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# GAS CHROMATOGRAPHIC DETERMINATION OF CARBARYL RESIDUES ON STORED WHEAT BY ON-COLUMN TRANSMETHYLATION

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## SUMMARY

A simple and rapid gas chromatographic method is described for the determination of carbaryl grain protectant residues down to 1 mg/kg on wheat during storage. Residues in acetone extracts are transmethylated on-column without extract concentration and are detected by a rubidium sulphate pellet alkali flame ionisation detector. Lower limits can be achieved by concentrating. Co-extracted phosphorothionates present on the grain are also detected.

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## INTRODUCTION

Carbaryl has been used extensively as a pre-harvest insecticide against pests of fruit, vegetables and field crops, and many methods have been devised for monitoring its residues[1]. For post-harvest application carbaryl has also been evaluated, with other new grain protectants, for control of organophosphorus (OP)-resistant grain storage insects in grain bulks in Australian field trials[2,3]. Although single insecticides controlled certain species, mixtures of fenitrothion or other OPs with either carbaryl or a pyrethroid were generally needed for full protection[4,5]. A screening method for carbaryl and fenitrothion residues on large numbers of wheat samples was required to monitor degradation during grain storage, and variation through bulks caused by possible incompatibility of spray mixtures.

Determination of carbamates has been reviewed extensively[6,7] and several chromogenic reagents have been utilised[8–11]. A semi-quantitative version of the diazo-coupling method[12] was adapted for monitoring carbaryl residues on wheat but this produced variabilities of up to 2 mg/kg units[13]. Direct gas–liquid chromatographic (GLC) procedures were inadequate at the concentrations present in wheat extracts, either because of low sensitivity and decomposition on column[14,15] or because of interference from co-extractives when using electron-capture detection (ECD)[16]. Most derivatives, for instance trichloroacetates[17], sulphonates[18] or bromoethylbenzamides[19] involved considerable work up and were unnecessarily sensitive for the purpose.

The present paper describes an adaptation of the on-column transmethylation procedure[20] by which carbaryl residues down to 1 mg/kg on whole wheat can be analysed rapidly as methyl N-methylcarbamate without the need for concentration of extracts. When required the method sensitivity can be extended to about 0.2 mg/kg without interference or clean-up, by prior extract concentration. Fenitrothion residues in the extract were also detected by the same method but not differentiated from other similar OP insecticides.

## EXPERIMENTAL

### *Insecticides*

Carbaryl formulation (Septene®) was provided by I.C.I. Australia (Groyden, Australia) as a 500 g/l folwable col, and fenitrothion formulation (Fenitrogard®) was provided by Wellcome Australasia (Concord, Australia) as a 1 kg/l emulsifiable concentrate. Analytical grade carbaryl 990 g/kg was provided by Roche-Maag Australia (Bankstown, Australia) and analytical grade fenitrothion 993 g/kg by Bayer Australia (Botany, Australia).

### *Wheat treatment and sampling*

Fresh residues on laboratory-sprayed wheat were obtained by applying a carbaryl emulsion with a continuous atomiser (100 kPa) on to 1 kg wheat which was allowed to empty from a funnel into a covered container. The wheat was sprayed twice for a total target application of 4 mg/kg. Eight replicates were taken from the bulk for extraction.

For field monitoring of fresh residues composite samples of six vertical cores of wheat treated with carbaryl 8 mg/kg in nine vertical concrete silo bins were taken shortly after the bins were filled. Samples of wheat with aged residues were drawn from a separate 15,000 tonne bulk which had been treated during normal harvest receival with a mixture of carbaryl 8 mg/kg and fenitrothion 12 mg/kg. At six months storage 20 separate 200-g grab samples were taken at 2 m depth and single extracts of each were analysed. At twelve months storage samples from six 500-g cores were mixed to form one composite sample which was replicated eight times for analysis. All samples were kept at 3°C or less until analysed.

