall be utilised to assess the concentration of semi-volatile compounds. Once the system is calibrated the calibration must be verified (tuned and 50 ng standard analysis) once per each 12 hour period of the operation. Recalibration will be required if the 50 ng standard fails to meet acceptable criteria.

Quality assurance in chemical sampling and analysis

5.1 Objectives

1. To control and document the laboratory processes in general use in each of the participating laboratories. This will be documented in a manual on sampling and analytical protocols. It is anticipated that the manual will be gradually improved up to the end of the joint program, but an advanced version of the manual is currently available.

2. To conduct an analytical program to verify the reproducibility and general good quality of data collected in each participating laboratory, by analysis of exchanged samples from the various experimental sites.

3. To administer the QA program and report the appropriate information in a manner enabling the end-users of the results of the joint program to assess the data quality.

5.2 Progress and conclusions to date

Each of the main analytical laboratories involved (BCRI (N. Ahmad) Rydalmere, NSW Agriculture; Agricultural Chemistry (B. Simpson) Indooroopilly, QDPI; Arncliffe NSW Water Resources (A. Awad) has provided information on laboratory capacity and performance, including methods of quality control (matrix effects, 'spikes', etc.). Inspections were made of each laboratory, standard laboratory protocols and primary data generation examined. This information is included elsewhere in this manual (eg. see Appendices).

An interchange program was successfully conducted between all three main laboratories. This resulted in improved procedures for sampling, storage and analysis, particularly considering the volatility of endosulfan. This program has verified that variations between these three laboratories is quite small (CV of mean values = 10–15% only), so that strong confidence in the accuracy of the data is justified. This degree of variation is much lower than in that found in most comparisons between commercial laboratories. In addition, immunoassays being arranged by the University of Sydney (CRC for Sustainable Cotton Production) and performed by CSIRO Plant Industry, have been included in this program and excellent agreement between chemical analyses and immunoassays has been demonstrated.

5.3 Schedule

The schedule used for the interchange of analytical samples is given in Table 5.1. It catered for the following aspects:

1. Analyses of samples included from all aspects of the joint program, including soil, water runoff including sediments, drift and volatilisation, and foliage. In addition, all projects within the joint program participated by supplying samples of an appropriate nature for interchange (including rain simulator and ecotoxicology studies).

2. It was expected that QA samples were given priority for analysis, to allow any corrections in procedures, indicated as necessary, to be made as soon as possible.

3. An intensive period of sample interchange occurred in October–December 1994 to prove the quality of current procedures.

Some variations to the original schedule were made after the first analyses proved to be very consistent in their results. Thus, only a few samplings marked with one asterisk in Table 5.1 were performed using standard analytical procedures (GLC/MS), and those marked with a cross were compared for both GC and immunoassay analyses. Some sample interchange continued throughout the life of the overall joint program.

Table 5.1
Initial sample interchange schedule

No.	Date	Sample (No.)	Source	Analyses	Laboratories
1*	1993–94	Soil (6)	Emerald	12	BCRI, QDPI
2	Nov-94	Runoff (6)	Emerald	12	BCRI, QDPI
3		Soil (6)	Emerald	12	
4^{+}		Rain simulator (6)	Warren	12	BCRI, QDPI
5	Dec-94	Runoff (6)	Warren	12	BCRI, QDPI
6*		Runoff (6)	Warren	12	BCRI, DLWC
7		Soil (6)	Warren	12	BCRI, QDPI
8^{+}		Drift (5)	Warren	10	BCRI, Gatton
9*	Jan-95	Runoff (6)	Emerald	12	BCRI, QDPI
10		Soil (6)	Emerald	12	BCRI, QDPI
11		Foliage (5)	Warren	10	BCRI, QDPI
12	Feb-95	Runoff (6)	Warren	12	BCRI, QDPI
13*		Soil (6)	Warren	12	BCRI, QDPI

5.4 Stability and homogeneity of samples

In conducting QA studies it was important that the analyte be kept stable under likely transport and storage conditions. Soil and water samples were stored in airtight brand new glass jars, sealed with aluminium foil or Teflon sheet (Ludowici), fitted with new plastic screw caps (see Australian Standard 20331.1, 1986), and chilled to less than 4°C as soon as they were taken. The samples were placed in an insulated box for transportation, and then held in a deep-freeze until the solvent extraction was performed. Dispatch of samples was made by express courier.

To ensure soil samples homogeneity, soil cores were thoroughly mixed and then split into two sets of samples, one for each laboratory. This step was of fundamental importance, without which QA results between laboratories could not be compared.

5.5 Analysis of data

Standard statistical procedures were used to compare the results form the different laboratories, and the results are expressed in a convenient graphical form. When comparing two types of analyses on the same sample, eg. GC and immunoassay, a correlation was calculated. When more than two laboratories were involved in the analysis an ANOVA was applied to the data. In the case for each pair of results (Youden pairs), the sum and the difference were tabulated. The sum value reflects the between-laboratory reproducibility of a participant's results compared to the other laboratory. The difference value reflects how well the within-laboratory repeatability compares.

5.5.1 Soil samples

Sets of six soil samples from Emerald and five samples from Warren were analysed at QDPI and BCRI and the results are shown in Figures 5.1 and 5.2. There was agreement for total endosulfan results and for each of the components (alpha, beta and sulphate), and the coefficient of variation for the replicated Emerald set was acceptable (22.9%), but slightly lower values were consistently obtained with this set by the BCRI. However, this discrepancy can be explained because those samples were stored for several weeks, partly at ambient temperature, before being sent to that laboratory, and the data are consistent with some volatilisation, particularly of the alpha-isomer. In the other set (Figure 5.2), the soil samples were analysed more promptly by both laboratories and no consistent difference between the laboratories was obtained.

Both endosulfan isomers, alpha and beta, showed more variable results than those of sulphate, confirming the difficulty in accurately analysing these volatile components in soil samples. The overall variation found among soil samples is higher than that of other type of samples, about 15% in the best case.

Figure 5.1
Soil samples from Emerald (September 1994)

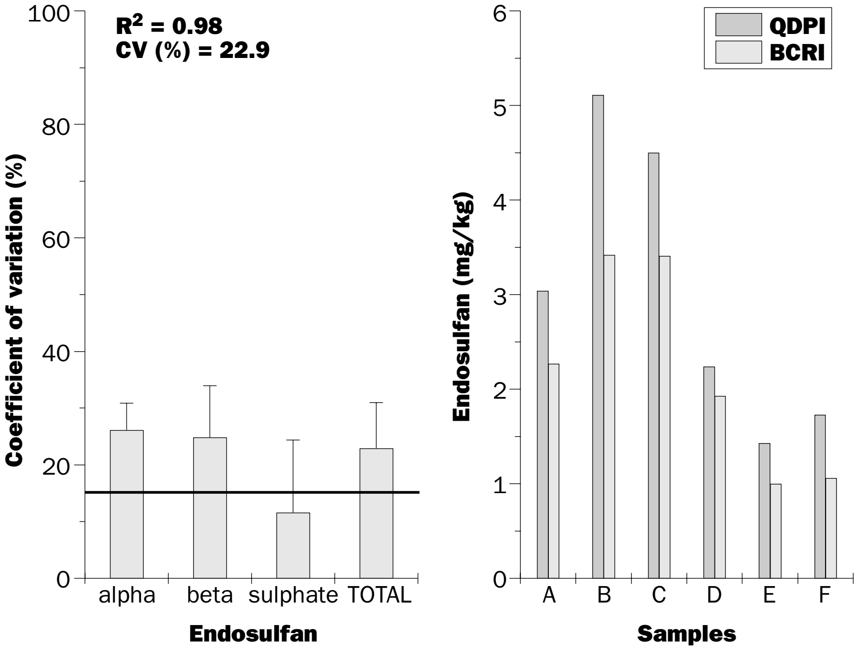


Figure 5.2
Soil samples from Warren (17 March 1995)

