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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: PS2529

2. Project title:
   Improving the Reproducibility and Cost Efficiency of the Analysis of Dithiocarbamates

3. Contractor organisation(s):
   Central Science Laboratory
   Sand Hutton
   York
   YO41 1LZ

4. Total Defra project costs (agreed fixed price): £ 46,518

5. Project: start date ..... 01 September 2006
   end date ..... 31 March 2007

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Overview

Dithiocarbamate fungicides are widely used in agriculture and residues are frequently detected in fruit and vegetables. The current 'PRC method' for the analysis of 'total dithiocarbamates' involves firstly cutting the sample into segments and then conversion of any dithiocarbamate compounds, present in the segments, to carbon disulphide (CS_2). Quantification of the CS_2 is undertaken by gas chromatography with either flame photometric or mass spectrometric detection. The quantification of dithiocarbamates as CS_2 can be questionable because of analytical variability associated, not with detection, but with the segmentation at sample processing stage. The segmentation procedure is employed to avoid degradation of dithiocarbamates that would occur if samples were homogenised at room temperature. Although the segmentation procedure reduces losses due to degradation it increases both; imprecision due to sub-sampling variability (the pesticides are not evenly deposited on the surface of samples) and; costs due to the need to analyse replicate analytical portions (to obtain acceptable analytical precision). Results reported to the Pesticide Residues Committee (PRC) are often based on the mean of the analysis of four analytical portions. This situation occurs when the first replicate analytical portion is found to contain a residue concentration at or above the reporting limit [or even the Maximum Residue Level (MRL)] but the second replicate analytical portion drawn from the same primary sample, does not contain a detectable residue. The analysis of two additional analytical portions is then undertaken to provide a better estimate of the concentration of the residue. It was considered a priority to develop procedures that address these issues.

Main objectives

The specific objective was to evaluate the use of cryogenic sample processing (homogenisation at low temperature, <-20℃) for the analysis of 'total dithiocarbamates'. The results from previous research demonstrated that the addition of dry ice during the comminution of frozen samples improved the homogeneity of the analytical portions and minimised losses ('improved stability') of a large number of pesticides [(1)]. However, cryogenic sample processing did not necessarily prevent losses of individual specific dithiocarbamates 'spiked' onto the surface of samples [(2)]. These losses are possibly caused by an unavoidable experimental error associated with the spiking & freezing procedure. Therefore, it was decided to assess if differential losses of 'field incurred' residues of dithiocarbamates occurred when using cryogenic sample processing compared to the sample segmentation procedure. If the precision resulting from the use of cryogenic sample processing is considered acceptable, and the mean results from cryogenic sample processing and segmentation are not statistically different, then it should be possible to also use the same cryogenically processed sample for the analysis of dithiocarbamates as well as other pesticides. The additional sample segmentation procedure and subsequent analyses would no longer be required. A single dithiocarbamate analysis of a cryogenically processed analytical portion, instead of quadruplicate analysis of segments, should suffice in most cases and thus provide significant cost savings to the PRC monitoring programme.

Secondary objectives were to assess the distribution of dithiocarbamate residues in individual heads of lettuce, a problematic commodity with respect to sampling, and to assess the stability of dithiocarbamate residues in cryogenically processed samples stored at -20℃.

Summary of research findings

Comparison of mean concentrations of dithiocarbamate residues (measured as CS_2) obtained using cryogenic sample processing and sample segmentation.

Duplicate laboratory samples of lettuce and pears withdrawn from the same sample lots (same grower identification) were analysed using either segmentation sample processing and/or cryogenic sample processing. Thirteen (24 %) of the 55 samples of lettuce and 27 (66 %) of 41 of pears were found to contain mean residue concentrations above the reporting limit of 0.05mg/kg. Only on a few occasions at concentrations close to the reporting limit did one of the methods detect a residue not found using the other method. Overall, statistical analysis of the qualitative data [residue detected at or above the lowest calibrated level (≥ 0.025 mg/kg) or not detected (<0.025 mg/kg)] showed there was no significant difference between the different sample processing methods for either lettuce or pears. Statistical analysis of the quantitative data (statistical analysis of samples containing residues at or above the reporting limit (0.05 mg/kg) again showed that there was no significant difference between the two methods.

The distribution of dithiocarbamate residues (as CS_2) in single heads of lettuce.

Ten individual heads from the same primary sample were each cut into 6 segments of approximately equal size. The residue concentrations for individual segments, from the same head, were highly variable with % CV in the range 38 – 172%. Individual segment concentrations across all 60 segments, taken from 10 heads varied by two orders of magnitude in the range 0.08 mg/kg (close to the reporting limit) to 8.96 mg/kg (in excess of the MRL).

Assessment of the stability of dithiocarbamate residues (measured as CS_2) in cryogenically processed samples stored at -20℃.

The concentration of dithiocarbamates (as CS_2) in cryogenically processed samples of lettuce and pear did not decrease significantly during 85 days and 92 days of storage at -20℃ respectively.

Conclusions

The overall objective to evaluate cryogenic sample processing for the analysis of dithiocarbamates (determined as CS_2) was met fully.

