JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 3693−3701 **3693**

Comparison of Accelerator Mass Spectrometry with Gas Chromatography for the Determination of Pesticide Residues in Individual Items in the Diets of Wild Birds and Mammals

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Methods to refine the assessment of exposure of wild birds and mammals to pesticides required measurement of pesticide residues in very small samples of their diets. Sample sizes were in the 1−100 mg range, and the target residue for measurement was 0.01 mg/kg. Gas chromatography−mass spectrometry (GC-MS) with large volume injection was compared with the use of an accelerator mass spectrometer (AMS) to measure residues of pesticide labeled at near-background levels with carbon-14. The GC-MS method was able to detect residues down to 0.1 ng per item of diet, and the AMS detected the radiolabel down to 1 mBq (0.06 disintegration per minute, 1 ng of pesticide at the specific activity used) per sample. The target residue level was achieved by the GC-MS method for samples down to 10 mg. The GC method appeared to be best suited to monitoring residues in field studies, and the AMS shows great potential for use in laboratory experiments concerning pesticide degradation.

KEYWORDS: Pesticide residues; GC-MS; accelerator mass spectrometry; seeds; insects; bird diets; mammal diets; radiolabeled pesticides; risk assessment

INTRODUCTION

Since the discovery of the adverse effects of organochlorine pesticides on wildlife, there has been public concern about the potential of agricultural chemicals to harm wildlife. Regulatory authorities address this concern by estimating the hazards from new pesticides. EU Directive 91/414/EEC (*1*) requires that risks to wild birds and mammals must be assessed before a pesticide can be authorized for sale. Risk estimates involve comparisons of pesticide exposure with the inherent hazard (toxicity) of the pesticide. Exposure is initially estimated by assuming that the animal feeds exclusively on food contaminated with pesticide at concentrations close to expected maximum levels. When this indicates unacceptable risk, the assessment may be refined using assumptions that are more realistic or by incorporating risk mitigation measures. These assumptions may be based on an estimate of the residues on the food (e.g., refs *1*−*3*), combined with the proportion of the daily food intake of a bird or mammal that comes from the treated area. This proportion is assumed to be equal to that of time spent in that area (*4*). However, the levels of pesticide residues may differ from those predicted, and the proportion of food that animals obtain in a treated area may not be equal to the proportion of time they spend there. It would therefore be desirable to test these assumptions with measurements of actual exposure in the field, as a research study to help improve regulatory approaches. Direct measurements could be obtained by recovering samples of diet from nestling birds in the wild, using neck collars (e.g., ref *5*), but such samples are often very small and may sometimes comprise only one or a few items of food.

Measurement of low levels of pesticide residues on small samples (e.g., 1−100 mg) requires a suitable method of analysis. Accelerator mass spectrometry (AMS) is a technique that measures isotopes in a sample on the basis of their mass-to-charge ratio, and when used to measure ^{14}C, it is up to a million times more sensitive than decay counting (AMS limit of detection is $\sim 7 \times 10^{-5}$ dpm/mg of C). With this sensitivity, a pesticide labeled with ^{14}C even at a low specific activity might be detectable at residue levels (e.g., 0.01 mg/kg). Prior to mass spectrometric determination of the isotopes, samples undergo drying under vacuum and "graphitization", which consists of oxidation to carbon dioxide followed by reduction to carbon. The AMS measures residues of ^{14}C-radiolabeled pesticide by comparing the ratio of ^{14}C to ^{13}C in the sample (*6*). Three approaches to sample preparation prior to graphitization (no preparation, enzymatic digestion followed by methanol extraction, and diethyl ether extraction followed by Florisil cleanup) have been compared. The AMS technique was compared to the conventional residue analysis technique of gas chromatography (GC) with a quadrupole mass spectrometer as detector together

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10.1021/jf040012u CCC: $27.50
Published on Web 05/13/2004

with a large-volume injector and a syringe of 100 μL capacity (*7*). Only the ether extraction with Florisil cleanup was used to prepare samples for GC. This study aimed to assess the suitability of these approaches as research tools for measuring low levels of pesticide on wildlife food items (individual seeds and insects). A fungicide not currently used in the United Kingdom was selected for use as a model compound.

MATERIALS AND METHODS

Instrumentation. The GC analysis equipment was a Voyager gas chromatograph−quadrupole mass spectrometer (Thermoquest, San Jose, CA) consisting of a GC8000, a Trace MS, an AS800 autosampler, and Xcalibur data system software. This was fitted with an Optic 2-200 multimode inlet (ATAS, Hardwick, Cambs., U.K.). The standard 10 μL syringe in the autosampler was replaced with a 100 μL syringe, to inject 100 μL (programmed as "10 μL") with one 100 μL (programmed as "10 μL") solvent wash before and after injection and with a 10 times slower plunger speed setting. The Optic inlet was programmed on the standard large volume injection program with an equilibration time of 1 min, initial temperature of 40 °C, vent time set to automatic (based on concentration of vented solvent vapor), ramp rate set to 4 °C/s, final temperature of 250 °C, end time of 22 min, split open time of 1 min and 20 s, purge pressure of 45 kPa, transfer pressure of 70 kPa, transfer time of 1 min and 30 s, initial run pressure of 50 kPa, final run pressure of 100 kPa, and solvent monitor threshold set at 50. The GC column was a 30 m × 0.25 mm i.d. fused silica column with a DB1701 stationary phase with film thickness of 0.25 μm (J&W, Folsom, CA). The GC oven was programmed to start at 40 °C with an equilibration time of 2 min. The temperature was held at 40 °C for 4 min (during transfer of sample from the inlet), then increased at 40 °C/min to 240 °C and then at 8 °C/min to 280 °C, and held at this temperature for 8 min. The quadrupole mass spectrometer was set to record electron impact chromatograms for selected ions *m*/*z* 216, 214, and 175 from 10 to 15 min (hexaconazole) and ions *m*/*z* 208, 181, and 97 from 15 to 20 min (fenpropathrin internal standard). Of the ions selected, those giving the best response and not affected by endogenous interferences were used for measurement (*m*/*z* 214 for hexaconazole and *m*/*z* 181 for fenpropathrin).