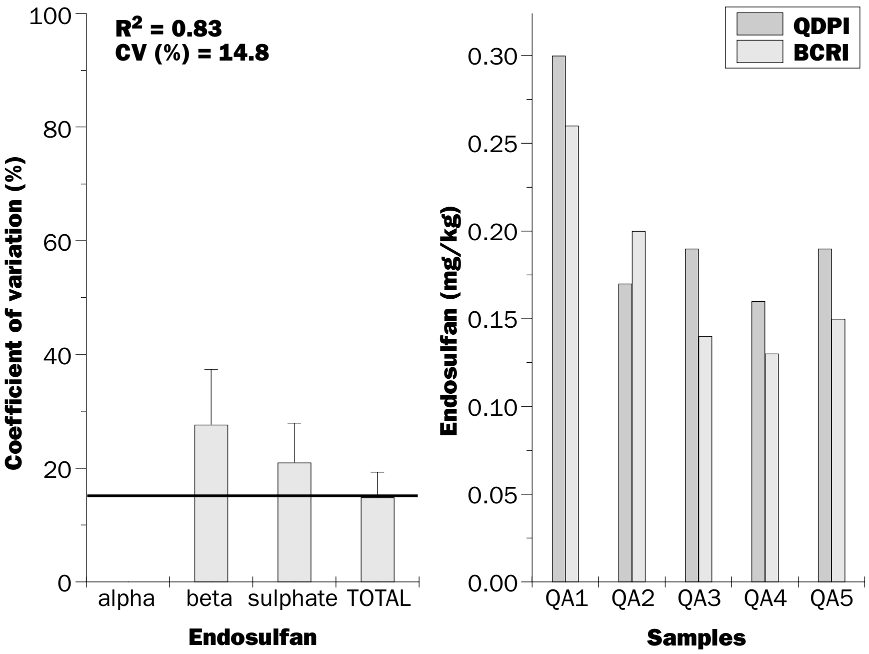


Figure 5.3a
Water runoff from Warren (24 November 1994)

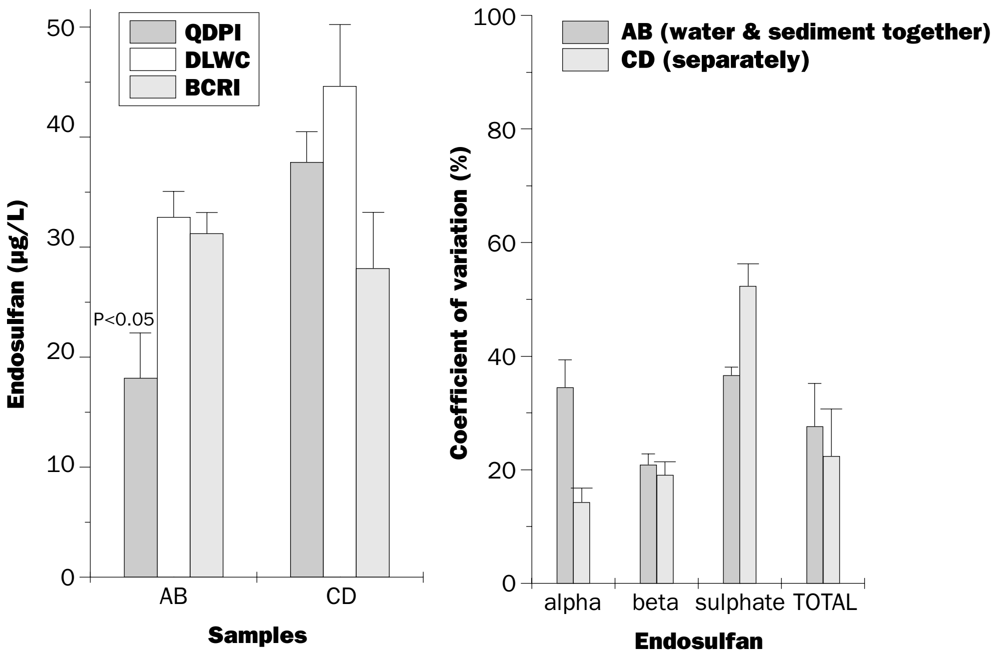
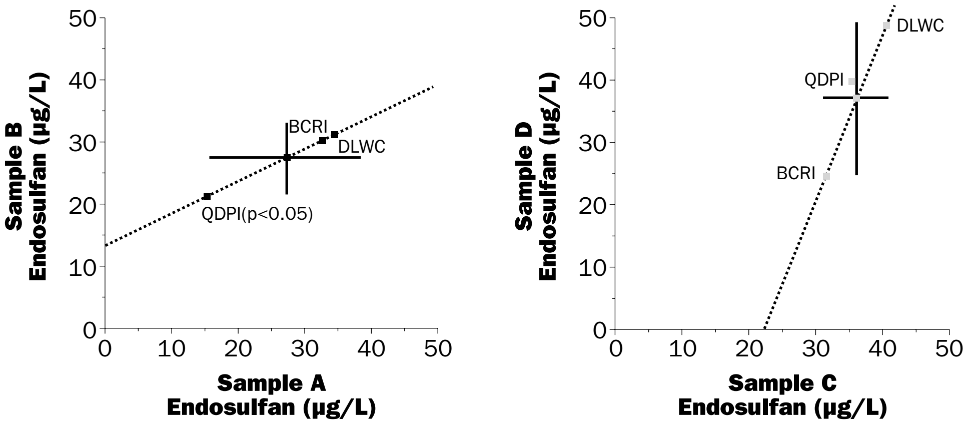


Figure 5.3b
Youden plots for analysis of water runoff from Warren (24 November 1994)

(i) Extracting the entire sample, water and sediment, together (A,B);
(ii) Extracting water and sediment fractions separately (C,D)



(i)

(ii)

Figure 5.4a
Water runoff from Warren (24 November 1994)

Results of the water and sediment fractions analysed separately (C,D)

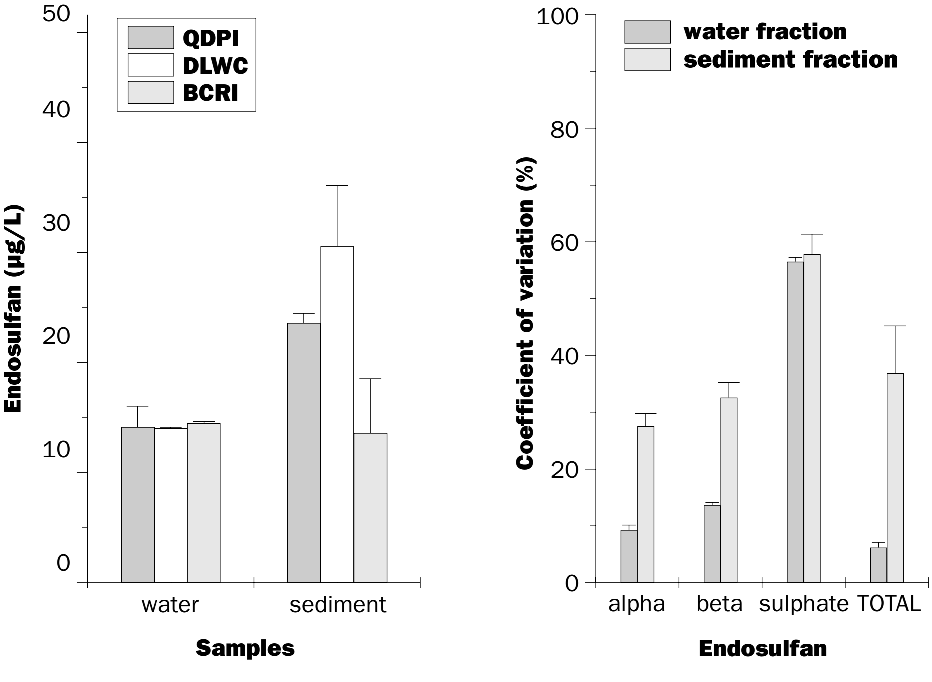
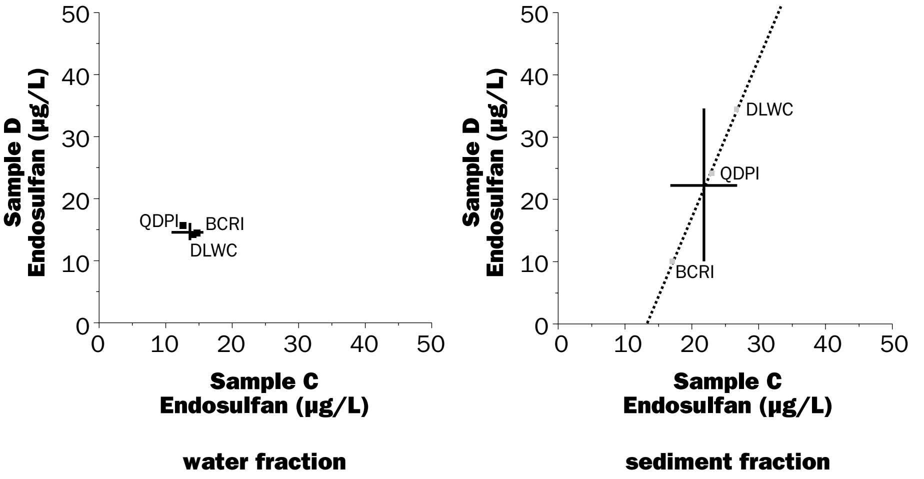


