R7528

Report of a Visit to Kutsaga Research Centre to Assess Pesticide Residue Analytical Quality, 18 January - 3 February 2002. Project A0918,Visit No.

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1. Background

1. The natural Resources Institute manages a DFID Crop Post Harvest Research Programme project concerned with food safety issues associated with horticultural produce grown by smallholders in peri-urban areas around Harare (A0918). The analysis of samples (tomato and kale) collected in support of this project is contracted to Kutsaga Research Station (KRS), Harare who test for microbial contaminants and for pesticide residues. Dr Andrew Graffham, the project leader, visited KRS in November 2001 to review their microbiological capabilities and to express some concerns over the delays and quality of the pesticide residue data produced by KRS. It was subsequently agreed by KRS that a programme of analytical quality assessment would be developed by NRI with their pesticide laboratory which would include some comparative analytical studies between KRS and the NRI analytical laboratories. A number of tomato and kale samples were returned to NRI in November 2001 for analysis to allow this comparison to be made during a later visit to KRS when an assessment of the quality of analysis being undertaken would also be carried out. This report details the findings of this assessment.

2. Objectives

2. The objectives of the visit and of the comparative analysis exercise were to:

- Examine and evaluate all aspects of the sampling and laboratory procedures used for the collection and analysis of samples from the NRI/DFID Food Safety Project,
- Assess the quality/skills of the staff involved in the analysis and management of the analytical programme agreed with NRI,
- Make recommendations concerning analytical and data management procedures as considered appropriate.

3. Summary and Recommendations

3. The visit was successful in that a full review of the analytical procedures and working practices was completed. Disappointingly, by the end of the visit, the KRS pesticide laboratory had only completed the analysis of 25% of the agreed number of samples taken in November 2001 for comparative analysis at KRS and NRI. However, the analysis of quality assurance samples prepared by the NRI team at KRS, using reference standard mixtures brought from the UK, was largely completed by the KRS analytical team and these results together with observation of all working practices allowed for a full assessment of operations to be conducted.

4. Areas of weakness were identified and reviewed with the Head of the Pesticide Department, Mrs Rudo Mbulawa, and two of the staff primarily involved in the work. Procedural changes were recommended that would help to minimize the

potential for analytical error. It was stressed that these recommendations must be implemented if Kutsaga were to continue their involvement in the project and this was accepted. The following paragraphs summarise the key concerns and recommendations made.

5. The age of the sample prior to analysis was an initial concern with many of the analyses conducted to date being on material that had been stored for approximately six months due to various laboratory problems (equipment breakdowns etc.). It is difficult to predict whether the integrity of the sample has been compromised after such an extended storage period. Where such delays are expected or have been observed it is normal practice to run residue storage stability trials. This involves the use of test samples which have been laboratory treated with a range of potential analytes and then storedunder identical conditions to the analytical samples. The proportion of residue remaining on analysis, compared with the proportion remaining on samples treated and then analysed immediately, indicates the degree of storage breakdown. In this case, such "recovery" experiments had not been conducted.

6. A second area of concern was with regard to initial sample preparation and mixing and the size and selection of the analytical sub-sample. The procedure for sample mixing, particularly for tomato samples, was inadequate and did not ensure homogeneity of sample. Similarly the size (10g) and the way in which the analytical sub-sample was selected from the frozen, incompletely mixed bulk sample, did not reflect good practice and could contribute to a significant and unquantifiable sampling error.

7. The use of a food processor to improve the quality of the sample preparation/mixing step was strongly recommended together with an increase in the size of the analytical sample. These analytical team accepted these points and in a separate discussion with the Director of KRS, Dr A Masuka, funds were authorized for the immediate local purchase of an appropriate processor. Should project funds permit, it is recommended that a hand-held homogenizer also be purchased specifically to help emulsify tomato extracts.

8. The sample extraction method in use was an old version of the ethyl acetate extraction method and not the revised version that had been provided to KRS before the analytical exercise commenced. It was recommended that the revised version (suitable for a wider range of analytes) be introduced immediately and this recommendation was also accepted.

9. The sample clean-up procedure in use seemed acceptable although it did not appear to have been validated for all of the potential analytes (compounds reported to have been used by farmers and derived from the questionnaires used by KRS when collecting samples). An example of the clean-up apparatus was obtained and returned to NRI for further investigation.