The AMS was an NEC 15SDH-2 Pelletron instrument. Graphite-containing cathodes were placed in a sample wheel that was inserted into the ion source of the AMS. The multicathode negative ion source (MC-SNICS) generated a cesium (Cs^+) ion beam that was accelerated onto the graphite surface. The resulting negative carbon ion beam was preaccelerated, passed through a spherical electrostatic analyzer, and then progressed toward the injection magnet. The magnet was set to inject $^{12}C^-$ (150 μs), $^{13}C^-$ (600 μs), and $^{14}C^-$ (0.1 s) ions sequentially at normally 68 keV; one combined measurement on each isotope in turn corresponded to one cycle. The carbon ion beam was accelerated toward the positive center terminal of the tandem Pelletron accelerator through an Einzel lens. The terminal voltage used for this series of analyses was 4.5 MV, with a particle energy of ∼22.5 MeV. At the central terminal electrons were stripped from the carbon atom to yield positively charged carbon ions ($^{12,13,14}C^{1+-6+}$). C^{4+} ions were selected for measurement as these were the most abundant at this energy. These ions were accelerated away from the center terminal and on toward the electrostatic quadrupole triplet and analyzing magnet. Immediately past the postanalyzing magnet, $^{12}C^{4+}$ and $^{13}C^{4+}$ ions were measured as an ion current in offset Faraday cups. $^{14}C^{4+}$ ions were passed down the high-energy beam line, through an electrostatic quadrupole doublet and a cylindrical electrostatic analyzer. From here, the ions entered a gas ionization detector where they were collected on anodes (four in total) that measured the energy loss and total energy of each ion. Other interfering non-$^{14}C^{4+}$ ions were generally prevented from entering the gas ionization detector by the combinations of electrostatic analyzers, magnets, slits, and charge state separation. Vacuum pressures of approximately 10^{-9} Torr were maintained in the beam line and 10^{-6} Torr in the ion source. Ion transmission through the instrument was between 30 and 60%. The burn-in time for each sample was 600 cycles (60.4 s) and the sample analysis time usually 3500 cycles (352.5 s). The cathode loading list, raw and processed data, and machine parameters were recorded.

Table 1. Hexaconazole Applied to Individual Seeds and Mealworms (Six Replicates for AMS, Three Replicates for GC)

item and mean mass (mg)	hexaconazole solution (μg/mL), 0.1 μL applied	activity per item (mBq)	hexaconazole per item (ng)	hexaconazole concn (mg/kg)
canary seed				
7	0.00	0.00000	0.000	0.000
7	0.75	0.00017	0.075	0.011
7	7.5	0.00168	0.75	0.107
7	75	0.0168	7.5	1.07
7	750	0.168	75	10.7
7	7500	1.68	750	107
mealworm				
30	0.0	0.00000	0.00	0.000
30	7.5	0.00168	0.75	0.025
30	75	0.0168	7.5	0.25
30	750	0.168	75	2.5
30	7500	1.68	750	25

An electronic digital pipet (Rainin) with a 10 μL disposable tip (Gilson C10) was used to treat each individual diet items with 0.1 μL of hexaconazole solution. Before use, the pipet was calibrated with a solution of [^{14}C]chlorothalonil in toluene. This calibration was done using a Packard Tri-Carb liquid scintillation counter. Samples for AMS analysis were dried under vacuum using a Savant AES2010 Speed Vac pump. The carbon content of samples for AMS was measured using a C,H,N analyzer (CE Instruments C,H,N NA2100 Brewanalyser). A water bath (37 °C) was used for proteinase incubations.

Reagents and Glassware. Laboratory glassware, including glass mortars (75 mm diameter) and pestles, was supplemented with disposable polypropylene items (funnels, syringes, etc.). Luer-fitting 0.45 μm PTFE syringe filters (Waters, Milford, MA) were used to separate sample extract from solid matrix. Disposable glassware used for the AMS graphitization process (York Glassware, York, U.K.) was prebaked at 500 °C for 2−4 h. The organic solvents and water were of HPLC grade from Fisher Chemicals, Loughborough, U.K., or of glass-distilled grade from Rathburn Chemicals, Walkerburn, Scotland. Anhydrous sodium sulfate, granular form (Fisher Chemicals "Certified"), was used in the extraction step. Cartridges used for extract cleanup were LC-Florisil (1 g, 6 mL) "Supelclean" SPE tubes (Supelco, Bellefonte, PA). Proteinase K, used to digest samples, was obtained from Aldrich Chemical Co. (Milwaukee, WI). Materials for the AMS graphitization process, tributyrin, copper oxide wire (ACS), cobalt powder (100 mesh, 99.9%), zinc powder (100 mesh, 98.98%), and titanium (II) hydride (325 mesh, 98%) were all from Aldrich Chemical Co. The aluminum cathodes and poco graphite rods (1 mm diameter) were obtained from National Electrostatics Corp. The ANU sugar ^{14}C standard for AMS had a certificated value of 1.5061 Fraction Modern Carbon (Quaternary Dating Research Centre, Australian National University, Canberra, Australia), and synthetic graphite (200 mesh, 99.9999%) was "Alfa Aestar" (Johnson Matthey PLC). Liquid scintillation counting materials were supplied by Packard Corp. C,H,N Analyzer materials, tin capsules, and Chromosorb W were provided by Elemental Microanalysis Ltd.; urea was provided by Thermoquest.

Pesticide Solutions. Hexaconazole [(*RS*)-2-2,4-dichlorophenyl-1-(1*H*-1,2,4-triazol-1-yl)hexan-2-ol], a fungicide, was selected as the model pesticide because of its low volatility and low toxicity. Furthermore, it is not registered for use in the United Kingdom (so that, should the study reach the field trial stage, there are unlikely to be residues already present). Radiolabeled hexaconazole with a specific activity of 2.16 GBq/mmol (6870 Bq/μL) was a gift from Zeneca (now Syngenta) together with 1 g of technical hexaconazole. A stock treatment solution was prepared by diluting the labeled compound with the technical substance in toluene to give a solution that contained 7500 μg/mL hexaconazole (0.75 μg and 0.0168 Bq in 0.1 μL). Individual sample items were spiked using dilutions of this solution in toluene. Further stock solutions of labeled hexaconazole were prepared to give a