Figure 5.4b
Youden plots for above analyses of water runoff



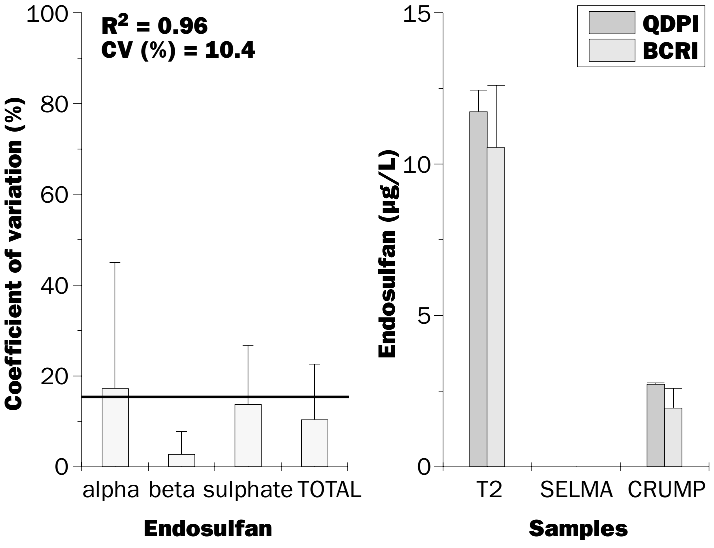
5.5.2 Water runoff samples

Two pairs of water runoff with a moderate sediment load from rain simulator studies conducted at Warren were analysed by all three laboratories. The results are shown in Figures 5.3 and 5.4.The laboratories were asked to analyse these samples using two procedures: (I) the standard EPA method simply of three dichloromethane extractions in a separating funnel, followed by combinations of the extracts (samples A and B); (ii) with a preliminary separation of the sediment by filtration or centrifugation, followed by extraction of the water by the EPA method and extraction of the sediment separately (samples C and D).

The first procedure showed a significant difference between the results of QDPI and the other two laboratories, for both alpha and beta isomers as well as for the total endosulfan. This discrepancy, however, disappeared when the laboratories used the second procedure (Figure 5.3, ii). In this case the results for water fraction are similar for all three laboratories, except those of endosulfan sulphate which varied considerably. The same problem was found in the analysis of the sediment fraction (Figure 5.4). It seems that endosulfan sulphate is harder to extract reproducibly from water samples than the two isomers, contrary to what happens in soil, no matter what procedure is used by the laboratories. This might explain the relatively higher variation found between the three laboratories (22.4% and 27.6%), although the overall agreement of the mean values was satisfactory.

These comparisons (and others) suggest that runoff (or river) samples can be analysed quite accurately by the standard EPA method provided the sediment load does not significantly exceed 0.1% (w/v). Thus, the routine analysis of river samples by this EPA method (as used by the NSW Department of Land and Water Conservation), provided it is conducted carefully, yields accurate values. However, for runoff in storms or simulated storms (sediments of 1% (w/v) or greater) separation beforehand is probably essential for adequate recovery of residues from the sediment fraction. A second test with three pairs of water samples from Emerald analysed at the Queensland Department of Natural Resources (Brisbane) and NSW Agriculture in Sydney showed better results, with a variation of only 10.4% (Figure 5.5). Again, most of the variation was found in the alpha isomer.

Figure 5.5
Water samples from Emerald (December 1994)



5.5.3 Drift and volatilisation samples

A set of 10 drift samples were compared using two analytical procedures, immunoassay and GC, for residue analysis. The results showed a good agreement ($R^2 = 0.77$, Figure 5.6), though immunoassay analysis gave higher values than GC analysis (about 10%). The variation within immunoassay results is larger than that of GC, but this could be due to the smaller quantity of analyte used by this technique. In other comparisons of volatilised endosulfan trapped in water trays, using both GC analysis and immunoassay, the trends of residue concentrations were established to be very similar (data not shown here).

Figure 5.6
Drift samples from Warren (21 December 1994)

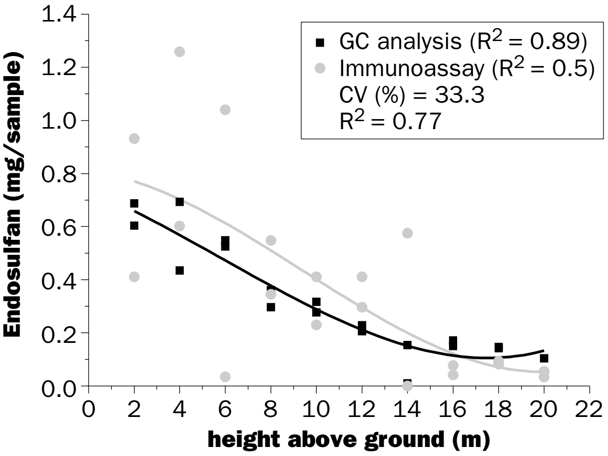
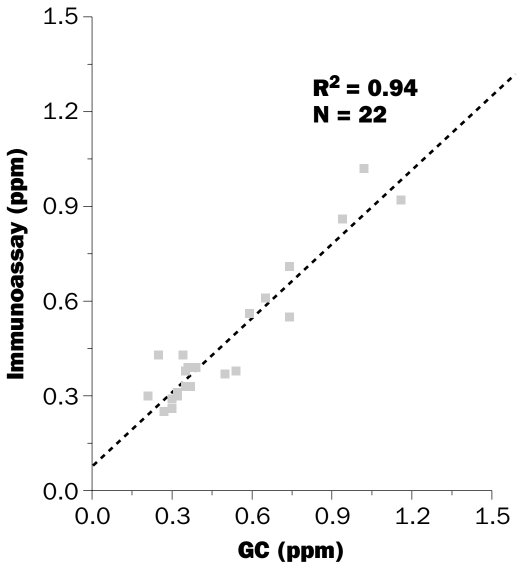


Figure 5.7
Immunoassay validation of soil samples from Warren (1994–95)



5.5.4 Immunoassays

Immunoassays are being widely applied to this research program (Kennedy *et al.*, 1997). Quality assurance for this technique has been established by comparing analytical data obtained by gas chromatography and that obtained by immunoassays. It needs to be understood that the endosulfan immunoassay provides the sum of the toxic forms (ie. both isomers and endosulfan sulphate), with slightly differing sensitivities for each form. However, excellent agreement is being obtained (see Figure 5.7 for data obtained with soil samples, R^2=0.94) between the two methods, verifying the validity of the immunoassay technique. Provided water samples are extracted in the field or are transported chilled very promptly and extracted immediately on receipt, excellent agreement is also being obtained with runoff samples (Lee *et al.,* 1997; M. Silburn, N. Ahmad, pers. com.).

5.5.5 Foliage samples—Helix

Methods for Helix (chlorfluazuron) analysis are poorly developed. No reliable method using gas chromatography is currently available. HPLC methods, however, produce satisfactory results—although the limits of detection are at least ten times less sensitive. A replicated set of six foliage samples from the fieldsite at Warren were analysed both by AnalChem Bioassay (Lilyfield, NSW) as well as at the BCRI at Rydalmere and the data communicated independently to the University of Sydney. As shown in Table 5.2, satisfactory agreement between the two laboratories (apart from one sample) was obtained.