The 'total dithiocarbamate' residue concentrations in individual segments of lettuce were extremely variable. Statistical analysis shows that the variability of 'total dithiocarbamate' concentrations within lettuce heads was much greater than the variability in concentrations between the heads.

The precision of residue results for both lettuce and pear samples were better following cryogenic sample processing compared to segmentation. Although there is no statistical difference between the overall mean residue concentrations, the mean residue concentrations in lettuce (but not pears) were generally lower using cryogenic sample processing. It is not known if the lower values are simply due to the greater distribution variability of residues or the lower stability of specific individual dithiocarbamates in lettuce, or a combination of the two factors.

The final decision to employ cryogenic sample processing for dithiocarbamates in the PRC programme will be considered by the Analytical Sub-Group of the PRC. The results obtained in this report support the use of cryogenic processing for the determination of dithiocarbamates in pears but use for lettuce is more subjective.

The excellent stability of dithiocarbamate residue concentrations in cryogenically processed samples stored at -20ºC should make it possible to obtain additional data by the analyses of samples cryogenically processed in the PRC 2007 programme.

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the scientific objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

Objectives

To evaluate the use of cryogenic sample processing for the analysis of dithiocarbamates in order to improve the precision and reduce the cost of this analysis.

Experimental

A definition of the terminology used in the following text is included to assist the reader in understanding the experimental protocol;

Sample lot;

A quantity of produce with the same packaging codes and/or reputedly from the same grower.

Primary sample;

The sample obtained from the retailer before further sub-sampling procedures are applied in the laboratory (for this project the primary sample comprised either 20 or 30 heads of lettuce or 20 units of pears).

Laboratory sample;

A sample comprising of 10 heads of lettuce or 10 units of pears, randomly withdrawn from the primary sample.

Analytical sample;

A sample produced after segmentation or comminution of the laboratory sample.

Analytical portion;

A sub-sample or portion of the analytical sample used for extraction.

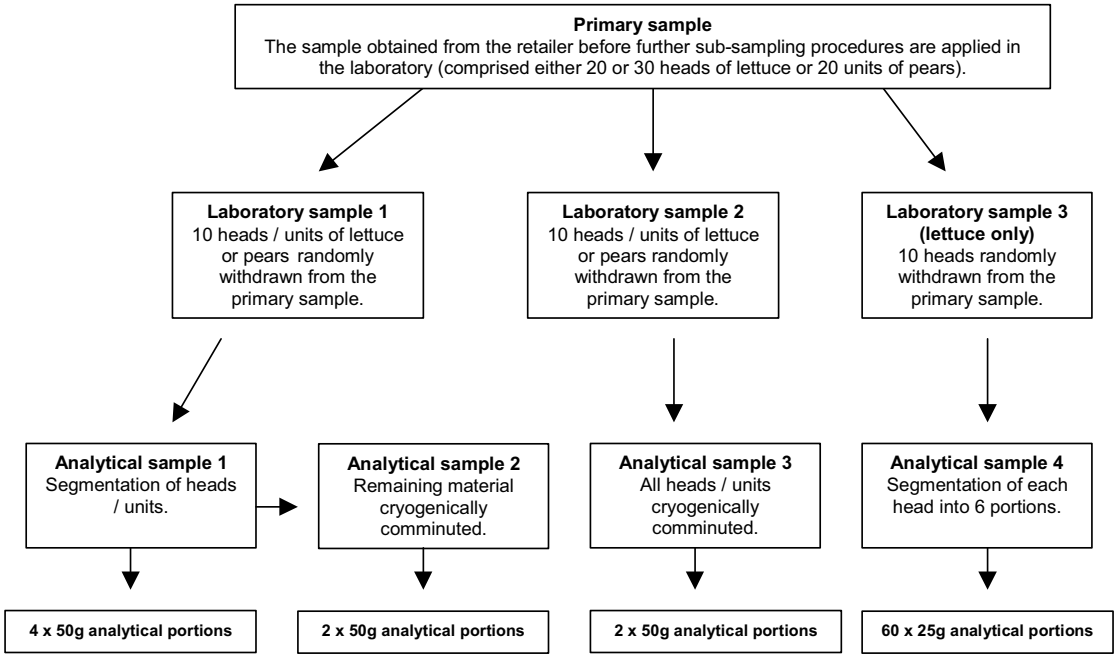
Primary samples of lettuce (where possible comprising 30 heads of lettuce) and pears (20 units) were obtained from local wholesalers and retailers between October 2006 and February 2007. The primary samples represented different varieties of lettuce and pears from both UK growers and imports. The samples were analysed for ‘total dithiocarbamates’ using a method

based on conversion of dithiocarbamates to CS_2 and quantification of the concentration of CS_2 by gas chromatography with flame photometric detection [3] as described later in the text.

Duplicate laboratory samples of lettuce (each comprising 10 lettuce heads, minimum 1 kg) or pears (each comprising 10 pear units, minimum 1 kg) were withdrawn from each primary sample. In each case, one of the laboratory samples was sampled using a segmentation procedure and the second sample comminuted using cryogenic processing as illustrated in Figure 1.