10. The analysis of the crop samples by Gas Liquid Chromatography was seen to be conducted in a conscientious manner by the laboratory staff using the procedures that they had been taught. It was evident, however, that there were deficiencies in these procedures such that there was scope for the mis-identification of analytes (particularly organophosphorous compounds) because of analytes eluting with the

same retention time. The staff also demonstrated an unwavering, but sometimes misguided, faith in the ability of the automatic integration systems to identify and quantify any eluted peaks. Although the chromatographs were calibrated with reference standard mixtures (not always appropriate mixtures for this particular analysis) these were almost always at a single concentration and did not seek to test, for example, the lower limits of analytical determination. These practices demonstrate a naivety in the procedures being used and the in - experience of those involved.

11. One of the problems at Kutsaga is the lack of a qualified senior chemist with the relevant experience to support the wide range of operations with which they are involved. This has restricted the development of expertise by the junior staff and is reflected in their limited knowledge of the requirements of multi-residue pesticide residue analysis. It is thus recommended that a two-week duration training course be run at Kutsaga for key members of the analytical team; further training can be "cascaded down" to other members of staff by those participating in the primary training. It is also recommended that should DFID CPHP funds be available for this institutional support, that the offer of training should be extended to staff at the Department for Extension and Research (DRE) and the University of Zimbabwe. This would involve staff involved in the analysis of samples for pesticide residues in support of a second CPHP food safety project. A suggested, draft training programme is attached as Annex 1 to this report.

12. In the light of the areas of weakness summarized above and the *potential* for significant error, one continuing area of uncertainty is with regard to the quality of the results reported to date by Kutsaga. On balance, and subject to further consideration/discussion with Dr Graffham, it is recommended that these analytical results be discarded as unreliable.

4. Laboratory and Procedural Assessment

13. The assessment of the laboratory operations affecting the capacity and quality of the procedures used for the analysis of the NRI food safety project samples was conducted in three stages;

- Observation of all stages in sample processing using the exact procedures used by Kutsaga in the analysis of previous samples,
- the analysis of prepared quality assurance samples using the Kutsaga analytical procedure,
- the comparative analysis of 28 samples (14 kale, 14 tomatoes) collected in November 2001.

14. This schedule was quite demanding within the relatively short duration of the visit but sufficient was seen on which to draw conclusions and to make a series of recommendations. Each of the three work areas is considered separately.

4.1 Review of current analytical process/procedures.

(i) Sample mixing and blending

15. Sample mixing and blending was observed on a set of samples specially obtained to support the quality assurance programme. At KRS, all sample chopping and preparation is done by hand using a sharp knife and a thick glass chopping board. The chopped sample, or an aliquot of it, is then transferred to a glass jar (tomato samples) or a wrapped in aluminium foil (kale samples) prior to storage in a chest – freezer. The chopping and mixing of all samples is carried out by the same person who adopts a consistent and conscientious attitude to a very demanding job. Every attempt is made to minimize the risk of sample contamination/cross-contamination with the working surface and cutting knife being washed between samples with water, acetone and hexane as are the gloves worn by the operator.

16. Kale samples (average 12 – 15 leaves) are chopped into slices up to about 1cm in width and then mixed by hand, scooping up the material and mixing as best as could be expected by this method. Tomato samples are cut into four sections and then cut again across the middle, crudely giving eight sections from each tomato (may be more sections on larger fruits). Because of the amount of fluid released, the mixing of the tomato samples is more difficult and the chopped pieces are then transferred to a glass jar without any homogenization or significant mixing.

17. Particularly with the tomato samples, this process of mixing is inadequate and the sample is not homogeneous. The use of a domestic food processor/blender would significantly improve this operation and procurement of such a unit was recommended. This was discussed with Dr Masuka, in a round – up meeting and the local purchase of an appropriate unit was instantly authorized. Mr King toured various electrical retailers with Mr Clement Chiringi to review what was available and a unit will be purchased in the week beginning 4 February. It is further recommended, however, that a hand – held unit be purchased, particularly for use with tomato samples. These units (an emulsifying head on a shaft) are not available locally and would have to be sent/carried from the UK.

(ii) Selection of the analytical sub-sample

18. It was observed that the analytical sub-sample is removed from the frozen bulk without de-frosting and re-mixing the sample. With kale samples, the material is broken from the mass, but the more aqueous tomato samples have to be chipped out of the jar. The portion of tomato sample taken depends on what has settled near the top of the jar (solid material or aqueous) and is wholly unrepresentative of the bulk. Such practices are based on expediency rather than science and must be stopped. **The principle must be that the analysis is only as good as the sample and if the sub-sample is not representative of the bulk sample then the analysis is wasted.** It is recommended that for future samples, and assuming a revised procedure for primary sample maceration/homogenization, that for each sample, two portions are frozen. The first is a weighed sub-sample such that this sample can be taken straight from the freezer, defrosted and then extracted without any further sub-division. The second sample is a larger bulk sample to act as a reserve in case of the need for re-analysis. In the event that this sample is required, it must be fully defrosted and re-homogenized before sub-sampling. Ideally a second weighed sub-sample should be retained for each sample (as well as the bulk sample) although for logistical reasons it is accepted that this may not be possible.