Table 5.2
Foliage samples from Warren (1995) analysed for chlorfluazuron.

	Chlorfluazuron (mg/kg)	
Sample ID	**AnalChem**	**BCRI**
A	15.17	13.65
B	4.06	2.06
C	3.08	2.46
D	10.49	3.09
E	5.73	3.57
F	<0.1	<0.005

5.5.6 Other laboratories—sediments

Quality assurance data was obtained for the University of Queensland (Gatton, N. Woods) using field samples obtained at Warren; these were also analysed at the BCRI, and by immunoassays/Mass Spectrometry at the University of Sydney. Data was also requested from projects on the ecotoxicology of endosulfan and pyrethroids conducted by NSW EPA (J. Chapman/R. Hyne). The latter program does not involve large numbers of field samples, but it was desirable that a similar level of quality assurance also be exercised here. Validation of the analytical data on pesticide residues in sediments of the ecotoxicology project was obtained from analysis conducted by the NSW Department of Land and Water Conservation laboratory at Arncliffe and the EPA laboratory at Lidcombe. In Table 5.3 an example of comparisons for endosulfan sulphate in sediments for data obtained from the two laboratories is shown—apart from one sample (possibly a false positive) there was good agreement.

Table 5.3
Endosulfan sulphate (mg kg^{-1}) found in sediment samples

Sample ID	EPA Lab	NSW L&W
2 I	8.0	<2
3 II	3.5	4
5 II	8.5	6
6 II	9.1	10
6 III	8.0	9
Pian Creek	4.4	4

5.6 Conclusion

As a result of the QA program and discussions arising from it, improved procedures have been adopted in all the participating laboratories. In general however, no significant disagreement in analytical data has been observed in comparisons between the laboratories.

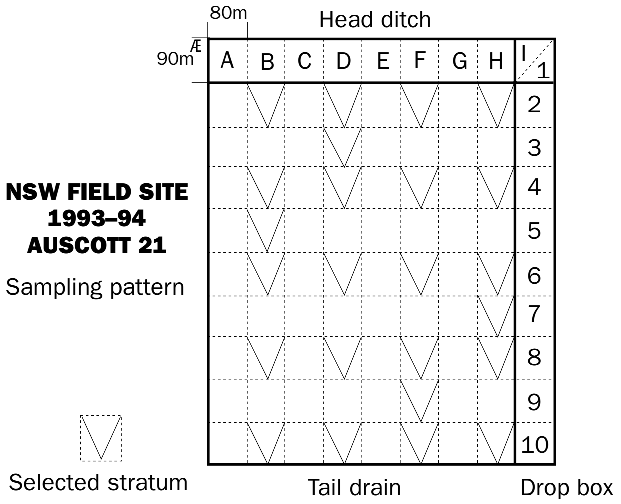
It is concluded that the data being obtained in all laboratories examined in the joint program is both accurate and sufficiently precise. Thus, the conclusions from the research program are based on data assured to be of high quality. The methods used here may also prove to be useful to other research workers interested in the environmental fate of pesticides.

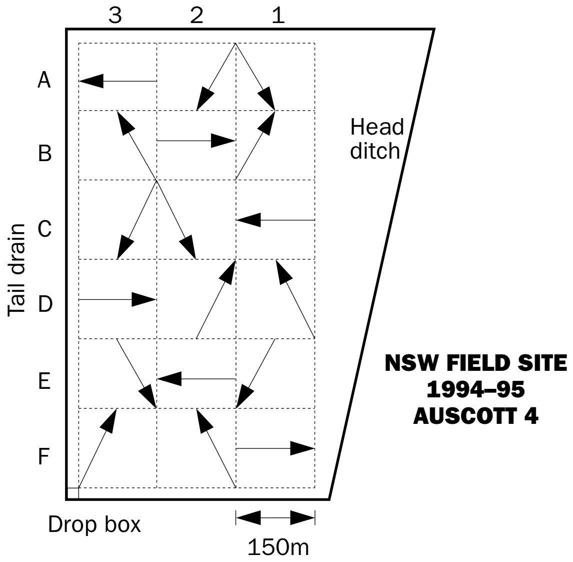
The results of the QA program continue to be used to monitor and improve methods. In this manual, presented by the end of the joint program by the University of Sydney, QA data from all the laboratories involved is presented, as part of an upgraded protocols manual.

APPENDIX 1

Sampling designs at the Narrabri and Warren field sites

1. The NSW field site on the Auscott property at Narrabri, Field 21 (1993–94 season) was marked into 90 approximately square strata each of 80–90 m sides, with surrounding buffer zones. For the 1994–95 season, Field 4 on the Auscott property at Warren was marked into 18 square strata of 150 m sides. In both sites a sub-sampling design for the strata has been selected (see diagram) to allow representative values and spatial variation to be assessed statistically.
2. As indicated above, soil cores can be collected from the tops, edges of planting beds or in the furrows.
3. Initially, to reduce the variance of soil samples, composite samples of 20 soil cores were collected, using a well-mixed sub-sample of each core representative of its full depth. Subsequent statistical analysis indicated that a composite of 10 soil cores was sufficient.
4. Soil cores were selected at regular intervals (alternate beds) on a V-intersection of each stratum at the Narrabri site, or at random lines across each stratum at the Warren site (see diagrams).
5. It is anticipated that soil analysis will be less sensitive to spatial trends than alternative procedures such as analysis of runoff water and sediments. However, it is considered essential to perform both kinds of analyses to establish correlations and seasonal and management practices.





APPENDIX 2

Dichloromethane extraction of water samples

A.2.1 Scope

The procedure is suitable for the extraction of the classes of organic chemicals listed in Table A.2.1

Table A.2.1
List of the classes of organic compounds for which the method is applicable.

Chemical Class	Analytical Method WELOR101	Extraction pH*	Final Solvent
Organochlorine	201	A, B or C	Hexane
Polychlorinated Biphenyls	202	A, B or C	Hexane
Pyrethroid	203	A, B or C	Hexane
Organo-Nitrogen and Phosphorus	204	B	Hexane
Organophosphates	205	B	Hexane
Organosulphur	206	B	Hexane
Pesticides by GC/MS	301	B	Dichloromethane or Hexane
Phenols	302	C	Propan-2-ol
Polycyclic Aromatic Hydrocarbons	303, 402	A, B or C	Dichloromethane, Acetonitrile or Hexane

* The following sample pH requirements are dependant on the mixture of analytes to be determined and the analytical method used:

A: Extraction as received

B: Extraction after addition of phosphate buffer (pH 6–8)

C: Adjust pH 1–2 with sulphuric acid before extraction.

NOTE: Sample treatment, and extraction solvents are entered onto a worksheet.

A.2.2 Precautions

Major sources of error are contamination, in particular, phthalate esters from plastics. Teflon or glass containers should be used. All glassware should be solvent rinsed after use, then washed. After washing, contaminated glassware should be heated in a furnace to 400°C before re-use.

Pesticide grade solvents (or all-glass distilled solvent) are used to minimise interferences. New batches of solvents, reagents and reagent water must be checked for any interference problems. Reagent water is prepared using a Milli-Q system.

Solvents, such as dichloromethane can have toxic vapours whilst others such as ether, hexane and acetone are highly flammable. All extraction and concentration steps are to be conducted in a well ventilated fume cupboard.

Some reagents and pesticides may be carcinogenic. All samples and reagents should be treated as potentially toxic. Neutralise and clean up any spills immediately. In addition to protective clothing and safety glasses, wear gloves when handling any suspected toxic materials.

A.2.3 Reagents

Unless otherwise specified, all reagents are of AR grade or equivalent. The calibration standards, check, spike and surrogate samples are prepared from certified reference materials/solutions.

A.2.3.1 Chemicals

Sodium sulphate-granular (10–60 mesh) anhydrous: heat at 450°C for not less than four hours in shallow silica dishes before use.

Cotton wool (hexane washed): soak the cotton wool in a Schott bottle for 10 min with hexane, decant and rinse three times with hexane, then air dry.

NOTE: after washing, do not touch the cotton wool with hands or gloves, only use tweezers.