Table 2. Hexaconazole Concentrations in Bulk Canary Seed and Mealworm Extracts

hexaconazole solution (ng/mL) in extract	activity of solution (mBq/mL)	volume taken (mL) before dilution	mass of 8 items in 4 mL of extract (mg)	hexaconazole concn (ng/mL)	activity (mBq/mL)	volume[b] (mL) taken for GC or AMS (mL)
canary seed		diluted to 4 mL				
75000.0[a]	168.0[a]	0.08 (evaporated)	60.0	1500.0	3.353	0.5
1500.0	3.353	0.4	60.0	150.0	0.3353	0.5
150.0	0.3353	0.4	60.0	15.0	0.0335	0.5
15.0	0.0335	0.4	60.0	1.5	0.00335	0.5
1.5	0.00335	0.4	60.0	0.15	0.00034	0.5
0.0	0.00000	0.0	60.0	0.00	0.00000	0.5
mealworm		dilited to 4 mL				
75000.0[a]	168.0[a]	0.08 (evaporated)	262.4	1500.0	3.353	0.25
1500.0	3.353	0.4	262.4	150.0	0.3353	0.25
150.0	0.3353	0.4	262.4	15.0	0.0335	0.25
15.0	0.0335	0.4	262.4	1.5	0.00335	0.25
1.5	0.00335	0.4	262.4	0.15	0.00034	0.25
0.0	0.00000	0.0	262.4	0.00	0.00000	0.25
diethyl ether		diluted to 4 mL				
75000.0[a]	168.0[a]	0.08 (evaporated)	none	1500.0	3.353	0.5
1500.0	3.353	0.4	none	150.0	0.3353	0.5
150.0	0.3353	0.4	none	15.0	0.0335	0.5
15.0	0.0335	0.4	none	1.5	0.00335	0.5
1.5	0.00335	0.4	none	0.15	0.00034	0.5
0.0	0.00000	0.0	none	0.00	0.00000	0.5

[a] Standard in solvent only, not extract. [b] For AMS, six replicates at each level; for GC, four replicates at each level.

Table 3. Hexaconazole Concentrations in Bulk Linseed, Grass, and Sorrel Seed Extracts and in Solvent (One Set in Methanol for AMS, One Set in Diethyl Ether for GC)

hexaconazole solution (ng/mL) in extract	activity of solution (mBq/mL)	volume taken (mL) before dilution	mass of 6 items in 0.6 mL extract (mg)	hexaconazole concn (ng/mL)	activity (mBq/mL)	volume[b] taken for GC or AMS (mL)
linseed						
75000.0[a]	16800.0[a]	0.0687 (evaporated)	52.0	8591.0	1920.0	0.1
1500.0	1920.0	0.06	52.0	859.1	192.0	0.1
150.0	192.0	0.06	52.0	85.9	19.2	0.1
15.0	19.2	0.06	52.0	8.59	1.92	0.1
1.50	1.92	0.06	52.0	0.859	0.192	0.1
0.00	0.00	0.00	52.0	0.000	0.000	0.1
grass		diluted to 0.6 mL				
75000.0[a]	16800.0[a]	0.0144 (evaporated)	10.5	1800.0	402.4	0.1
1800.0	402.4	0.06	10.5	180.0	40.24	0.1
180.0	40.2	0.06	10.5	18.0	4.024	0.1
18.0	4.02	0.06	10.5	1.8	0.402	0.1
1.80	0.40	0.06	10.5	0.18	0.040	0.1
0.00	0.00	0.00	10.5	0.00	0.000	0.1
sorrel		diluted to 0.6 mL				
75000.0[a]	16800.0[a]	0.0084 (evaporated)	6.24	1050.0	234.7	0.1
1050.0	234.7	0.06	6.24	105.0	23.5	0.1
105.0	23.5	0.06	6.24	10.5	2.35	0.1
10.5	2.35	0.06	6.24	1.05	0.235	0.1
1.05	0.235	0.06	6.24	0.105	0.024	0.1
0.00	0.000	0.00	6.24	0.000	0.000	0.1
solvent		diluted to 0.6 mL				
75000.0[a]	16800.0[a]	0.06 (evaporated)	0.0	7500.0[a]	1680.0[a]	0.1
7500.0[a]	1680.0[a]	0.06	0.0	750.0[a]	168.0[a]	0.1
750.0[a]	168.0[a]	0.06	0.0	75.0[a]	16.8[a]	0.1
75.0[a]	16.8[a]	0.06	0.0	7.50[a]	1.68[a]	0.1
7.5[a]	1.68[a]	0.06	0.0	0.75[a]	0.168[a]	0.1
0.75[a]	0.17[a]	0.06	0.0	0.075[a]	0.017[a]	0.1
0.00[a]	0.00[a]	0.00	0.0	0.000[a]	0.000[a]	0.1

[a] Standard in solvent, not extract. [b] For AMS, four replicates at each level; for GC, three replicates at each level.

concentration of 75 μg/mL. Solutions in diethyl ether and in sample extract were obtained by careful evaporation of the solvent from aliquots of the concentrated solution and redissolving in a larger volume of either solvent or sample extract. These solutions were serially diluted with either solvent or sample extract with the addition of internal standard when appropriate. Fenpropathrin, a pyrethroid insecticide, was a convenient internal standard in the GC-MS method to correct for errors in the final volume of the extracts; it was obtained from QMX Laboratories Ltd., Thaxted, Essex, U.K.

Diet Items and Treatment with Pesticide. Untreated seeds of several types (canary, grass, linseed, and sorrel) and small mealworms as representative insects were used in this study. Seeds and mealworms were spiked individually with hexaconazole dissolved in 0.1 μL of toluene, using an electronic micropipet. Doses were based on the