(iii) Sample extraction

19. The current Kutsaga extraction procedure is for a 10g sample to be macerated with 50ml of ethyl acetate in the presence of sodium sulphate. This concept is based on an old NRI procedure although the sample weight has been reduced to 10g (from 25g). The procedure is efficient although a revised procedure (uses 60ml of ethyl acetate and the addition of sodium hydrogen carbonate at the extraction stage), which is more consistent and applicable to a wider range of neutral pesticides was provided to Kutsaga at the outset of this project and which was thought would be used.

20. It is strongly recommended that the updated procedure is used for all future samples. It is also essential that the sample size be increased from 10g to 30g as in the revised procedure. A 10g aliquot is not statistically representative with most pesticide analytical laboratories using a minimum 25g sample and most using a sample size in the range of 25 – 50g to minimize sampling errors.

(iv) Sample clean – up

21. Sample clean – up (the separation of the analyte from sample co – extractives such as plant pigments) is only used for organochlorine pesticides and pyrethroids where the electron capture detector is used and not for the organophosphorous/organonitrogen compounds where a thermionic-type detector is used (also discussed in *(v)* below).

22. The column clean – up system used at KRS is an old design originally used for organochlorine residues in tobacco and its range of use since extended. The design of the column is unusual - a modified test tube with a capillary tube at the base which extends up the outside of the tube in a "swan – neck" design and acts as a siphon. Solvent is fed into a reservoir attached to a capillary (approx. 1mm) tube which connects to the top of the column. The standard Kutsaga elution system comprises a 2g (1ml) sample loading on a 5g column of florisil deactivated by the addition of 5% water and eluted using a 100ml hexane fraction followed by 50 ml of a 5% mixture of diethyl ether in hexane and 50 ml of a 20% mixture of diethyl ether in hexane. These fractions are collected separately, evaporated to dryness and each re-dissolved in 2ml of hexane for GLC analysis. Overall clean – up time is approx. 10 minutes and the solvent flow rate is approx. 25ml per minute (compared with classical column flow rates of 4 – 5ml per minute). From observations of recovery levels produced by KRS, this column seems to work well for the organochlorine compounds and for the synthetic pyrethroids. We do not believe, however, that the column has been validated for Dicofol and for the endosulphan isomers. This must be done with the column/procedure being validated for all likely analytes suggested by farmer survey data.

(v) Sample residue determination

Nitrogen – Phosphorous compounds

23. The GLC analysis for Nitrogen – Phosphorous (NP) compounds is based on the injection of the primary, sample extract at a concentration of 2g/ml. This extract

is not cleaned – up and therefore not subject to clean – up losses although the GLC traces can be distorted, particularly with kale samples, and there is the potential for column damage and analyte masking.

24. Observation of the elution pattern of a mixture of NP compounds on a CP Sil 8 column showed a problem with peak co-elution such that positive identification of some compounds was not possible. For example diazinon and disulfoton had the same GLC retention time, as did parathion ethyl and chlorpyrifos. Fenitrothion, malathion and pirimiphos methyl also had similar retention characteristics. Standard technique requires confirmation of residue identity on two columns of different characteristics such that separation of co-eluting peaks can be obtained on one of the columns. This practice does not seem to be followed.

25. At the time of the visit, the NP detection system normally used was not working, but we were assured that the column in that instrument was the same as the one on the GLC used to demonstrate the KRS analysis, so it is unlikely that the situation observed was worse than normal. There were also observed problems with the analysis of dimethoate and methamidophos with peaks for these compounds not being detected. Earlier chromatograms had shown these peaks although they were prone to distortion and variable sensitivity, possibly because of column ageing and the continuous injection of impure extracts. It is recommended that the use of extract clean – up, pre – analysis, be considered; the extracts for electron capture analysis are already cleaned – up and these fractions should be examined to see how applicable the existing procedure is used for the NP compounds and determine what additional steps may be necessary.