A.2.3.2 Reagent Solutions

Phosphate buffer: prepared from 1M HCl (75 mL) and 1M dipotassium hydrogen phosphate (125 mL), made up to 2,000 mL.

A.2.3.3 Standard Solutions

Prepare stock solutions from certified reference materials at a concentration of 200 µg/mL. Acetone is generally used as the dilutant and the resultant solutions are stored in amber bottles with Teflon lined caps.

Prepare spike mixes and surrogates from the stock solutions and make up to volume with methanol or acetone as appropriate. Store all solutions in amber bottles with Teflon lined caps at or below 4°C; in a flame-proof refrigerator.

A.2.4. Apparatus

Table A.2.2
List of preparative apparatus used

Apparatus	Specifications
Separating Funnel	Glass, fitted with a Teflon stopcock and a glass stopper. Capacity appropriate to the sample volume to be extracted.
Filter Funnel	Glass, 75–100 mm diameter with a hexane-washed cotton wool plug.
Graduated Tube	10 mL, glass with a B14 socket
Kuderna-Danish Flask	500 mL, with a B14 lower joint and a B19 top joint and fitted with hooks to attach (by springs) to the graduated tube.
3 Ball Snyder Column:	Approx. 20 cm with B19 upper and lower joints.
Water Bath	Six hole, with temperature control to ± 5°C.

A.2.5 Quality control protocol at sample extraction

Each batch of samples includes: reagent blanks (10% of samples, minimum one) recovery blanks and recovery samples (5–10% of samples, minimum one). The samples for recovery data are **spiked with a mixture of certified standards** appropriate to the samples being analysed. Similar mixtures of certified standards (10% of samples) are analysed with each batch of samples to **cover the range and concentration levels** expected in the samples tested.

Prior to sample extraction, the Section Head must record on liquid-liquid extraction worksheets the appropriate information. Ensure that all of the above quality control requirements are fulfilled. Enter onto the worksheet the spiking mixes and surrogates to be used with the volume and concentration levels. Determine any special requirements of the client (analytes, detection levels) and/or analytical method, and record these details on the worksheet.

A.2.6 Procedure

The following procedure is routinely followed for water quality samples. Any difference in the sample treatment or extraction solvent must be recorded on a worksheet.

1. Add a 500 mL aliquot of the water sample to the separating funnel. Prepare a batch as per worksheet list (to include reagent blanks, duplicates, replicates, recovery blanks and recovery samples).

2. Depending on the analytical method, check the pH of the sample, adjust the pH or add phosphate buffer (25 mL) as indicated in Table A.2.1.

3. Add surrogate standards, if indicated on the worksheet, to all of the samples and spiking mixes.

4. Add dichloromethane (30 mL) to the separating funnel seal, then shake for two minutes with frequent venting at the stopcock.

CAUTION: Vent immediately after the initial shake to prevent build-up of pressure.

5. Allow the two layers to separate and collect the organic layer in an Erlenmeyer flask. If an emulsion forms, and cannot be separated, transfer the organic phase plus the emulsion to a smaller separating funnel.

6. Repeat steps 4 and 5 twice and combine the three extracts. If an emulsion has formed, combine all extracts into a small separating funnel, remove the organic phase then add saturated aqueous sodium chloride to break up the remaining emulsion. Add additional dichloromethane to ensure quantitative extraction of the organic compounds.

7. Consult the analytical method to determine the extraction conditions for the relevant group of analytes. If a second extraction is required at a different pH, adjust the pH level and repeat steps 4 to 6. Combine the three extracts.

8. Depending on the analytical method, extracts obtained at different pH values are combined or treated separately. These modifications are noted in the procedures for individual methods.

9. Attach by springs (or clips), a 10 mL graduated tube to a 500 mL Kuderna-Danish (K-D) flask. Filter the organic extract collected in the Erlenmeyer flask (step 5) into the K-D flask through a filter funnel containing anhydrous sodium sulphate. Rinse the Erlenmeyer flask three times with 10 mL of dichloromethane into a filter funnel.

10. Add carborundum boiling chips, attach a Snyder Column which was prewet with dichloromethane (about 1 mL). Place on the water bath at 80–90°C and concentrate to about 5 mL.

11. For solvent exchange, leave the apparatus on the water bath, add 3 x 5 mL portions of the exchange solvent and concentrate to about 5 mL before each addition.
12. Remove from the water bath and allow to cool. Rinse the walls of the K-D with a minimal volume of solvent.
13. If no further treatment is required before the analysis, adjust the volume in the graduated tube to the required volume (usually 5 mL). This can be accomplished by the addition of solvent or removal of the excess of solvent (evaporation with a stream of nitrogen over the surface). The normal procedure is to place a 1 mL aliquot into a vial for analysis and the remainder into a suitable amber vial with a Teflon lined cap. Store this reserve sample below 4°C.
14. If further clean-up or derivatisation is required, proceed from step 11 as required for the analytes or class of analytes to be determined.

APPENDIX 3

Extraction of soil and foliage

A.3.1 Extraction procedure for endosulfan residues in soil

A.3.1.1 NSW Agriculture, BCRI

The method employed for extraction of soil by NSW Agriculture Biological and Chemical Research Institute (BCRI-120) is a modification of that of Sission *et al.* (1968) and de Faubert Maunder (1964). Organics are extracted by shaking with a solvent mixture of Nanograde dichloromethane and acetone (80:20, v/v). The coextractives are removed from the concentrated extract on an alumina column and the eluate is chromatographed on a 30 m capillary column and quantitated by electron capture detection.

A.3.1.2 University of Sydney

The method developed at the University of Sydney (CRDC project, US2C) involves the following procedure given in detail.

1. Thoroughly mix soil samples with a clean spatula before sub-sampling. Sieving of moist cracking soils to remove debris is impossible and drying to facilitate this would result in losses of volatile chemicals such as endosulfan (Spencer and Cliath, 1975).
2. Weigh a 25 g sample accurately into a clean 250 mL conical flask sealed with a ground-glass stopper. Weigh accurately a further 10 g sample onto a watch-glass for moisture determination by drying for two days at 110°C. Re-weigh and determine moisture content by difference.
3. Extract the soil in the conical flask with 150 mL of 25% acetone in dichloromethane by shaking on an orbital shaker at 165 rpm overnight (Braun Certomat provides reliability).
4. Decant the solvent carefully through a fluted filter paper containing 1–2 g of anhydrous Na_2SO_4 and collect 75.0 mL for analysis.
5. Transfer 75.0 mL to a Kuderna-Danish flask and evaporate to 5 mL.
6. Prepare a clean-up glass chromatography column using a cotton wool plug to retain 7 g of alumina (7% H_2O, w/w), overlaid with 1 g of anhydrous Na_2SO_4.
7. Apply the concentrated sample to the clean-up column and elute with 60 mL hexane followed by 60 mL 25% acetone in hexane. Collect and discard the first 10 mL of eluate (this fraction contains principally DDE) and collect the remaining eluate in a Kuderna-Danish apparatus, ready for concentration.
8. Concentrate the eluate to less than 10 mL and rinse with hexane. (It is essential to ensure that all dichloromethane is removed because it has a high electron capturing ability.)
9. Reduce the sample to less than 5 mL under a flow of nitrogen gas, transfer quantitatively to a 10 mL volumetric flask and make up to volume with Nanograde hexane.

Alternatively, the evaporation steps using the Kuderna Danish apparatus may be replaced with TURBO-VAP equipment using automatic evaporation with heat and nitrogen gas to a specific volume. Such TURBO-VAP equipment is now available including collection of evaporated solvent, avoiding the use of a fume cupboard with environmental dispersion into the atmosphere.

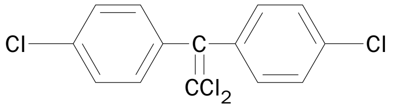
It should be noted that endosulfan lactone must be analysed independently and that endosulfan diol, although recovered well, is analysed with consistently low sensitivity, apparently as a result of degradation on-column, even with capillary columns using low temperature injection with temperature ramping. Fortunately, diol peak heights are constant with a given set of analytical conditions, but a method involving derivatisation could be necessary to obtain comparable sensitivity.