### *Sample preparation*

Wheat samples (60 g) were extracted in analytical reagent grade acetone (50 ml) overnight, or longer, after grain dust had been removed by light sieving. Extracts were centrifuged at 1500 *g* for 5 min after which methanol (0.4 ml) was added to the supernatant (2.0 ml). Just prior to analysis the solution was made alkaline with 1 *M* KOH (40 $\mu$l) and mixed with a vortex stirrer.

### *Gas chromatography*

The prepared solutions (0 5 $\mu$l) were injected directly onto a 50-mm reaction bed of KOH-treated glass beads at 215°C which formed the initial part of a glass column of 1.8 m × 2 mm I.D. containing 100–120-mesh Porapak PS at 200°C. The Porapak column was silanised *in situ* with Silyl-8 (50 $\mu$l) before the glass beads were put in place. A Varian 2700 all glass column system was used with a rubidium sulphate pellet alkali flame ionisation detector (AFID) at 235°C. Flow-rates of nitrogen 31 ml/min (320 kPa) and hydrogen 46 ml/min were used with air set to 270 ml/min as a basis for later adjustment. Prepared columns were purged overnight at operating conditions of temperature and carrier flow and were ready for use the next day. The methyl N-methylcarbamate peak at retention time 2.5 min was compared with spiked extracts or standards. The stability and life of the column depended on the number of samples analysed and the amount of charring on the reaction bed from co-extractives. Peak heights for calibration standards declined slowly as the column aged but satisfactory results could normally still be obtained after three weeks of moderate use or, on a new column, with loads of over 60 samples per day Septa were adjusted or replaced daily because of the high operating pressure, and when required the column was cleaned and repacked with new support and reaction bed

### *Low residue analysis*

Centrifuged extracts of low residue samples (0.2–1 mg/kg) were concentrated up to five-fold in a gentle air stream before methanol and alkali equivalent to the amounts for normal extracts were added. Peak heights were compared with spiked extracts concentrated to the same degree. In exhaustive extraction studies, residues were first determined on whole wheat extracts after various extraction times. The wheat was then dried and milled to flour before being further extracted in acetone (60 ml). These extracts were treated in the same way as for low-residue wheat but were allowed to stand for 10 min after being made alkaline.

## RESULTS AND DISCUSSION

### *Sensitivity and recovery*

The facility of the method for monitoring large numbers of samples depends on adequate sensitivity of the nitrogen-selective AFID to detect the carbaryl derivative without having to concentrate extracts. Sensitivity was proportional to hydrogen flow and optimum detection was achieved by balancing hydrogen and air flows so that the solvent tail returned to baseline, and not beyond, before the carbamate was eluted. With the older style rubidium sulphate salt tip used the hydrogen flow was limited to 46 ml/min and the air flow was adjusted in the range 270–300 ml/min. The response of carbaryl standards in acetone was linear in the range 0.6–12 ng, equivalent to 1–20 mg/kg of carbaryl applied to grain, and at optimum flow-rates typical peak heights per 0.6 ng were 5–7% of full scale deflection ($\times 16 \cdot 10^{-12}$ A). Recovery from wheat extracts spiked with carbaryl in the same range was 92% at 1 mg/kg and varied between 96 and 101% in the 2–20 mg/kg range.

### *Solvent extraction efficiency*

Methanol was tested initially as an extraction solvent because of its previous use in extraction of OP protectants from wheat[21] and its known compatibility with the detection method[20]. However, methanol extracted other wheat components which reacted on-column and severely interfered with the detection of the carbaryl derivative. Because chlorinated solvents such as methylene chloride recommended in a semi-quantitative method[12] were unsuitable for the AFID, acetone was used instead.