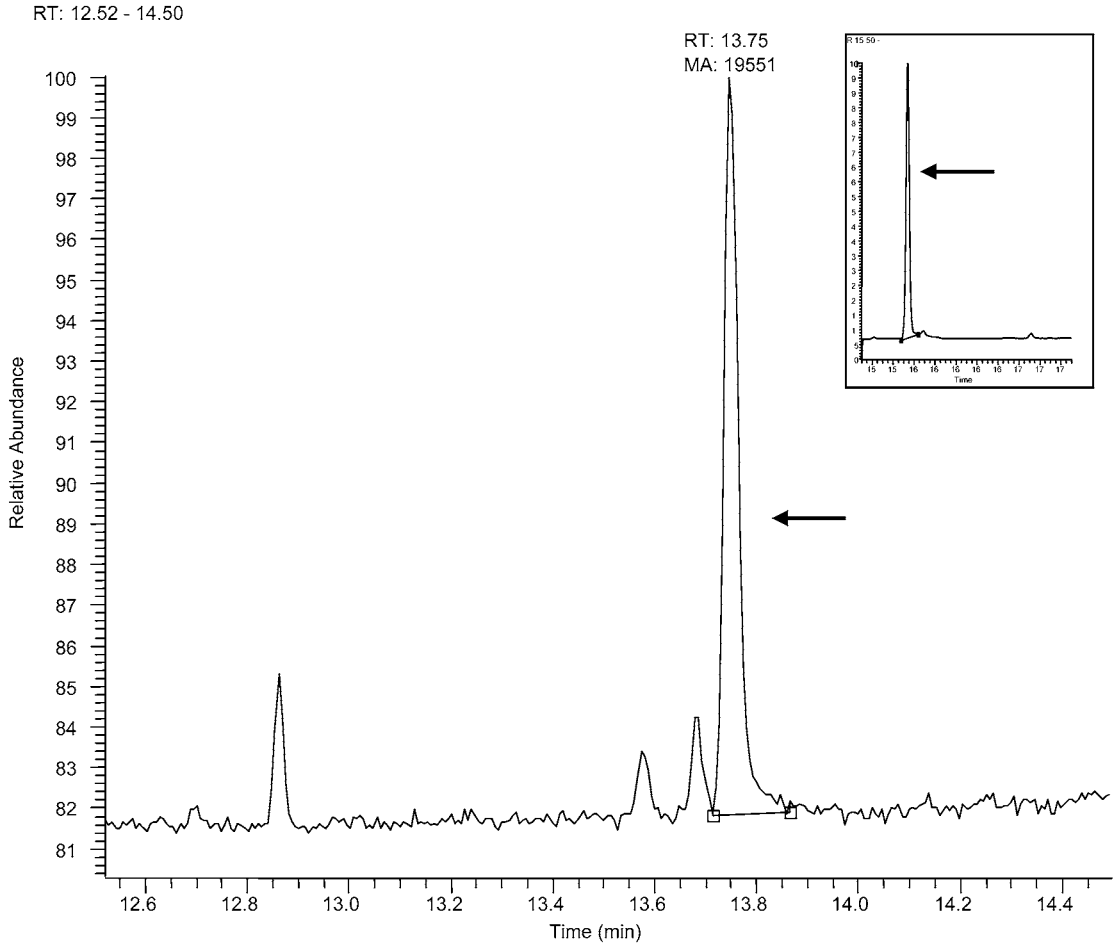

Figure 1. Ion chromatogram (*m*/*z* 214) of 0.001 μg/mL hexaconazole standard (0.1 mL injected) with retention time of 13.75 min. (Inset) Ion chromatogram (*m*/*z* 181) from 15.5 to 17.5 min. Internal standard retention time is 15.94 min.

average mass of items of each type, but individual seeds and mealworms were weighed so that the actual concentrations could be calculated. All samples were treated on the same day, left to dry for 10 min, then put into labeled plastic Eppendorf tubes, and stored at −80 °C. The treatment levels for the main study using spiked samples are shown in **Table 1**.

Analytical Procedures. The aim of the study was to compare different approaches to the measurement of background residues in small samples. One set of samples was taken straight into the graphitization procedure in preparation for AMS analysis. Another set of samples was digested with enzyme and extracted into methanol and then graphitized. A third set of samples was extracted with diethyl ether and cleaned up using Florisil solid phase extraction cartridges for both GC-MS and AMS. Additionally, the measurement techniques were compared directly by spiking bulk extracts.

Preparation of Individual Samples for AMS Analysis without Extraction. Two separate sets of samples were used to optimize radioactivity levels and sample preparation for AMS analysis. The first set contained too much activity for a result on the AMS equipment, so a second set of samples was spiked with a solution of $\sim^1/_{10}$ of the specific activity as shown in **Table 1**. The samples were added to 50 ± 10 mg of copper oxide wire and dried under vacuum for 60 min with 30 min of radiant cover heat using the Speed Vac pump. The dry samples (~2.8 mg per canary seed, ~5.5 mg per mealworm) were then oxidized to carbon dioxide, which was reduced to graphite and transferred manually to the cups that were inserted into a sample wheel to become cathodes for AMS analysis.

Enzyme Digestion/Methanol Extraction of Seeds and Mealworms Prior to AMS. Further samples from the second set were digested and extracted as follows. Mealworm samples were frozen in liquid nitrogen; weighed frozen mealworms and seed samples were then pulverized with a glass rod and reweighed. To each sample was added 100 μL of a 1 mg/mL solution of proteinase K in water, and the sample was vortex mixed and incubated overnight at 37 °C to digest the sample. Methanol (200 μL) was added, vortex mixed, and centrifuged for 5 min at 2100*g*. An aliquot (200 μL) was taken from the supernatant and added to 100 μL of 20 mg/mL tributyrin in methanol and 50 ± 10 mg of copper oxide wire in a sample tube. The tributyrin, from a petrochemical source low in ^{14}C, was added to increase the carbon content of the extract to provide sufficient graphite for manual transfer to the cathode. These samples were dried using the Speed Vac pump, for 1 h, with 30 min of radiant cover heat, prior to graphitization and AMS analysis.

Extraction and Cleanup Prior to GC-MS and AMS Analyses. An extraction and cleanup method was adapted for the analysis of hexaconazole on grain and then scaled down for use on individual seeds and mealworms. Individual blank or spiked samples of canary seed (or mealworm) were weighed and added to 0.25 g (or 0.8 g) of anhydrous sodium sulfate in a mortar and ground to a powder using a pestle. The powder was transferred via a funnel to a 4 mL HPLC vial followed by 2, 1, and 0.5 mL rinsings of the mortar and pestle; the vial was then capped, and the contents were thoroughly mixed. A 0.45 μm PTFE filter was fitted to the base of the barrel of a 2 mL disposable syringe, suspended above a clean 4 mL HPLC vial. The supernatant extract was transferred by Pasteur pipet from the extraction vial to the syringe barrel followed by two vortex-mixed rinsings with 1 mL of diethyl ether; for the second rinse, the funnel was placed in the top of the syringe barrel, and the contents of the extraction vial were tipped into the funnel. The vial was rinsed with 2 × 0.5 mL portions of diethyl ether, which were added to the funnel. The syringe now contained sodium sulfate and ether rinses so it was necessary to use the syringe plunger to push the rinses through the filter. The vial containing clear extract filtrate was capped, briefly vortex mixed, and then evaporated