A.3.1.2 Queensland Department of Primary Industries

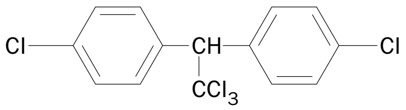
This method describes the procedure for the extraction, identification, quantisation and confirmation of the endosulfan and some related pesticides listed in Table A.3.1 in soil.

Table A.3.1
Chlorinated Pesticides

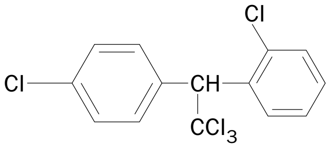
p,p¢-DDE
$C_{14}H_9Cl_4$
Mol. Wt. 319
1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene



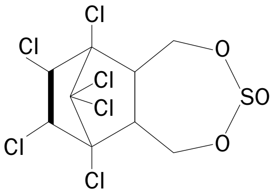
p,p¢-DDT
$C_{14}H_9Cl_5$
Mol. Wt. 354.5
l,l,l-trichloro-2,2-bis (4-chlorophenyl)
ethane



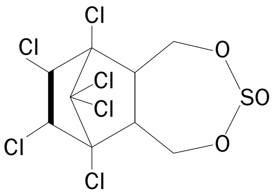
o,p¢-DDT
$C_{14}H_9Cl_5$
Mol. Wt. 354.5
l,l,l-trichloro-2
(2-chlorophenyl)-2(4-chlorophenyl) ethane



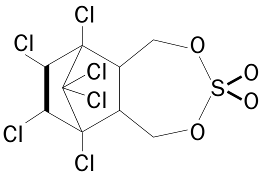
α-Endosulfan
$C_9H_6Cl_6O_3S$
Mol. Wt. 406.9
6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo[e]-dioxathiepin-3-oxide



β-Endosulfan
$C_9H_6Cl_6O_3S$
Mol. Wt. 406.9
6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo[e]-dioxathiepin-3-oxide



Endosulfan sulphate
$C_9H_6Cl_6O_4S$
Mol. Wt. 422.9
6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3,3-dioxide



A.3.1.3.1 Referenced documents

The following document is referred to in this method: *Pesticide Chemistry Quality Manual*

A.3.1.3.2 Definitions

The ‘Detection Limit’ is the lowest level of an individual compound. To quantify that the method can confidently confirm at least the presence of an individual compound.

Detection Limit for Method: 0.002 mg/kg (generally).

This figure is a guide only and varies from one pesticide to another as well as from sample to sample and depending on the manner of reporting.

A.3.1.3.3 Principle

The sample is extracted with methanol/water. The pesticides are extracted into *n*-hexane from aqueous methanol. The hexane phase is dried over sodium sulphate and cleaned up on a Florisil column. The chlorinated pesticides present in the resultant concentrate are determined by GLC and identity confirmed by GC-MS.

A.3.1.3.4 Reagents

Solvents: Methanol, *n*-hexane, diethyl ether, iso-octane

Chemicals: Deionised water, Sodium chloride (LR grade) in deionised water (saturated solution). Whatman glass microfibre filters (GF/A) (9.0cm). Anhydrous granular sodium sulphate (AR Grade), Florisil. (60–100 mesh P.R.)

Standard solutions: The following standard solutions are prepared and stored in accordance with procedures described in the Pesticide Chemistry Quality Manual.

1. *Bulk Solutions*: Prepare individual stock solutions in iso-octane of each of the following pesticides at a concentration of 200 mg/L in 50 mL volumetric flasks.

    a-endosulfan
    b-endosulfan
    endosulfan sulphate
    p,p´-DDE
    o,p´-DDT
    p,p´-DDT
    trifluralin.

2. *Intermediate solution:* Prepare the following composite solutions in iso-octane at a concentration of 2 mg/L of each standard. All intermediate solutions are stored in a freezer and discarded every six months.

    OC-1:
    a-endosulfan
    b-endosulfan
    endosulfan sulphate
    p,p´-DDE
    o,p´-DDT
    p,p´-DDT
    trifluralin.

3. *Working solutions*: Prepare working solutions from the intermediate standards at a concentration of 0.02, 0.10 and 0.2 mg/L of each standard material in hexane. Working solutions are stored in a refrigerator. Fresh standards are prepared and compared to the old standards before the old standards are discarded.

A.3.1.3.5 Apparatus

500ml Quick-fit Erlenmeyer flasks, Vacuum filtration apparatus, Rotary evaporator, Water bath, Gas chromatograph/EC detector, Glass column (OV-1), 500ml round bottom flasks, 500ml separating funnels, 100ml stoppered measuring cylinders.

A.3.1.3.6. Sampling and sample preparation

Refer to the Pesticide Chemistry Quality Manual.

A.3.1.3.7. Procedure

Preliminary screen

Because of the possibility that some soil samples could contain very high levels of pesticide residues, a preliminary screening is advisable for samples with no background information to minimise the possibility of contamination of the laboratory and/or of the analytical apparatus. Before commencing the analysis proper, weigh soil (5 g) into a stoppered test tube. Add hexane (10mL). Insert a soni-probe for three minutes and then filter a small quantity through glass fibre paper. Assess the likely level in the soil by GLC with a Hall detector.

Moisture content

In general, most soil samples submitted for analysis are analysed and reported on a ‘dry weight’ basis. In such cases, weigh sample (20 g) into a tared moisture dish. Dry overnight at 105°C. Allow to cool in a desiccator and reweigh. Calculate the % moisture.

Residue analysis

All soil samples are air dried at 40^{0}C for 48 hours and ground to a particle size, no greater than, 2mm.

Weigh sample (25 g) into a Quickfit conical flask (500 mL). Add methanol (100 mL) and water (25 mL). Record the volume of water added. Stopper and shake for one hour on a wrist-action shaker. Filter under vacuum through glass fibre filter paper and record the volume. Transfer the extract to a separating funnel (500 mL) and add hexane (50 mL).

Shake vigorously for two minutes and add sodium chloride solution (10 mL) and water (300 mL). Shake the mixture gently, but thoroughly, for two minutes and allow to settle.

Discard the aqueous layer after the layers separate. Transfer the hexane solution to a stoppered measuring cylinder (100 mL) and record the volume. Add sodium sulphate (» 5 g) and shake. Allow to stand for at least one hour to dry the hexane.

Prepare a Florisil column by packing a 2.0 cm i.d. column (fitted with a fritted glass disc and tap) to a height of 9 cm with Florisil (» 25 g) which has been dried at 105°C. Introduce a layer of sodium sulphate (» 1 cm) to cover the Florisil. Wash the column with hexane (50 mL) before use.

Transfer the sample solution to the column and allow to run at two drops/sec into a round-bottomed flask (500 mL). Elute (*See note*) the column with:

(a) ether/hexane (20+80 v/v) (100 mL) and

(b) ether/hexane (60+40v/v)(150mL) Collect the eluate separately and evaporate to »5mL on a rotary evaporator with water bath at 40°C. Transfer the concentrate to separate volumetric flasks (10ml) for determination.

NOTE

The second eluate [(60+40v/v) ether/hexane], contains any b-endosulfan and endosulfan sulphate.

A.3.2 Extraction of foliage

The extraction of foliage can be made by blending leaves with a mixture of dichloromethane and acetone (80:20) in an omnimixer. Extraction efficiencies should be established by spiking foliage samples at four different levels, one very close but slightly above the detention limit of the method with replication to establish the variation of the method. This method is currently in use at the BCRI.

Another method is extensively adapted from one developed by Chopra and Mahfong (1977) for extraction of endosulfan and metabolites from tobacco leaves. It has been employed for study of the rate of dissipation of endosulfan from cotton leaves (Coleman and Kennedy, 1993).

The steps of the procedure are:

1. Chopped leaves (ca. 15 g fresh weight) with 20 g Na_2SO_4 plus 5 g NaCl are homogenised in 50 mL of 25% (v/v) acetone in hexane (Nanograde) in a Sorvall Omnimixer equipped with a 250 mL cup for one minute ('spikes' can be added at this stage). The solvent is filtered through 125 mm filter paper containing 6 g anhydrous Na_2SO_4 into a 100 mL round-bottom flask. The homogeniser cup is rinsed with about 20 mL of 25% (v/v) acetone-hexane, with the plant material in the filter.