26. It would also improve the analytical assessment if the reference standard mixture in use better reflected the pesticides known to be used by small producers, rather than a more general mixture including some compounds which it is highly unlikely would be found such as fenamiphos (a granular nematicide) or parathion ethyl. In this regard, a useful mixture would be diazinon, dimethoate, pirimiphos methyl, chlorpyrifos and carbaryl. Note that the final compound, carbaryl does not appear to be sought in the KRS screening although KRS data sheets from the market survey report this pesticide as being used.

Organochlorine and synthetic pyrethroid compounds

27. The three fractions from the florisil clean – up column are analysed separately to ensure resolution of known analytes without an extended run time. The hexane column fraction is analysed for aldrin, DDT, HCH and HCB residues, the 5% fraction for dieldrin, endrin and beta endosulphan (presumably alpha endosulphan shows in the hexane fraction) and the 20% fraction is analysed for the synthetic pyrethroids.

28. In general these procedures work well although the analysis uses two Gas Liquid Chromatographs and is labour intensive. It is suggested that KRS work towards the development of a temperature programme which would allow two, or even all three, of the fractions to be combined and analysed in a single injection with separation of all of the potential analytes. This would save a considerable amount of time, even allowing for an extended analytical run time.

29. Dicofol, a compound reported to be used by small producers is not included in the normal screen and may be missed. This compound was included in the quality assurance exercise and was not initially detected.

Overall Conclusions on the analytical determination

30. The GLC analysis is conducted in a conscientious but naïve manner and needs to be improved for effective multi-residue analysis. The procedures in use are based on old methods used for screening tobacco for residues and have not moved on to meet the demands of screening other substrates (such as fruits and vegetables) where a different range of pesticides may be used. The analysis should ensure that as a minimum all analytes identified as of interest should be capable of determination, or where this is not possible it should be discussed with the client.

31. Most identifications are made using automatic integration systems or other data handling packages. This relies on the instruments being programmed with reference standard details; where the information is incomplete (such as where analyte peaks co-elute) this can lead to misidentification. Additionally, some integrators are triggered manually, rather than automatically upon injection, and this can lead to slight differences in retention time. Examples were observed where analytes were mis - identified or missed totally because of this rigorous adherence to the computer generated data. There needs to be greater human assessment of the data and a better understanding of the requirements of chromatographic analysis. As a starting point, tables of analyte retention times and relative retention times should be developed for each stationary phase in use in the laboratory. These tables should be made as comprehensive as possible and will show to inexperienced staff which peaks co-elute or elute so closely that they may be confused and which alternative columns could be used to effect difficult separations.

32. At the request of the Mrs Mbulawa, Mr Cox made a short presentation to staff to introduce the concept and practicalities of relative retention times, a subject of which they were apparently unaware.

4.2 Reporting

33. Reporting by KRS of analytical results has been of great concern to NRI with little data being released by KRS without specific requests being made. The quality of the released data has also been of concern; the results have not been clear, errors have been observed and method validation data has been withheld. These issues were discussed at length and it was agreed that although the procedure for data reporting (and general liaison with the client – NRI) had never been formally agreed, there were areas of weakness which needed to be addressed.

34. It was agreed that liaison/reporting required improvement and suggestions were made as to how this could be done (e-mail, fax etc) and suggestions also made as to how the quality of data reporting could be improved and standardized by the use of standard reporting formats. An example of an NRI pesticide residue report was used to illustrate what could be done and how it could be customized for use at KRS.

4.3 Analysis of quality assurance samples

35. Bulk chopped tomato and kale samples were provided and prepared by KRS. These samples were re-mixed by the NRI team and, in addition, the tomato sample was blended/homogenized using an Omni-Mix blender to ensure that sub-samplingfor the quality assurance exercise would be representative.

36. As the objective of the exercise was to validate the KRS procedure sample size was kept at 10g and it was requested that the sample extraction, clean – up and analysis was completed using the standard KRS procedure. In total, 12 samples were provided:

- 2 untreated kale samples
- 2 untreated tomato samples
- 4 treated kale samples; 2 treatment levels, 2 replicates of each
- 4 treated tomato samples; 2 treatment levels, 2 replicates of each

Details of the treatment mixtures are given in Annex 2.

37. When the revised sample extraction method was introduced, the opportunity was taken to add additional laboratory spiked samples:

- 1 untreated kale sample
- 1 untreated tomato sample
- 2 treated kale samples; 2 treatment levels, 1 replicate of each
- 2 treated tomato samples; 2 treatment levels, 1 replicate of each

The analysis of the first exercise was virtually completed by the end of the visit (although not fully tabulated/reported) but the result of the second exercise was not known. However, these results taken together with other observations, were adequate for a primary assessment of laboratory quality/capabilities to be made.