If available, and when necessary, the third laboratory samples of lettuce (comprising the remaining 10 heads of lettuce) were retained for an assessment of the within head variability of dithiocarbamates (expressed as CS_2). This experiment was specifically requested by PSD.

Figure 1: Summary of sample processing protocol



A total of 55 lettuce and 41 pear samples were analysed for 'total dithiocarbamates' using both sample-processing methods. In an attempt to obtain more samples containing higher concentrations of dithiocarbamate residues an additional 18 samples of lettuce were screened by the segmentation method. These additional samples were not subjected to cryogenic processing because the residues were close to the reporting limit and sufficient results at low concentrations had already been obtained. All of the analyses were conducted within 1 day of collection of the primary sample in an attempt to minimise the degradation of dithiocarbamate residues.

Segmentation procedure

The individual heads/units (10 per sample) were quartered and one quarter from each individual head randomly selected and then combined to form an analytical sample. The quarters comprising the analytical samples were sub-sampled (segmented) and a number of the segments randomly combined to form an analytical portion of approximately 50g. This sub-sampling procedure was repeated a further 3 times to produce a total of 4 analytical portions.

Two of the analytical portions were analysed immediately using the GC-FPD method described below.

The 3rd and 4th analytical portions were frozen rapidly in the presence of dry ice and then stored at -20ºC.

For a number of samples, the material remaining after removal of the analytical portions was frozen to allow comminution by cryogenic sample processing [(1)]. The comminuted analytical sample was stored at -20ºC and duplicate analytical portions (50 g) removed for CS_2 analysis when required.

Cryogenic sample processing procedure

The 10 heads/units in each laboratory sample were halved or quartered as appropriate and frozen (overnight at - 20ºC). The whole sample (approximately 1-5 Kg) was comminuted in the presence of dry ice using a Stephan UMC12 food processor. The comminuted sample, in the form of a flowable powder, was placed in a freezer (-20ºC) for a minimum of 16 hours to allow dissipation of CO_2. Duplicate analytical portions (50g) were withdrawn for dithiocarbamate analysis.

Distribution of dithiocarbamate residues (as CS_2) within and between individual heads of lettuce.

The individual lettuce heads (10 in total) from a primary sample found to contain a high mean concentration of CS_2 were each cut, vertically, into 6 segments (analytical samples) of approximately equal mass. The relative position of each segment was recorded and each of the sixty segments were analysed individually for total dithiocarbamates.

Analysis of total dithiocarbamate residues (as CS_2) by GC-FPD

Dithiocarbamate residues present in the sample were reduced to CS_2 in the presence of tin (II) chloride/hydrochloric acid at 80 ºC. The CS_2 formed was trapped in an upper layer of 2,2,4-trimethylpentane. Determinations were made using capillary gas liquid chromatography with FPD operating in the sulphur mode. Injection (1 ml) was splitless at 150 ºC and the detector temperature was set at 250 ºC. Chromatography was performed using a DB-1 column (30 m x 0.53 mm i.d. x 1.5 mm film thickness) with the carrier gas (helium) at a flow rate of 4.2 ml/min. All determinations were calibrated using multi-point, matrix-matched standards, which bracketed the extracts.

The lowest calibrated level (LCL) was equivalent to 0.025 mg/kg and the reporting limit set at 0.05 mg/kg.

Where necessary sample extracts were diluted to ensure that the detector response fell within the calibrated range.

The recovery of thiram (a specific dithiocarbamate) spiked at 0.1 mg/kg and 1 mg/kg was generally in the range 70% -110% demonstrating satisfactory analytical quality control.

Stability of dithiocarbamate residues (as CS_2) in cryogenically processed samples

The stability of dithiocarbamate residue concentrations in cryogenically processed samples (4 lettuce and 5 pears) stored at -20 ºC were measured (as CS_2) for a period of up to 13 weeks after sample processing. The stability of the concentration of CS_2 in a solvent extract of lettuce, stored at -20 ºC was measured over a period of 4 weeks after extraction.

Statistical methods of analysis

To compare the three processing procedures; (i) segmented heads/units, (ii) cryogenic processing of the material remaining after segmentation and (iii) cryogenic processing of 'whole' frozen heads/units), two statistical approaches were taken.

Statistical analysis of 'qualitative' data

Initially, to take into account those values that were less than the LCL, an indicator was calculated; being equal to 'one' if the residue was found to be 'positive' (≥ 0.025mg/kg) or a 'zero' (< 0.025mg/kg). This indicator was then analysed using a generalized linear mixed model with a binomial distribution. The sample identification number was used as a random effect to allow for repeated measures on the same sample and the method type was used as a fixed effect. This type of analysis compares the proportion of positive residues between methods, whilst taking into account the fact that the replicates have come from the same primary sample of lettuce or pears.

Statistical analysis of 'quantitative' data

For those samples found to contain residues (>0.05 mg/kg) the concentration values were then analysed using a linear mixed model (with a normal distribution) after $\log_{10}$ transformation. Again, the sample identifier number was used as a random effect to allow for repeated measures on the same sample and the method type was used as a fixed effect. The stability data was investigated in the same way.