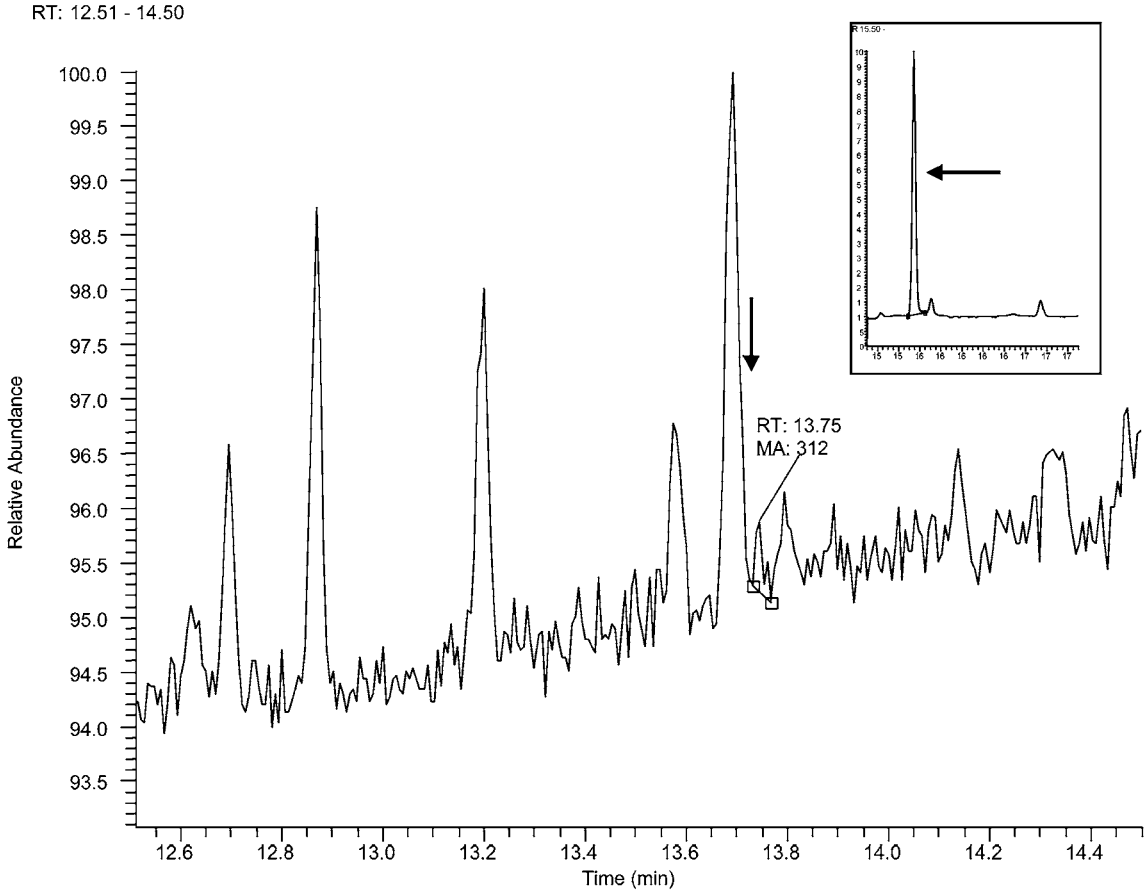

Figure 2. Ion chromatogram (*m*/*z* 214) of blank sorrel seed extract. Hexaconazole retention time is 13.75 min. (Inset) Ion chromatogram (*m*/*z* 181) from 15.5 to 17.5 min. Internal standard retention time is 15.94 min.

just to dryness under a gentle stream of nitrogen, and the residue was dissolved in 1 mL of 4% ethyl acetate and 96% hexane. The extract in 4% ethyl acetate and 96% hexane was applied to a prewashed Florisil (1 g) SPE cartridge followed by hexane rinses of the sample tube. The cartridge was eluted with 5 mL of 50% hexane and 50% ethyl acetate (discarded) and then with 10 mL of 2% acetone and 98% ethyl acetate (collected). The collected fraction was evaporated just to dryness, and the residue was redissolved in diethyl ether and made up to an appropriate volume (0.5 mL).

Individual sample extracts were transferred to 1 mL HPLC vials, then evaporated just to dryness, and made up to 0.2 mL by the addition of 0.1 mL of a 0.01 μg/mL fenpropathrin solution and 0.1 mL of the same solvent. Approximately 160 μL of each extract was transferred to a 250 μL vial insert supported in a 2 mL GC vial.

Preparation of Simulated Samples from Bulk Extracts. To eliminate the variability from the application, extraction, and cleanup stages when the measurement techniques were compared (AMS against GC), bulk extracts were prepared from untreated seeds and mealworms as described for individual items but using 5 times the amount of extraction solvent. The cleaned up bulk extracts were used to prepare standard hexaconazole solutions to simulate extracts of individual spiked seeds and mealworms. The cleaned up bulk extracts were made up to the following volumes: 50 canary seeds in 25 mL, 50 mealworms in 25 mL, and 100 linseed, grass, or sorrel seeds in 10 mL. To spike these bulk extracts, aliquots of stock hexaconazole standard solution were evaporated just to dryness and redissolved in extract or solvent as shown in **Tables 2** and **3**. These solutions were serially diluted with extract or solvent as shown in the tables. Aliquots equivalent to one seed or half a mealworm were added to glass sample tubes containing 50 $\pm$ 10 mg of prebaked copper oxide wire and then dried under vacuum and graphitized for AMS analysis. For GC analysis, aliquots equivalent to one seed or half a mealworm were evaporated just to dryness and dissolved in an internal standard solution such that the amount of fenpropathrin was 3.2 ng per seed or half mealworm and the volume of solution in a 250 μL insert in a GC vial was at least 150 μL.

Calibration Standards and Controls for AMS. The two standards used to calibrate the AMS instrument were ANU sugar (5−7 mg) and synthetic graphite (2−3 mg). These were added to copper oxide as for the samples. As a blank for the methanol extracts of proteinase digests, a combustion tube containing copper oxide and 100 μL of tributyrin solution was prepared. Several replicates of each were dried, graphitized, and analyzed by AMS. Sample extracts were accompanied by standard solutions of equivalent concentrations in the appropriate solvent.