TABLE I
EXTRACTION OF CARBARYL RESIDUES FROM FRESHLY TREATED AND AGED WHEAT SAMPLES

| *Sample and application* | *Storage time* | *Extraction time (days)* | *Mean residue* (mg/kg)* | *Proportion* ** extracted (%)* | *Replicates or number of samples* |
|---|---|---|---|---|---|
| Fresh | Negligible | 1 | 3.8 (0.4) | 98 (5) | 8 |
| 4 mg/kg | | 2 | 3.7 (0.3) | 95 (3) | 8 |
| | | 5 | 3.8 (0.4) | 100 | 8 |
| Field | 6 Months | 1 | 2.9 (0.6) | 74 (10) | 20 |
| 8 mg/kg | | 22 | 3.8 (0.9) | 100 | 20 |
| Field | 12 Months | 1 | 0.95 (0.06) | 74 (4) | 8 |
| 8 mg/kg | | 2 | 1.06 (0.11) | 81 (7) | 8 |
| | | 5*** | 1.29 (0.11) | 98 (9) | 8 |
| | | 11*** | 1.34 (0.14) | 100 | 8 |

* Standard deviation in brackets

** Mean of the proportions calculated from each replicate or sample

*** Two replicates at both days re-extracted as flour gave additional residues of 0.1 mg/kg (5 days) or none detectable (11 days)

Fresh and aged residues were extracted and analysed for carbaryl over extended periods following the criterion of Watts[22]. Results in Table I indicate that the freshly applied residue was completely extracted (*i.e.* > 95%) from the wheat within one day. For aged residues, however, after both six and twelve months, about 70% of the total extractable residue was detected after one day, with the proportion increasing to near completion between two and five days. Since wheat re-extracted after milling contained negligible additional residue, less than the standard deviation for wheat residues at five days, there appeared to be no advantage in extending extractions beyond this time. The residues found for both bulks of aged wheat were consistent with predicted values generated from a degradation model of carbaryl on grain[13].

TABLE II
CARBARYL RESIDUES OF SILO BULK SAMPLES DETERMINED BY TWO METHODS
Carbaryl residue determined as described in this paper. Figures in brackets are ranges determined by Dr. J. M. Desmarchelier using the TLC method adapted from Horwitz[12].

| *Site* | *Tonnes treated* | *Carbaryl residue (mg/kg)* |
|---|---|---|
| A | 600 | 5.5 (4–6) |
| B | 200 | 3.4 (2–4) |
| C | 200 | 5.6 (4–6) |
| D | 180 | 4.2 (4–6) |
| E | 780 | 7.1 (6–8) |
| F | 840 | 9.1 (6–8) |
| G | 1500 | 12.5 (6–8) |
| H | 1400 | 6.8 (6–8) |
| I | 1490 | 4.7 (4–6) |

*Field monitoring and comparison of method*

Representative samples from the upper 1-m layer of wheat bulks in nine country storage silo bins were taken shortly after completion of treatment. Nominal applications to the total bulk in each bin ranged between 7.2 and 8.3 mg/kg, but there was considerable variation within and between bins attributable in part to localised pockets of over- and under-treated wheat[23]. The levels were determined by the present procedure and compared with a semiquantitative method based on Horwitz[12] by an independent analyst. Residue results from the seven samples in the 3–7 mg/kg range were consistent using either method, but two samples estimated in the 6–8 mg/kg range by the thin-layer chromatographic (TLC) procedure gave figures exceeding 8 mg/kg by the present procedure (Table II).

*Detection of organophosphorus insecticides*

Six OP grain protectants were tested for possible interference with the carbaryl derivative. Under the conditions of analysis phosphorothionates [P(S)OR] such as fenitrothion were slowly hydrolysed on standing in the alkaline extract and the resulting acid was methylated on-column. Because the AFID was more sensitive to phosphorus than to nitrogen compounds a relatively large trimethylthiophosphate peak was detected at 8 5 min. The other protectants of this type tested, pirimiphos-methyl, methacrifos and chlorpyrifos-methyl, all generated peaks at this retention time but at different rates determined by their stability. The separation from the carbaryl derivative ($t_R$ 2.5 min) was sufficient to allow extracts to be injected every 4 min without interference if only the carbaryl residue was to be determined. A phosphorodithioate [P(S)SR], malathion, and a phosphate [P(O)OR], dichlorvos, produced small peaks at 8.5 min and 7.0 min respectively. Other grain protectants such as bioresmethrin (pyrethroid) synergised with piperonyl butoxide were not detectable.