The Bland and Altman[(4)] method for assessing agreement measurement analysis, as proposed in the research proposal, was not pursued because of the unequal replication between methods for each sample. A sample mean could have been calculated for the residue values and subsequent pairing could have been made, but this would ignore the information on the variation between replicates.

The distribution results were assessed by analysis of variance to enable a comparison between lettuce head variation and within lettuce head variation.

All statistical analyses were performed using GenStat® 9.2.

Results

The results obtained from the analysis of 55 samples of lettuce and 41 samples of pears are summarised in Tables 1 & 2 respectively. Residues of dithiocarbamates (expressed as CS_2) were detected at or above the reporting limit (0.05 mg/kg) in at least one analytical portion taken from 13 (24 %) of the 55 primary samples of lettuce and 27 (66 %) of the 41 primary samples of pears. Segments labeled C & D were only analysed if the difference between the A & B segments exceeded a value equal to 50% of the highest of the two concentrations or the residue was at a significant concentration (e.g. >MRL).

One sample of lettuce (No. 35) contained a mean residue (5.33 mg/kg) that exceeded the UK MRL of 5 mg/kg. No samples of pears contained residues at or above the UK MRL of 3 mg/kg.

Table 1:
Summary of results for dithiocarbamates (as CS_2) in analytical portions of samples of lettuce

	Segment Analysis Results (mg/kg CS2)					Cryogenic Processing Analysis Results (mg/kg CS2)					
						Segment			Whole units		
Lettuce	Replicate					Replicate			Replicate		
Samples Nos.	A	B	C	D	Average	E	F	Average	a	b	Average
2	**0.06**	**0.68**	**0.16**	**0.19**	**0.27**	n/a	n/a		**0.37**	**0.37**	**0.37**
6	**0.17**	<0.05	**0.19**	<0.05	**0.09**	n/a	n/a		**0.06**	**0.07**	**0.07**
10	**0.06**	<0.05	**0.14**	<0.05	**0.05**	n/a	n/a		**0.05**	**0.05**	**0.05**
12	**0.06**	<0.05	**0.07**	<0.05	<0.05	n/a	n/a		<0.05	<0.05	<0.05
13	**0.33**	n/a	**0.53**	**0.10**	**0.32**	n/a	n/a		**1.40**	**1.21**	**1.31**
22	<0.05	**0.15**	**0.06**	<0.05	**0.05**	**0.05**	**0.06**	**0.06**	**0.11**	**0.10**	**0.11**
27	**0.80**	**0.08**	**0.05**	**0.06**	**0.25**	**0.17**	**0.18**	**0.18**	**0.26**	**0.25**	**0.26**
32	<0.05	<0.05	n/a	n/a	<0.05	<0.05	<0.05	<0.05	**0.06**	**0.07**	**0.07**
34	**0.37**	**<0.05**	<0.05	**0.06**	**0.11**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
35	**8.03**	**6.13**	**3.33**	**3.82**	**5.33***	**1.31**	**1.45**	**1.38**	**1.65**	**1.71**	**1.68**
37	**0.21**	**0.32**	**0.43**	**0.45**	**0.35**	<0.05	<0.05	<0.05	**0.15**	**0.14**	**0.15**
43	**1.07**	<0.05	<0.05	**0.07**	**0.29**	**0.07**	**0.06**	**0.07**	<0.05	<0.05	<0.05
46	<0.05	**0.09**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
48	**0.1**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
50	<0.05	**0.27**	<0.05	<0.05	**0.07**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
51	<0.05	**0.07**	**0.05**	<0.05	<0.05	**0.16**	**0.10**	**0.13**	<0.05	<0.05	<0.05
53	<0.05	<0.05	n/a	n/a	<0.05	**0.06**	**0.06**	**0.06**	**0.06**	**<0.05**	<0.05
55	**0.76**	**1.30**	**1.31**	**1.14**	**1.13**	**0.57**	**0.56**	**0.57**	**0.66**	**0.64**	**0.65**

Notes: a) samples not found to contain residues of dithiocarbamates (<0.05 mg/kg CS_2) in any analytical portions are not shown, b) n/a = not analysed, c) * nominally exceeds UK MRL of 5 mg/kg d) retail samples containing 30 lettuce heads from the same lot/grower were difficult to obtain. As a result the distribution study could not be undertaken for all samples containing significant residues (e.g. No. 35). The distribution study was carried out using sample number 55.

Table 2:
Summary of results for dithiocarbamates (as CS_2) in analytical portions of samples of pears