2. After evaporation to 5 mL, the sample is transferred quantitatively to a 10 mL volumetric flask and made up to volume with hexane.

4. Clean-up is achieved on a glass column (7 mm ID) containing 1.90 g Florisil overlaid with 2–3 cm anhydrous Na_2SO_4. The packed column is rinsed with 10 mL hexane and 5 mL of fresh hexane placed directly in a 50 mL pear-shaped flask placed under the column. 1 mL of the leaf extract is loaded onto the column and allowed to settle into the Na_2SO_4. 30 mL of diethyl ether is flushed through the column.

5. The material in the flask is evaporated to 2–3 mL (rotary vacuum evaporator, eg. Buchi or under a flow of N_2) and transferred quantitatively to a 5 mL volumetric flask and made up to volume with hexane. The solution may be diluted with hexane if necessary for gas chromatographic injection (see below).

This treatment results in removal of most of the plant pigments, but some yellow colour may remain, depending on the carotenoid content of leaves. For samples that will be undiluted before gas chromatographic analysis, additional clean-up from pigments may be obtained by use of a 12 cm column containing 2.50 g Florisil, with about 5–10% less recovery of endosulfan and metabolic products. The procedure provides better than 85% recovery for endosulfan isomers and all breakdown products.

APPENDIX 4

Gas chromatographic analysis

A.4.1. Determination of pesticides using gas chromatography—mass spectrometry

A.4.1.1 Preface

This method (WELOR301) was provided by NSWDLWC and is based on USEPA Method 625, 'Bases/neutrals and acids'. The procedure can be used for the determination of a range of organic classes, including pesticides in complex matrices such as soils, sludges and waste water. It can also be used to complement or to confirm results using a more selective detector. The range of chemicals include the organochlorines and other pesticides which are of concern to the Department. These pesticides are generally used in the agricultural areas of NSW where cotton and rice are harvested.

Modifications to this method are:

a) The primary GC column is a 30m x 0.25mm x 0.25µm DB5 column and a mass selective (MS) detector.

b) Range of analytes.

A.4.1.2 Scope

This procedure has been validated for the pesticides listed in Table A.4.1. The laboratory maintains certified reference materials/standard solutions for the analytes reported. The method is applicable to the determination of pesticides in soils, sediments, sludges and waste water and is complementary to the conventional methods for the determination of pesticides in groundwater, surface water, potable water, and marine water. Other classes of organic compounds for which this method is also applicable are the phenols (WELOR302), polycyclic aromatic hydrocarbons (WELOR303), petroleum hydrocarbons (WELOR304) and organic acid herbicides (WELOR305).

A.4.1.3 Principles

Samples are extracted using an appropriate method (WELOR101,102). The dried extracts are concentrated using a Kuderna-Danish flask and the solvent is exchanged to hexane (or a suitable solvent). The final volume is initially equivalent to a 1:100 fold concentration for water samples and a 1:20 fold concentration for soil samples. Further concentration or dilution is dependent on the level of detection required.

A.4.2 Determination of chlorinated pesticides

Chlorinated pesticides are determined by Electron Capture Detector using an OV-1 GLC column (injection volume 1–5mL).

Instrument Parameters (Varian 3600)

Column (1): 3% OV-1 on Chromosorb WHP 100/120 mesh (7' 5" x 2mm i.d.).

GLC Conditions (Varian 3600)	Column (1) (3% OV-1)
Column Temp.	195°C
Injector Temp.	230°C
Carrier Gas	Nitrogen
Carrier Gas(flow rate)	25 mL/min
Detector	ECD
Column Pressure	7.2 kPa
Detector Oven Temp	350°C

A.4.2.1 Calculations:

(a) The concentration of the pesticide in the sample (on a dry weight basis) is—

$$\text{level(mg/kg)} = \frac{C \times A_1 \times (W_1 + 125) \times 50}{W \times A_2 \times V_1 \times V_2}$$

where C = concentration of the standard (mg/mL)

A_1 = peak area of the sample

A_2 = peak area of the standard

W_1 = weight (in g) of water contained by the test portion of soil

V_1 = recorded volume of the methanol/water filtrate

V_2 = recorded volume of *n*-hexane recovered

W = weight (in g) of the test portion of air dried soil

assuming 100 mL of methanol and 25 mL of water are used to extract the soil sample, and 50 mL of *n*-hexane is used in the partitioning step.

A.4.2.2 Reporting of results:

Examples of the various formats of test reports used are given in the Pesticide Chemistry Quality Manual.

Table A.4.1
List of pesticides for which WELOR301 is applicable.

Compound	**Retention time (min)**	**Primary ion**	**Secondary ion**	**Group**	**Detection Limit *(ng/L)**
Dichlorovos	9.73	109	TI	1	100
Molinate	13.86	126	187	1	50
Demeton-S-methyl	14.91	88	60	2	100
Trifluralin	15.62	306	264	3	50
Phorate	15.83	75	121	3	100
a-BHC	15.95	219	181	3 & 4	100
Thiometon	16.00	88	125	4	100
Dimethoate	16.22	87	93	4	100
Atrazine	16.45	200	215	4	50
Hexachorobenzene	16.55	284	286	4	50
b-BHC	16.57	219	181	4	100
d-BHC	16.73	219	181	4	100
Diazinon	17.12	179	304	4	100
Disulfoton	17.25	88	90	4	100
g-BHYC	17.30	219	181	4	100
Parathion methyl	18.39	263	109	5	100
Heptachlor	18.67	272	274	6	200
Prometryn	18.72	241	226	6	100
Bromacil	19.25	205	207	6	100
Malathion	19.54	125	173	7	200
Aldrin	19.73	66	263	7	100
Chlorpyrifos ethyl	19.90	314	316	7	100
Metolachlor	19.90	162	238	7	100
Pendimethalin	21.00	252	281	8	100
a-Chlordane	21.90	373	375	9	50
a-Endosulfan	22.38	237	265	9	200
g-Chlordane	22.50	373	375	9	50
p,p-DDE	23.33	246	318	10	100
Dieldrin	23.40	79	263	10	200
Oxyfluorofen	23.75	300	252	10	100
Fluazifop butyl	24.43	282	383	11	200
Endrin	24.53	67	345	11	400
b-Endosulfan	24.66	237	265	12	200
p,p-DDD	25.09	235	165	13	200
Endrin Aldehyde	25.48	345	67	13	400
Heptachlorepoxide	20.90	353	357	7	100
Parathion ethyl	21.54	109	291	8	100
Sulprofos	25.84	156	322	13	100
Endosulfan Sulphate	26.55	235	272	14	200
p,p-DDT	26.78	235	237	14	100
Haloxyfop	28.24	302	433	15	100

* The detection limits were determined from reference standards and not recovery samples.

A.4.2.3 Confirmation of reportable residues:

GC-MS Confirmation:	Results determined by GLC with EC detection may be confirmed by GC-MS. Use splitless 1 or 2mL injections. For the MS method, use the SIM mode for 48 ions in three time groups, representing 21 compounds (3 ions per compound).
GLC column	J & W Scientific DB-1 capillary, 30 m x 0.25mm i.d. x 0.25 mm.
Temperatures:	
Injector	250°C
Column	60°C for 2 min, 60°C to 240°C @ 30°C/min 240°C for 10 min
Interface	280°C
Precision:	Information on detector linearity is to be included.
Method Recovery:	Information on the recovery of organochlorine residues from soil is to be included.

A.4.3 Determination of nitrogen and phosphorus containing chemicals using gas chromatography

A.4.3.1 Preface

This method (NSWDLWC WELOR204) is based on USEPA Method 507 for the determination of nitrogen and phosphorus containing pesticides. A gas chromatograph fitted with a nitrogen-phosphorus detector is used.

Modifications from this method are:

a) a 500 mL aliquot volume of sample is extracted

b) methyl tert-butyl ether is replaced by hexane as the exchange solvent

c) primary GC column is 30m x 0.25mm x 0.25µm DB1 column. Confirmation is by use of a 30m x 0.25mm x 0.25µm DB5 column and a mass selective (MS) detector, or a 30m x 0.25µm DB 1301 column and a flame photometric (FP) detector for phosphorus/sulphur containing chemicals.