Estimates of the fenitrothion residue were obtained by analysing samples and appropriate standards when hydrolysis was near completion after 20 h Extracts from a group of 20 wheat samples taken from the bulk stored for six months were treated in this way, and a mean residue equivalent to 5.6 mg/kg fenitrothion (S.D. 1.1 mg/kg) was found. Subsamples of the same 20 wheat samples which were extracted in methanol and analysed directly for fenitrothion by a standard method[21] indicated a mean residue of 4.9 mg/kg (S.D. 1.0 mg/kg). The reason for the slight variation between results was not determined but it could be related to extraction of dimethylthiophosphoric acid.

*Interference and low residue detection*

Carbaryl residues below about 1 mg/kg, which are no longer effective for grain protection, were not adequately determined with normal extracts because of interference from a small background peak at 2.7 min. When required, however, residues down to 0.2 mg/kg could be readily determined without interference by concentrating the extract and analysing within 1–2 min of being made alkaline. In these conditions, where the concentration of protein and other co-extractives was considerably increased, the carbaryl was still derivatised completely, but the hydrolysis producing the interfering peak was virtually inhibited. A related effect was observed with the fenitrothion derivative which also developed more slowly in wheat extracts after concentration. When large amounts of co-extractives were present, as in extracts of flour, even the carbaryl derivative required up to 10 min for full development. The nature of the compound responsible for the 2.7 min peak was not determined but it appeared to be associated with the alkaline acetone. Of the other derivatives of carbaryl from higher alcohols the ethyl-N-methylcarbamate at retention time 3.6 min has the advantage of better separation, but the sensitivity was reduced to about 40% of that of the methyl derivative. The propyl (6.1 min), isopropyl (4.4 min) and butyl derivatives (10.4 min) provided much less sensitivity.

Interference from dimethylformamide, which might possibly be used as a formulating agent for carbaryl, was also examined because of the sensitivity of the detector to nitrogen compounds. Under normal analytical conditions it was adequately resolved (2.8 min) from the carbaryl derivative (2.5 min) and was detected with comparable sensitivity to that of carbaryl. Test extractions of residue amounts from wheat treated with an experimental carbaryl formulation utilising this solvent indicated that both compounds were readily detected in the presence of the other to a lower limit of less than 1 mg/kg for dimethylformamide. As most other nitrogen-containing solvents available for use are less volatile it is likely that their retention times would be greater, and hence would not give rise to interference.

## APPLICATIONS

The procedure as developed would apply to N-methylcarbamates generally as well as to carbaryl, and to phosphorothionates generally as well as to fenitrothion. At the present time carbaryl is the only carbamate available for use as a grain protectant in Australia so the possibility of other N-methylcarbamates contributing to the derivative should not arise in this situation. Mixtures of different OPs are also not normally used. Carbaryl is normally applied to grain at 8 mg/kg in admixture with fenitrothion 12 mg/kg to protect grain from storage insects for up to nine months. The analytical procedure described would serve as a useful screen for incorrectly treated wheat and for rapid monitoring of variations in residue in bulks, such as locating pockets of under-treated grain which would have less protection. During storage both insecticides degrade predictably[13], but in cases where unforeseen early outloading of particular bulks was required the procedure would be useful to determine accurately the level of residue which may be close to the maximum residue limit of 5 mg/kg acceptable for carbaryl. It is also likely to be useful on grades of wheat other than Australian Standard White, which formed the basis of these tests, and on different wheat varieties and other grains subject to the absence of abnormal co-extractives.

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