	Segment Analysis Results (mg/kg CS2)					Cryogenic Processing Analysis Results (mg/kg CS2)					
						Segment			Whole units		
Pear	Replicate					Replicate			Replicate		
Samples Nos.	A	B	C	D	Average	E	F	Average	a	b	Average
3	**0.07**	**0.16**	**0.07**	**0.06**	**0.09**				**0.10**	**0.10**	**0.10**
4	**0.15**	**0.13**			**0.14**				**0.21**	**0.20**	**0.21**
5	<0.05	**0.16**	**0.15**	**0.25**	**0.14**				**0.08**	**0.08**	**0.08**
7	<0.05	<0.05	n/a	n/a	<0.05	n/a	n/a		**0.07**	**0.07**	**0.07**
8	**0.14**	**0.21**	n/a	n/a	**0.18**	n/a	n/a		**0.15**	**0.15**	**0.15**
11	**0.09**	**0.06**	n/a	n/a	**0.08**	n/a	n/a		<0.05	<0.05	<0.05
14	<0.05	<0.05	n/a	n/a	<0.05	n/a	n/a		**0.08**	**0.11**	**0.10**
17	**0.49**	**0.65**	n/a	n/a	**0.57**	**0.23**	**0.22**	**0.23**	**0.46**	**0.45**	**0.46**
18	**0.08**	**0.08**	n/a	n/a	**0.08**	**0.05**	**0.06**	**0.06**	<0.05	<0.05	<0.05
20	**0.26**	**0.26**	n/a	n/a	**0.26**	**0.31**	**0.29**	**0.30**	**0.34**	**0.35**	**0.35**
21	**0.14**	**0.06**	**0.06**	**0.12**	**0.10**	**0.15**	**0.15**	**0.15**	**0.32**	**0.30**	**0.31**
22	**0.25**	**0.30**	n/a	n/a	**0.28**	**0.55**	**0.53**	**0.54**	**0.48**	**0.49**	**0.49**
23	**0.07**	**0.07**	n/a	n/a	**0.07**	**0.10**	**0.11**	**0.11**	**0.15**	**0.14**	**0.15**
24	**0.32**	**0.28**	n/a	n/a	**0.30**	**0.41**	**0.43**	**0.42**	**0.25**	**0.25**	**0.25**
26	**0.12**	**0.06**	**0.07**	**0.07**	**0.08**	**0.09**	**0.10**	**0.10**	**0.13**	**0.13**	**0.13**
27	**0.08**	**0.11**	n/a	n/a	**0.10**	**0.10**	**0.10**	**0.10**	**0.14**	**0.13**	**0.14**
28	**0.18**	**0.27**	n/a	n/a	**0.23**	**0.58**	**0.62**	**0.60**	**0.52**	**0.50**	**0.51**
29	<0.05	**0.62**	**0.14**	**0.11**	**0.22**	**0.13**	**0.14**	**0.14**	**0.09**	**0.09**	**0.09**
30	**0.30**	**0.12**	**0.14**	**0.24**	**0.20**	**0.19**	**0.21**	**0.20**	**0.21**	**0.18**	**0.20**
31	**0.12**	**0.45**	**0.32**	**0.20**	**0.27**	**0.39**	**0.30**	**0.35**	**0.34**	**0.37**	**0.36**
32	<0.05	<0.05	n/a	n/a	<0.05	**0.05**	**0.05**	**0.05**	**0.06**	**0.06**	**0.06**
33	**0.25**	**0.32**	n/a	n/a	**0.29**	**0.24**	**0.26**	**0.25**	**0.24**	**0.23**	**0.24**
34	**0.18**	**0.08**	**0.23**	**0.06**	**0.14**	**0.09**	**0.09**	**0.09**	**0.12**	**0.11**	**0.12**
35	**0.13**	**0.12**	n/a	n/a	**0.13**	**0.14**	**0.14**	**0.14**	**0.10**	**0.10**	**0.10**
38	<0.05	<0.05	n/a	n/a	<0.05	<0.05	<0.05	<0.05	**0.08**	**0.08**	**0.08**
40	**0.60**	**0.30**	**0.18**	**0.15**	**0.31**	**0.16**	**0.16**	**0.16**	**0.19**	**0.24**	**0.22**
41	**0.22**	**0.19**	n/a	n/a	**0.21**	**0.23**	**0.25**	**0.24**	**0.38**	**0.37**	**0.38**

Notes: a) samples not found to contain residues of dithiocarbamates (<0.05 mg/kg CS_2) in any analytical portions are not shown, b) n/a = not analysed.

Analysis of dithiocarbamate residue results obtained for lettuce

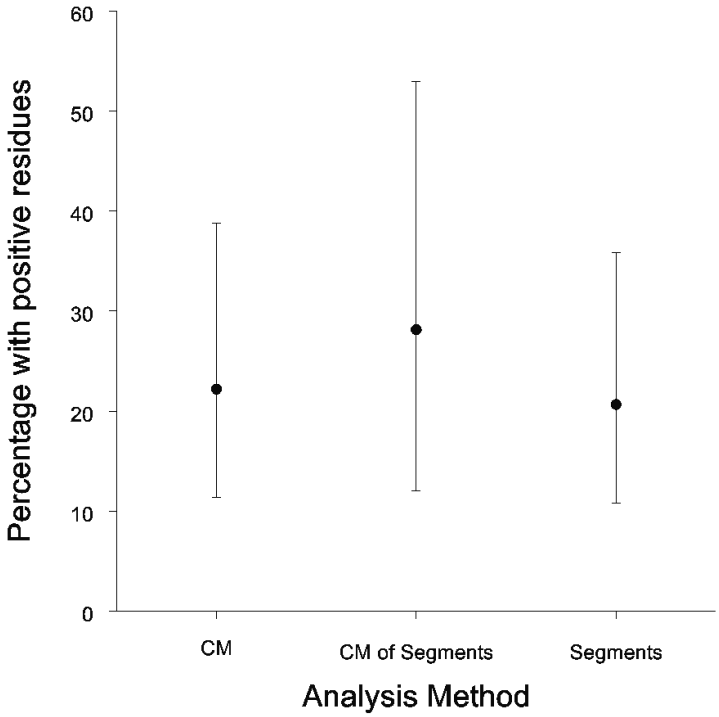
In 3 (5 % of) samples the mean residues detected at low concentrations in segmented analytical portions were not detected in cryogenically processed analytical portions taken from the same primary sample; see Table 1. For each of these 3 samples residues were not detected in all of the 4 segmented analytical portions; indicating that the differences are more likely to be due to the variability from the segmentation procedure, rather than losses of dithiocarbamates during the cryogenic processing procedure. The reverse situation; where a residues detected after cryogenic sample processing were not detected after segmentation, occurred in 1 sample.