Calibration Standards for GC. Sample extracts and spiked blank extracts were analyzed in sequences each containing standard solutions in a mixture of ethyl acetate and hexane (typically 1:9 by volume and each containing fenpropathrin internal standard at a concentration of 0.005 μg/mL) spread through the sequence at concentrations covering the expected range of hexaconazole concentrations.

AMS Analysis. The sample wheel, in which the graphite-containing cathodes were placed, was inserted into the ion source of the AMS instrument. The cesium (Cs^+) ion beam was accelerated onto the graphite surface, and the resulting negative carbon ion beam contained $^{12}C^-$, $^{13}C^-$, and $^{14}C^-$ and other ions such as $^{16}O^-$ (the isobar $^{14}N^-$ is unstable and therefore cannot interfere with the ^{14}C measurement). One combined measurement on each carbon isotope in turn corresponded to one cycle. The upper limit of measurement was taken as 150 counts per cycle. There were typically 3500 cycles per sample.

Gas Chromatography. Each 100 μL sample injection onto the packing of the injection port liner was preceded by a noninjected 100 μL syringe wash with solvent (but no sample wash) and followed by a further 100 μL solvent wash. Most of the solvent from the injected sample was evaporated by passing helium carrier gas through the packed liner at a temperature of 40 °C until the solvent vapor detector in the open injection port split outlet registered a low level. The split outlet was then closed automatically and the injection port heated rapidly to

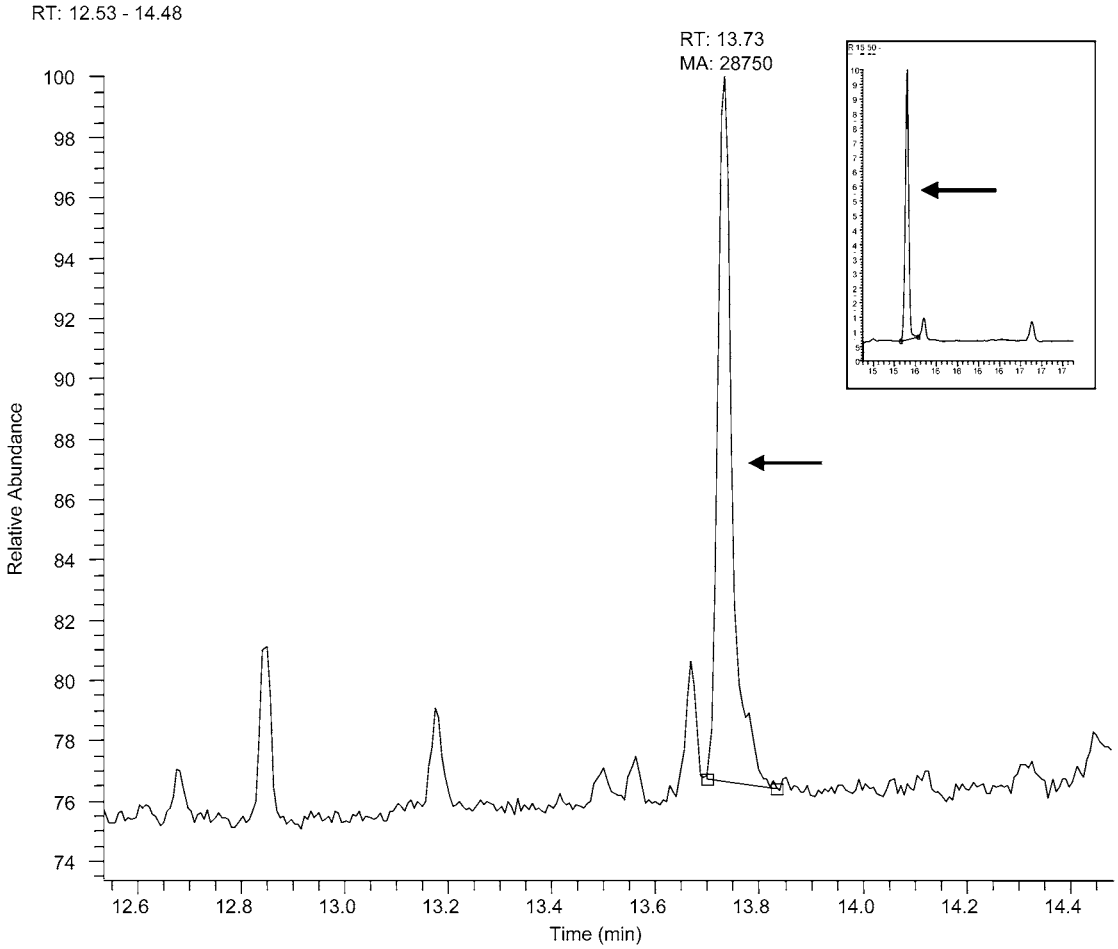

Figure 3. Ion chromatogram (*m*/*z* 214) of spiked sorrel seed extract equivalent to 0.1 ng of hexaconazole added to a 1 mg seed. Hexaconazole retention time is 13.73 min. (Inset) Ion chromatogram (*m*/*z* 181) from 15.5 to 17.5 min. Internal standard retention time is 15.92 min.

Table 4. Results from AMS Analysis of Individually Treated Seeds and Mealworms

sample	hexaconazole added (ng)	activity added (mBq)	activity found (mBq)	coefficient of variation (%)	hexaconazole found (ng)[a]
canary seed	0.000	0.00000	<0.899[b]		<102.9[b]
mean 7 mg	0.075	0.00017	<0.899[b]		<102.9[b]
(6 replicates	0.750	0.00168	<0.899[b]		<102.9[b]
at each level)	7.50	0.0168	<0.899[b]		<102.9[b]
	75.0	0.168	1.243	8	256
	750	1.68	3.364	20	1203
mealworm	0.000	0.00000	<2.003[b]		<11[b]
mean 30 mg	0.750	0.00168	<2.003[b]		<11[b]
(6 replicates	7.50	0.0168	<2.003[b]		<11[b]
at each level)	75.0	0.168	<2.003[b]		166
	750	1.68	4.983	22	1558

[a] Hexaconazole calculated after subtraction of mean of blanks. [b] Less than mean of blanks + 2 standard deviations.