A.4.3.2 Scope

This procedure has been validated for the analytes listed in Table A.4.2. The laboratory maintains certified reference materials/standard solutions for all analytes reported. The method is applicable to the determination of nitrogen and phosphorus containing chemicals in groundwater, surface, potable, marine and waste waters. Soil and sediment samples can be analysed by this method, or by the use of selective detection (WELOR301).

A.4.3.3 Principles

The aqueous sample is extracted with dichloromethane in a separating funnel. The dried extract is concentrated using a Kuderna-Danish flask and solvent exchanged to hexane (or suitable solvent) to a final volume equivalent to a 1:100 fold concentration.

The final extract is analysed by gas chromatography using a nitrogen phosphorus detector. Confirmation is carried out by GC with MS detection, or by the use of a GCV column of different polarity with an FP detector.

Table A.4.2
Nitrogen and phosphorus containing pesticides for which WELOR204 is applicable.

Compound[a]	CAS Number	Method Detection Limit (μg/L)	Acceptable Recovery Range (%)
Acephate	30560–19–1	0.1	70–130[d]
Atrazine	1912–24–9	0.1	62–122[b]
Bromacil	314–40–9	0.1	61–121[b]
Diazinon	333–41–5	0.1	85–145[b]
Dichlorvos	62–73–7	0.1	67–127[b]
Disulfoton	298–04–4	0.1	59–119[b]
Demeton-S-methyl	919–86–8	0.1	75–135[b]
Chlorpyrifos-ethyl	2921–88–2	0.1	62–122[c]
Metolachlor	51218–45–2	0.1	63–123[b]
Malathion	121–75–5	0.5	40–115[c]
Parathion-methyl	298–00–0	0.1	70–130[d]
Parathion-ethyl	56–38–2	0.1	70–130[d]
Phorate	298–02–2	0.1	70–130[d]
Prometryn	41198–08–7	0.1	63–123[b]
Molinate	7287–19–6	0.1	68–128[b]
Trifluralin	2212–67–1	0.1	51–140[c]
Pendimethalin	1582–09–8	0.1	59–119[c]
Sulprofos	40487–08–7	0.1	68–128[c]
Profenofos	41198–42–1	0.1	57–117[c]
Dimethoate	35400–43–2	0.1	70–130[d]
Thiometon	60–51–5	0.1	70–130[d]

[a] Certified reference standards are maintained for all analytes.

[b] Range obtained using procedure described in USEPA Method 507, (Section 10.3.2. plus results from Table A.4.2). Range quoted is Mean ± 30% or Mean ± 3 x S.D. (whichever is the larger).

[c] Range obtained from results in this laboratory.

[d] When no recovery data are available, the recovery range used is 70–130.

A.4.4 Analysis of endosulfan using gas chromatography

A.4.4.1 Preface

The following protocol was submitted by the Gatton College Analytical Laboratory, University of Queensland, where studies on pesticide drift as part of the overall program are being conducted (contact, Nicholas Wood). This is modified from the USEPA Method 608, to include use of more modern capillary columns. It is reproduced here in full, although it is expected that individual laboratories will use procedures differing slightly in details such as choice of capillary column. It is anticipated that all laboratories concerned in the program will employ capillary columns for routine analyses, however, in case a packed column method is required, an effective column packing previously used at the University of Sydney is SE-30 (5%):DC-200 (5%) on Gaschrom Q, 80–100 mesh, a liquid phase allowing separation of endosulfan isomers, endosulfan sulphate and the full range of degradation products (Guerin *et al.*, 1993). The identity of all peaks will be verified by inclusion of some analyses on columns with a different separation pattern and using internal or external standards for all compounds separated. Alternatively, as indicated here, mass spectrometric verification of peaks can be performed.

USEPA Method 608 prescribes packed columns. The following procedure recommended by Hewlett-Packard (Klee, 1989) substitutes a high resolution capillary column without compromising the specified criteria for EPA-608.

A.4.4.2 Equipment and conditions

Instrument:	Hewlett-Packard 5890A gas chromatograph with HP7673A automatic injector and an HP 3396A integrator.
Detector:	Electron capture detector with N_2 make-up gas.
Detector temperature:	325°C
Carrier gas:	CIG Ultra-High Purity Helium, with indicating moisture and oxygen traps.
Injector port temperature:	190°C
Injection mode:	Splitless
HP 5890A split-vent flow rate:	50 mL/min
HP 5890A split-vent On time:	1 minute
Column:	25 m x 0.2 mm, 0.11 um Ultra-2 capillary column (5% phenyl methyl silicone, part no.19091B-002) at a column head pressure of 30 psi (to maximise effective resolution. Any other column giving good resolution (see section 4.1–4.2).
Injection volume:	0.5 mL
Standards:	Supelco (Bellefonte) or other suppliers and diluted in iso-octane to concentrations of 0.5–3.0 mg/mL.
Analysis time:	For EPA-608 pesticides, 18 min.

A.4.4.3 Optimised GC analysis conditions

Multiple temperature ramp:	80–175°C @ 30°C/min
	175–225°C @ 2.5°C/min
	225–275°C @ 10°C/min

This gives optimum separation of endosulfan isomers from other EPA-608 pesticides. The starting temperature of 80°C is a compromise between stationary-phase focussing and a short analysis time. In this protocol, there should be no 'splitless effect' and sample volume, solvent type and starting oven temperature are not critical.

A.4.4.4 Confirmatory analysis

A separate Hewlett Packard bench-top GC/MS (5890 Series II coupled to a 5790 MSD) will be used as a confirmatory tool.

A.4.4.5 Extraction solvent

Solvents to be tested to extract metal and inert surfaces (targets) include isopropanol and iso-octane (hexane should be suitable for endosulfan). Previously, ethylene digol has been used to remove spray drift dyes from leaf surfaces. A solvent of the type discussed in Section 2.3 should prove superior for this purpose with endosulfan isomers, which can be expected to be absorbed into the leaf tissue.

APPENDIX 5

Immunoassay of endosulfan and diuron

A.5.1 Endosulfan immunoassay

Antibodies were raised as described (Lee *et al.,* 1994).

A.5.1.1 Preparation of endosulfan standard

A stock solution of 100 ppm endosulfan (isomer mixture: 70% alpha and 30% beta) is prepared in methanol. From this stock solution, 100 ppb is prepared by 1/1,000 dilution in purified water and then serially diluted to obtain 100 ppb, 30 ppb, 10 ppb, 3 ppb, 1 ppb, 0.3 ppb and 0.1 ppb in borosilicate glass tubes for the standard curve.

A.5.1.2 Laboratory analysis ELISA

To each antibody-coated plate, 100 µL of endosulfan standard or sample followed by 100 µL HRP-conjugate diluted in PBS or PBS with 0.5% fish gelatin (w/v), are added and incubated for one hour at 20°C. After this incubation, plate contents are removed and the plate is then washed in distilled water. Hydrogen peroxide substrate/chromogen (3,3',5,5"-tetramethylbenzidine-hydrogen peroxide in acetate buffer, pH 5.5, 150 µL) is added and incubated 30 minutes at 20°C. The colour development is stopped by adding 50 µL 1.25M sulfuric acid, and the plate is read at 450 nm.

A.5.1.3 Field analysis ELISA

Four drops (160 µL) of sample and four drops (160 µL) of HRP-conjugate are added to a tube and incubated for 10 minutes at ambient temperature. The tube is washed with tap water four times and shaken vigorously. Then four drops of substrate (300 mL) followed by four drops of chromogen (150 µL) are added to a tube for colour development. After five minutes, four drops of stopping solution (0.625M sulfuric acid) are added, and the absorbance is read with a portable photometer.

A.5.2 Diuron immunoassay

A.5.2.1 Preparation of Diuron Standard

A stock solution of 100 ppm diuron is prepared in methanol. From this stock solution, 100 ppb is prepared by 1/1,000 dilution in purified water and then serially diluted to obtain 5 ppb, 1 ppb, 0.5 ppb, 0.1 ppb, and 0.05 ppb in borosilicate glass tubes for the standard curve.

A.5.2.2 Laboratory and field analysis ELISA

Both laboratory and field analysis for diuron are exactly the same as for endosulfan (see above), using diuron antibody-coated plates and diuron-HRP conjugate.

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