The analysis of the 'qualitative data' (i.e. results considered to be positive or negative if > or < than 0.025 mg/kg respectively), show no significant difference between the methods of analysis with a *p*-value of 0.713. The predicted mean percentage of samples with positive residues detected by the different processing methods is not significantly different as shown in Table 3 and illustrated in Figure 2.

Table 3: Mean percentage lettuce samples with residues (>0.05 mg/kg) by analytical method with 95% confidence interval

		95% Confidence Interval	
Method	Mean	Lower	Upper
Cryogenic processing (CM)	22.18	11.36	38.80
Cryogenic processing - segmented	28.14	12.01	52.91
Segmented	20.66	10.83	35.83

Figure 2: Mean percentage lettuce samples with residues (>0.05 mg/kg) by analytical method with 95% confidence interval



The results from the same statistical analysis but after exclusion of the additional replicates from the segment procedure (to enable a comparison of variation on the same number of replicates) again show that the methods were not significantly different, p=0.537.

The analysis of the 'quantitative data' (inclusion of only those residues >0.05 mg/kg) again showed that there is no significant difference between the methods of analysis, with a *p*-value of 0.692. Table 4 shows the predicted $\log_{10}$ (residue concentration) by analytical method.

Table 4: Mean $\log_{10}$ (residue concentration) of lettuce by analytical method, with 95% confidence interval.

Method	Mean	Standard Error	95% Confidence Interval	
			Lower	Upper
Cryogenic Milling	-0.97	0.193	-1.356	-0.591
Cryogenic Milling Segmented	-1.02	0.242	-1.503	-0.543
Segment	-0.82	0.160	-1.141	-0.505

Applying the same statistical analysis but after exclusion of the additional replicates from the segment analysis (to enable a comparison of variation on the same number of replicates) again shows that the methods were not significantly different, *p*=0.678.

Using the segmentation procedure the mean residue concentration in sample (No. 35), was much higher (5.33 mg/kg) than the mean residue concentration (1.38 mg/kg) obtained after cryogenic comminution of the sample material (same laboratory sample) remaining after segmentation. The concentration of 1.38 mg/kg was in good agreement with the concentration (1.68) obtained after cryogenic processing of a different analytical sample derived from the same primary sample. The reasons for the differences (around 75 %) are not known but degradation of specific dithiocarbamates during cryogenic processing cannot be ruled out. The unknown identity of the specific dithiocarbamate fungicide(s) present in the sample was a limitation identified at the outset of the project. To provide information on the stability of specific dithiocarbamates in lettuce will require further research and development to treat cultivated lettuce with individual dithiocarbamates. The difference (possible degradation) of around 75% between segmentation and cryogenic processing observed for sample (No. 35, Table 1) is higher than differences of 0 - 50 % observed for other lettuce samples in this study and losses of around 50 % of dithiocarbamates spiked onto the surface of lettuce in a previous study [(2)]. It is suspected but cannot be proven (additional heads were not available for this sample) that variability in residue concentrations between individual segments in this particular sample contributed to the large difference. If the variability factor could be proven in such cases then implementation of cryogenic processing for the analysis of dithiocarbamates in the PRC monitoring programme could more easily be justified.

Dithiocarbamate residue distribution data within and between individual lettuce heads

Table 5 shows the distribution of residues across 10 individual lettuce heads from the same laboratory sample (primary sample number 55, Table 1). The residue concentrations for individual segments from the same head were highly variable with % RSDs in the range 38 –172%. Individual segment concentrations across all 60 segments varied by 2 orders of magnitude in the range 0.08 mg/kg (close to the reporting limit) to 8.96 mg/kg (in excess of the MRL).

Table 5: Summary of the distribution of dithiocarbamate (as CS_2) within and between individual lettuce heads.