Table 5. Results from AMS Analysis of Methanol Extracts of Individually Treated Seeds and Mealworms (Corrected for Blank Results)

sample	hexaconazole added (ng)	activity added (mBq)	activity found (mBq)	standard deviation (mBq)	hexaconazole found[a] (ng)
canary seed	0.000	0.00000	<0.046[b]		<20.6[b]
mean 7 mg	0.075	0.00017	<0.046[b]		<20.6[b]
(6 replicates	0.750	0.00168	<0.046[b]		<20.6[b]
at each level)	7.50	0.0168	<0.046[b]		<20.6[b]
	75.0	0.168	0.234	0.044	105.7
	750	1.68	2.283	1.046	1032
mealworm	0.000	0.00000	<0.029[b]		<12.7[b]
mean 30 mg	0.750	0.00168	<0.029[b]		<12.7[b]
(6 replicates	7.50	0.0168	0.089	0.033	39.7
at each level)	75.0	0.168	0.315	0.067	140.6
	750	1.68	2.852	0.676	1273

[a] Calculated after subtraction of mean of blanks. [b] Less than 2 standard deviations above mean of blanks.

transfer the solute in the carrier gas stream from the injection port packing onto the GC's capillary column, which was held at a temperature of 40 °C for 4 min from injection until the start of the first column oven temperature ramp.

The quadrupole mass spectrometer recorded individual chromatograms for three ions obtained from each compound by electron impact fragmentation. The hexaconazole concentrations were calculated using the ratio of hexaconazole *m*/*z* 214 ion peak height to that of fenpropathrin *m*/*z* 181 peak height. Ion chromatograms for a standard, a blank, and a sample are shown in **Figures 1**−**3**. A typical calibration graph of peak height ratio (hexaconazole/internal standard) against hexaconazole concentrations between 0.16 and 8.0 ng/mL was fitted by a regression line of the equation $0.0215x - 0.0023$ with an R^2 value of 0.9977 (**Figure 4**). Having established linearity, to compensate for any response changes, the ratio in samples was compared to the average of that in standards of similar concentration injected before and after.

RESULTS AND DISCUSSION

The electronic pipet used to apply hexaconazole to individual items was found to be inaccurate when operated at its lowest volume and the applied solution ran off some samples. To avoid

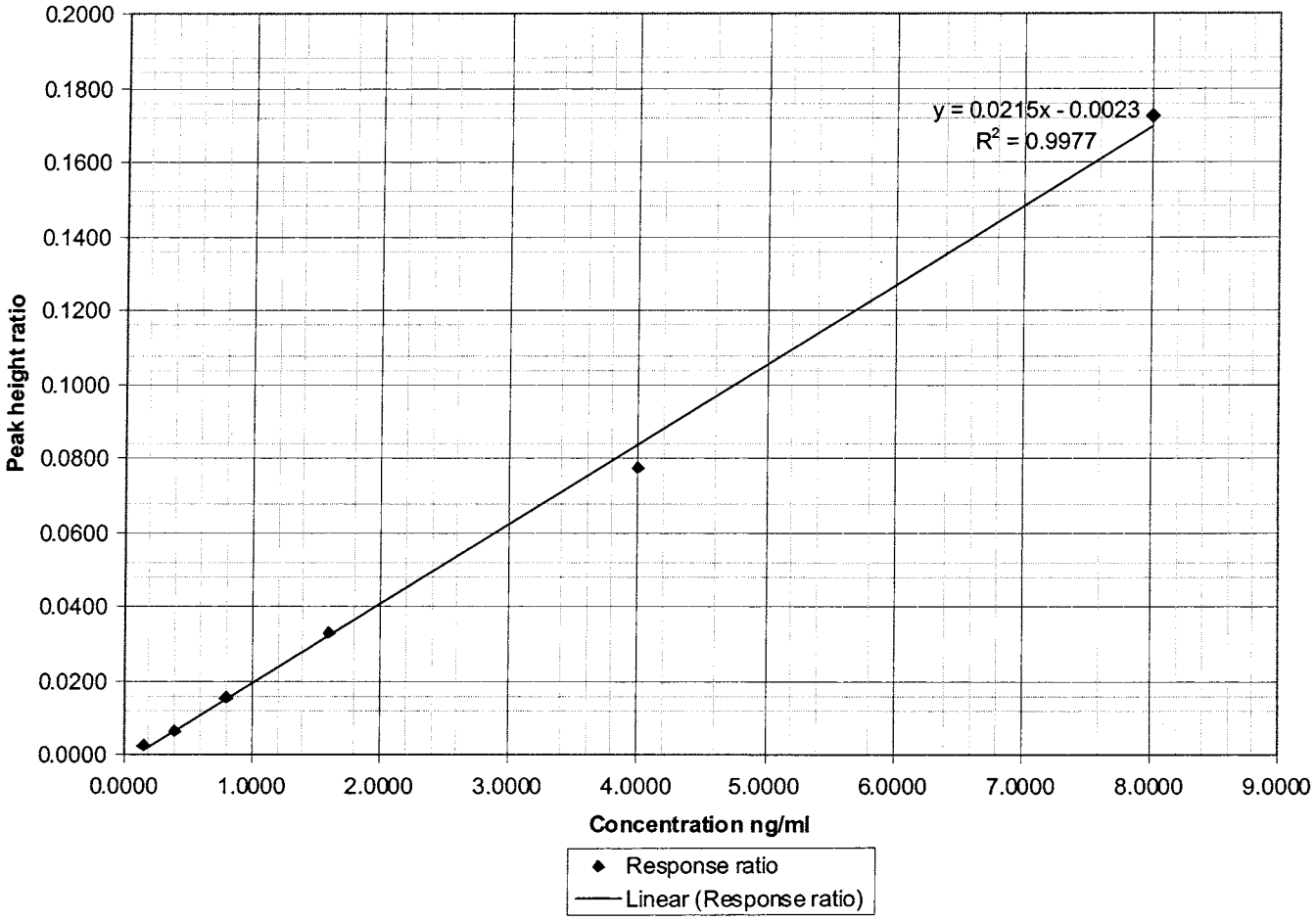

Figure 4. Typical calibration curve for hexaconazole using the ratio of its peak height from the *m/z* 214 chromatogram to the height of the *m/z* 181 fenpropathrin internal standard peak.

this source of error when the two instrumental techniques were compared, untreated samples were extracted in bulk and the extracts were spiked at the appropriate levels.