4.4 Analysis of comparative samples

38. By the completion of the visit, only seven of the 28 samples used for comparative analysis had been completed. This was disappointing as the intention to undertake the analysis was agreed during Dr Graffham's visit in November 2001. When Mr Cox first visited KRS on Monday 21 January, he too stressed the importance of completing this analysis. In the event, sample analysis did not start until later that week and due to some misunderstanding of the number of samples involved, only a proportion of the agreed number of samples were analysed. When this became clear, KRS started the remainder of the work although the sample analysis had not been completed by the end of the visit. It was agreed that the results would be e-mailed to NRI as soon as they were available (likely during the week beginning 4 February 2002).

39. The number of comparative samples completed is inadequate for comprehensive analysis to be undertaken and a separate report of thatwork will be provided to the project leader, Dr Graffham, when all the results are available.

Further comparative samples (sub – samples of those collected on 1 February) were taken and returned to the UK for analysis should funding permit.

5. Other Sampling Issues

40. KRS staff collected market samples from Mbare on 30 January as part of the scheduled sampling programme. The NRI team did not accompany the sampling team because of other commitments but examined the samples on their return to Kutsaga when it became clear that the individual samples were significantly smaller in size than on previous occasions. It was found that only 3 or 4 tomatoes had been taken as a sample from three field boxes instead of 12 – 15 (4 – 5 per box) as originally agreed. This was attributed to a shortage of produce (seasonal variation, political instability with growers reluctant or unable to travel to Harare market) causing many sellers to be reluctant to allow larger sample to be taken from a box in case it reduced its market value. In normal circumstances the proportionately high price paid for the few tomatoes collected has been sufficient to overcome the sellers caution.

41. It was obvious that the samples so collected could not be considered as representative and as such were unacceptable. This was discussed with the sampling team and Mrs Mbulawa and it was agreed that the sampling would be repeated on the 1 February when whole boxes of tomatoes, made up of tomatoes from three separate boxes would be purchased. This was further discussed and agreed with the project manager, Dr Graffham, prior to re-sampling. Kale was also re-sampled at this time with whole bunches being purchased rather than a sub-sample of 12 – 15 leaves from a bunch.

42. It was accepted that there would be a cost implication to this change in procedure but that it would not apply at all times, but would be dependant upon the supply situation.

6. Training requirements

43. Staff at KRS are given training in their immediate duties and to accommodate their normal work programme. There is a weakness, however, with regard to their pesticide analytical expertise and particularly so with multi – residue analysis. The expertise required is not available at KRS and the staff are frequently not fully aware of the correct procedures to adopt and of the problems and pitfalls which can only be recognized through experience. The result of this is that there is an uncertainty with regard to the quality of some analysis (not all) and therefore with the validity of the analytical result.

44. To address this problem, it is recommended that a two week training programme be developed for KRS staff to supplement and build upon their existing skills/knowledge. This recommendation was discussed with Dr Masuka who gave it his full support.

45. The CPHP has a complementary food safety project in Zimbabwe, managed by Wye College and based at the University of Zimbabwe and with links to the

Department of Extension and Research, Harare. It is additionally recommended that staff from these Institutions also be invited to join in any training programme. This would not only help to provide a consistency of analytical approach across the CPHP projects but provide additional Institutional support in developing a broader pesticide analytical capability in Zimbabwe.

Acknowledgements

46. The help and co-operation of the Director, Dr Anxious Masuka and of the Head of Department, Mrs Rudo Mbulawa, in allowing the review to be successfully completed is gratefully recognized. Similarly the efforts of the pesticide laboratory staff in demonstrating their procedures for sample preparation and sample analysis was much appreciated.