Individual Head	Position of Replicate	Concentration (mg/kg)	Concentration (mg/kg)	Position of Replicate	Average Concentration (seg concns / no. of segments analysed)(mg/kg)	RSD (%)	Total Residue Measured (mg)
1	SHA1 SHA2 SHA3	8.96 0.80 1.14	0.17 0.20 0.68	SHB1 SHB2 SHB3	1.99	172	0.29
2	SHA1 SHA2 SHA3	0.27 0.13 1.14	1.07 0.52 0.37	SHB1 SHB2 SHB3	0.58	73	0.08
3	SHA1 SHA2 SHA3	2.45 0.28 0.08	4.36 9.62 0.58	SHB1 SHB2 SHB3	2.90	127	0.74
4	SHA1 SHA2 SHA3	0.24 0.60 0.08	0.29 0.17 0.35	SHB1 SHB2 SHB3	0.29	62	0.04
5	SHA1 SHA2 SHA3	3.14 0.75 0.33	0.36 0.41 0.74	SHB1 SHB2 SHB3	0.96	114	0.18
6	SHA1 SHA2 SHA3	0.47 1.76 1.30	1.58 1.88 1.16	SHB1 SHB2 SHB3	1.36	38	0.18
7	SHA1 SHA2 SHA3	4.53 0.21 0.38	0.20 0.14 0.75	SHB1 SHB2 SHB3	1.04	167	0.17
8	SHA1 SHA2 SHA3	1.81 0.09 0.26	0.05 0.96 0.07	SHB1 SHB2 SHB3	0.54	132	0.06
9	SHA1 SHA2 SHA3	0.09 0.11 0.24	0.68 0.26 0.62	SHB1 SHB2 SHB3	0.33	77	0.04
10	SHA1 SHA2 SHA3	0.24 1.13 0.14	0.18 0.18 0.10	SHB1 SHB2 SHB3	0.33	120	0.04

Notes: segment SHA1 = segment No.1 from the lettuce half denoted 'A'. The coding A1 –A3 are in the same relative positions in each head. B coding refer to corresponding segments from the lettuce half denoted 'B.

Table 6 shows the results of analysis of variance from the distribution data. The variability of dithiocarbamate residue concentrations within the lettuce heads was much greater than the variability between individual lettuce heads, with respective sums of squares 4.35 and 12.00. There is also a marginally significant difference between the mean residue concentrations in the individual heads samples, p=0.0567.

Table 6: Analysis of variance results of dithiocarbamate residue (*as CS_2*) distribution data within and between individual lettuce heads.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F Probability
Between Lettuce Head	9	4.35	0.48	0.057
Within Lettuce Head	50	12.00	0.24	
Total	59	16.35		

Stability of dithiocarbamate residues (measured as CS_2) in lettuce.

The analysis of dithiocarbamate residues in cryogenically comminuted samples of lettuce showed that there was no significant decrease in CS_2 concentration after 85 days storage at -20ºC, with a p-value of 0.298. The slope had a value of 0.0008 with a standard error of -0.0008.

Statistical analysis relatively few number of results for the determination of the stability CS_2 in 2,2,4-trimethylpentane (from an extract of lettuce) stored at -20ºC tentatively indicate that there is no significant decrease in CS_2 concentration 28 days after extraction, with a *p*-value of 0.373. The slope had a value of -0.00143 with a standard error of 0.0095. Additional results are required to confirm this finding.

Analysis of dithiocarbamate residue results (measured as CS_2) obtained for pears

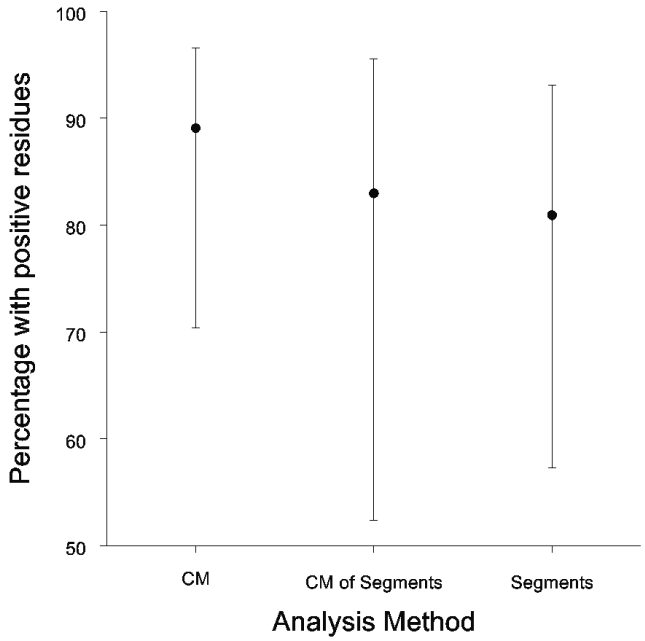
In 2 (5 % of) samples, residues at low concentrations detected following the segmentation procedure were not detected in analytical portions taken from either the same primary sample or the same analytical sample and processed cryogenically (non segments), see Table 2. The reverse situation of residues detected following cryogenic processing but not segmentation occurred in 4 (10 % of) samples.

The analysis of all of the 'qualitative data' (i.e. results considered to be positive or negative if > or < than 0.025mg/kg respectively) showed no significant difference between the methods of analysis, with a *p*-value of 0.502. The predicted mean percentage of samples with positive residues detected by the different processing methods is not significantly different as shown in Table 7 and illustrated in Figure 3.

Table 7: Mean percentage pear samples with positive residues (>0.05 mg/kg) by analytical method, with 95% confidence interval.