OUTLINE PROPOSED TRAINING PROGRAMME

Location:	Kutsaga Research Station, Harare
Duration:	Two weeks
Participants:	KRS staff, DER staff, University of Zimbabwe representatives
Objectives:	Building on existing skills, to develop a greater awareness of the requirements for pesticide multi – residue analysis and appropriate techniques to meet those requirements.
Teaching method:	Approx. 20% tutorials, 80% practical studies
Proposed timing:	April/May 2002

Outline Programme :

Week 1:

Tutorials:

1. Concept of multi-residue analysis and examination of multi - residue analytical procedures
2. Reference standards; preparation and storage
3. Sample blending and storage
4. Analytical quality assurance
5. Sample clean - up

Practical sessions:

1. Sample preparation.
2. Ethyl acetate and acetone extraction procedures for multi – residue determination.
3. Sample clean – up; comparison of procedures
4. Recycling of solvents, management of reagents

Week 2:

Tutorials:

6. GLC analysis: Establishment of multi – residue screening conditions.
7. Data interpretation
8. Data reporting
9. Round – up; open discussion

Practical sessions:

5. Development of relative retention time (RRT) data
6. Analysis of unknown solutions; identification and quantification of analytes
7. Analysis of laboratory treated samples

Note: This outline programme is open to discussion and change to fine tune it to the requirements of the participants. It has been developed, however, to address identified weaknesses at KRS which may be common to others involved in residue analysis who are less experienced in the subject and who have not received formal training.

ANNEX 2

QUALITY ASSURANCE APPRAISAL: KUTSAGA

Evaluation samples:

2 x Untreated kale
2 x Untreated tomato
2 x kale treated with test mixture 1
2 x kale treated with test mixture 3
2 x tomato treated with test mixture 2
2 x tomato treated with test mixture 3

Total 12 samples: 4 untreated, 8 treated

	Component	Concentration	Treatment level (10g sample/1.0ml of test solution)
Test Mixture 1:	Deltamethrin	5µg/ml	0.5mg/kg
	Fenvalerate	2µg/ml	0.2mg/kg
	Dicofol	1µg/ml	0.1mg/kg
Test Mixture 2:	Diazinon	1µg/ml	0.1mg/kg
	Dimethoate	4µg/ml	0.4 mg/kg
	Cyhalothrin	2.5µg/ml	0.25 mg/kg
Test Mixture 3:	Dimethoate	1µg/ml	0.1 mg/kg
	Lindane	0.5µg/ml	0.05 mg/kg
	Deltamethrin	1µg/ml	0.1 mg/kg
	Metalaxyl	5µg/ml	0.5 mg/kg

REFERENCE STANDARDS

	Supplier	Lot No.	Expiry date	Prepn. date
Cyhalothrin	QM_x	00302	03/2004	7.1.02
Deltamethrin	QM_x	81112	11/2004	7.1.02
Diazinon	QM_x	00406	05/2003	7.1.02
Dicofol	QM_x	90727	08/2003	7.1.02
Dimethoate	QM_x	90305	3/2002	7.1.02
Fenvalerate	QM_x	90507	07/2003	7.1.02
Lindane	QM_x	00628	06/2006	7.1.02
Metalaxyl	QM_x	70717	08/2003	3.12.01

Stock solution of each prepared in acetone; all subsequent dilutions in acetone.

Nominal preparation schedule:

Test Mixture 1:	Deltamethrin	5µg/ml	5ml of 100ppm +
	Fenvalerate	2µg/ml	2ml of 100ppm +
	Dicofol	1µg/ml	10ml of 10ppm
	Diluted to 100ml.		
Test Mixture 2:	Diazinon	1µg/ml	10ml of 10ppm +
	Dimethoate	4µg/ml	4ml of 100ppm +
	Cyhalothrin	2.5µg/ml	2.5ml of 100ppm
	Diluted to 100ml		
Test Mixture 3:	Dimethoate	1µg/ml	10ml of 10ppm +
	Lindane	0.5µg/ml	5ml of 10ppm +
	Deltamethrin	1µg/ml	10ml of 10ppm +
	Metalaxyl	5µg/ml	5ml of 100ppm
	Diluted to 100ml		

Actual values: (for details see Preparation of working level solutions Form 7.3(D) for test mixes 1 – 3)

Test Mixture 1:	Deltamethrin	4.74µg/ml	5ml of 94.7ppm +
	Fenvalerate	2µg/ml	2ml of 100.4ppm +
	Dicofol	1µg/ml	10ml of 10ppm
	Diluted to 100ml.		
Test Mixture 2:	Diazinon	1.04 µg/ml	10ml of 10.37ppm +
	Dimethoate	4µg/ml	4ml of 100.1ppm +
	Cyhalothrin	2.51µg/ml	2.5ml of 100.2ppm
	Diluted to 100ml		
Test Mixture 3:	Dimethoate	1µg/ml	10ml of 10ppm +
	Lindane	0.5µg/ml	5ml of 10ppm +
	Deltamethrin	0.95µg/ml	10ml of 9.47ppm +
	Metalaxyl	5µg/ml	5ml of 100ppm
	Diluted to 100ml		