		95% Confidence Interval	
Method	Mean	Lower	Upper
Cryogenic Milling (CM)	89.08	70.36	96.55
Cryogenic Milling Segmented	82.98	52.38	95.58
Segment	80.93	57.32	93.06

Figure 3: Mean percentage pear samples with residues (>0.05 mg/kg) by analytical method with 95% confidence interval



The results from the same statistical analysis but after exclusion of the additional replicates from the segment procedure (to enable a comparison of variation on the same number of replicates) again show that the methods were not significantly different, *p*=0.392.

The analysis of the 'quantitative data' (positive residues only) again showed that there was no significant difference between the methods of analysis, with a *p*-value of 0.972. Table 8 shows the predicted $\log_{10}$ (residue concentration) by analytical method.

Table 8: Mean $\log_{10}$ (residue concentration) of pear by analytical method, with 95% confidence interval.

Method	Mean	Standard Error	95% Confidence Interval Lower	Upper
Cryogenic Milling	-0.89	0.127	-1.145	-0.643
Cryogenic Milling Segmented	-0.80	0.158	-1.107	-0.483
Segment	0.93	0.116	-1.153	-0.697

Applying the same statistical analysis but after exclusion of the additional replicates from the segment analysis (to enable a comparison of variation on the same number of replicates) again show that methods were not significantly different, *p*=0.793.

Using cryogenic processing the concentration of dithiocarbamate residues were generally equal or higher compared to those obtained using segmentation. This could indicate improved stability of dithiocarbamates in pear as opposed to lettuce matrix but could simply reflect the use of different more stable dithiocarbamate compound in pears. The distribution of dithiocarbamate residues in individual pear units was not undertaken (not requested). The precision of the results obtained from duplicate analysis of segments is much improved compared to the analysis of lettuce and perhaps is not surprising considering the different morphology of the two commodities.

Overall, the results for pears support the implementation of cryogenic sample processing for the analysis of dithiocarbamates (as CS_2) in the PRC programme.

Stability of dithiocarbamate residues (measured as CS_2) in cryogenically processed samples of pear

There was no significant decrease in dithiocarbamate residues (measured as CS_2) in cryogenically comminuted samples of pear stored for 92 days at -20ºC with a *p*-value of 0.850. The slope had a value of -0.0002 with a standard error of 0.0009.

Conclusion

The overall outcome of the project was that all of the objectives to evaluate different sample processing methods were met fully.

The mean concentrations of incurred residues in lettuce and pears were generally close to the reporting limit and as a consequence there is limited data for the comparison on the effect of sample processing methods on the determination of residues at high concentrations (around MRL values).

The residue concentrations in segments of lettuce samples were extremely variable. Statistical analysis showed that the variability of dithiocarbamate residue concentrations within the lettuce heads was much greater than the variability between individual lettuce heads. There was a moderately significant difference in the mean residue concentrations between individual lettuce heads.

Overall, the mean residue concentrations determined following segmentation were *not significantly* different from the mean residue concentrations obtained using cryogenic sample processing for either lettuce or pears. The precision of dithiocarbamate residue concentrations in both commodities was better using the cryogenic sample processing procedure compared to the segmentation procedure.

The mean residue concentrations of dithiocarbamates in lettuce, but not pears, were generally lower using cryogenic sample processing compared to segmentation. It is likely that these lower residues occur in lettuce because of some losses during cryogenic sample processing as reported previously [(2)]. The results in this report support the implementation of cryogenic processing for the analysis of dithiocarbamates in pears but the application for lettuce is more subjective. Further research using lettuce containing incurred residues of individual dithiocarbamates would help to confirm the suitability/reliability of cryogenic sample processing for samples of lettuce.

There was no significant decrease in dithiocarbamate residues (measured as CS2) in cryogenically processed samples of lettuce and pears stored for 85 days and 92 days at -20ºC respectively. This stability means that it should be possible to obtain data from the 2007 PRC samples in order to increase the statistical data set; also to cover more matrices, higher residue concentrations and possibly more different dithiocarbamate fungicides. This data could be available during 2007 with a view to providing a recommendation to the PRC in time for the 2008 programme. If it is possible to introduce cryogenic processing for dithiocarbamate residue analysis then not only would this action provide significant cost savings and more consistent results but it should be possible to provide an archive sub-sample in cases where results are disputed. This is not possible using the segmentation procedure because the precision is not sufficient to allow meaningful comparisons of results generated in different laboratories.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

1) Fussell, R.J., Hetmanski, M.T., Macarthur, R., Findlay, D., Smith, F., Ambrus, A., and Brodesser, P.J., (2007) Measurement uncertainty associated with sample processing of oranges and tomatoes for pesticide residue analysis; J. Agric. Food Chem., 55, 1062-1070.

2) Hetmanski, M.T., Fussell, R.J., and Smith, F., (2003) Assessment of the Stability of Dithiocarbamate Pesticides during Cryogenic Sample Processing of Lettuce; CSL Report FD 0243.

3) Harrington, P., Horner, J., Hird, S., Griffiths, T., and Reynolds, S.L., (1998), Modification of the method for measurement of dithiocarbamate residues as carbon disulphide in fruit and vegetables; CSL Report FD 98/46.

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