Samples for AMS Analysis, Not Extracted. Most of these samples in the second (lower activity) set were analyzed by graphitization of the whole sample, and the results are shown in **Table 4**. The lower limit of detection was limited by the intrinsic radioactivity of the untreated sample (estimated at approximately 0.92 mBq for a canary seed and 1.18 mBq for a mealworm). Although this background could be subtracted, even a 1% variation in the estimation of the carbon content of the sample (due to the presence of different moisture content, etc.) would mask all but the top two levels of added radioactivity from the lower specific activity application.

Samples for AMS Analysis Digested by Enzyme, Then Extracted. Some of the samples of the second set were digested with proteinase and then extracted into methanol, which was evaporated prior to AMS analysis. **Table 5** shows the results for the methanol extracts, corrected as far as possible for the radioactivity in the added tributyrin and in the residual carbon from coextractives as indicated by the difference between blank extract and tributyrin alone. This procedure shows mean recoveries of radioactivity closer to the amounts applied, but the variation between samples (as measured by standard deviation) is proportionately larger than in samples that were analyzed without extraction.

Samples for AMS and GC, Extracted and Cleaned Up. Individually treated canary seed and mealworm samples, extracted with diethyl ether, cleaned up, and analyzed by GC, showed a variable recovery of hexaconazole (**Table 6**) that was higher than expected due to the inaccuracy of the application device, but these results have been included as they show that the GC method was capable of detecting hexaconazole down to the lowest treatment level and at the correct order of magnitude. The extraction and cleanup method used here would be prohibitively expensive for the analysis of large numbers of samples, but it should be possible to adapt it to a 96-well plate format in which up to 96 samples (including procedural recoveries, etc.) could be prepared simultaneously at a lower cost.

Table 6. Results from GC Analysis of Individually Treated Seeds and Mealworms

sample	hexaconazole added (ng)	hexaconazole found (ng)	standard deviation	coefficient of variation (%)
canary seed	0.000	<0.063[a]		
mean 7 mg	0.075	0.268	0.015	6
(3 replicates at	0.75	1.21	0.035	3
each level)	7.5	14.2	2.71	19
	75	96	49.1	51
	750	1420	248	17
mealworm	0.00	<0.089[a]		
mean 30 mg	0.75	1.00	0.092	9
(3 replicates at	7.5	8.4	3.29	39
each level)	75	113	55.3	49
	750	1250	558	45

[a] Less than 2 standard deviations above the mean of the blanks.

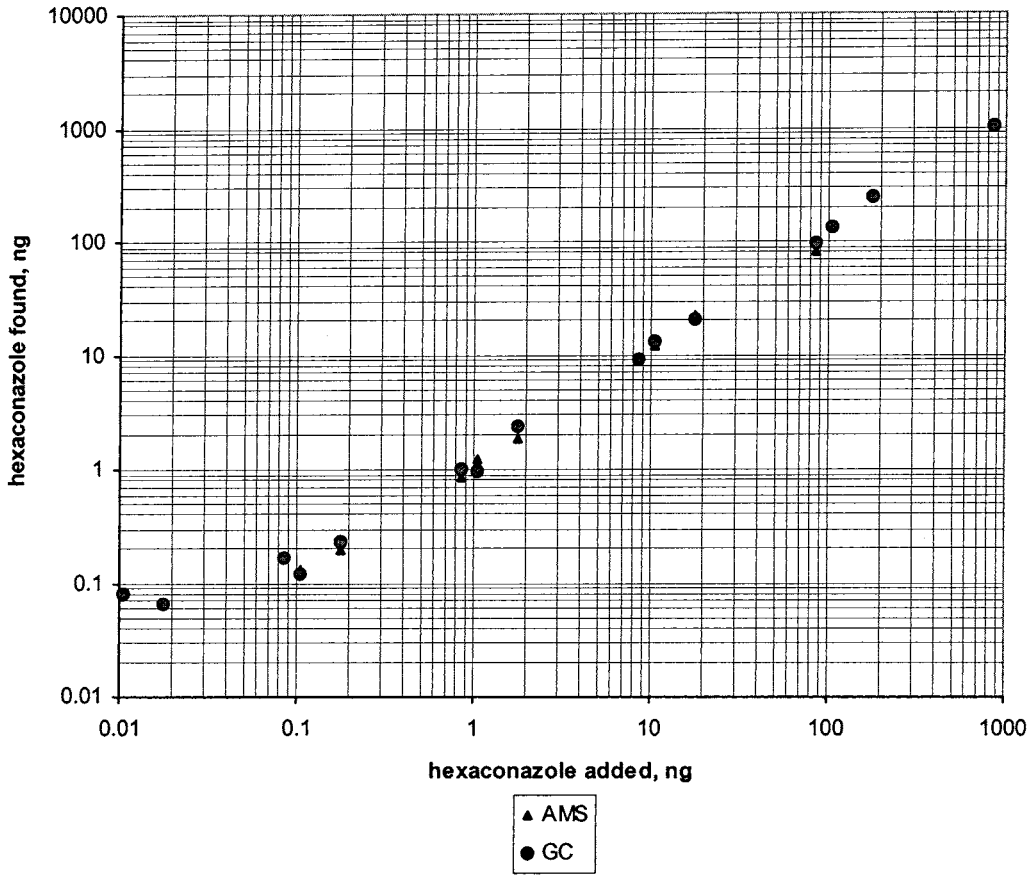

Figure 5. Hexaconazole measured in bulk extracts of five sample types, by AMS and by GC, as presented in **Tables 7** and **8**.

Analysis of Bulk Samples. To compare the measurement techniques but avoid other sources of variability, cleaned extracts were prepared from untreated samples and then spiked with hexaconazole, and aliquots equivalent to individual seeds or half-mealworms were taken for analysis by AMS and GC. These extracts should contain fewer coextracted nonvolatile carbon compounds than the uncleaned methanol extracts previously used for AMS analysis. The specific activity of the radiolabeled hexaconazole solution used to spike linseed, grass, and sorrel seed extracts was deliberately raised in order to detect the smaller residues, but this meant that the top level of treatment saturated the AMS detector and failed to give quantitative results. **Table 7** shows the results of all the AMS analyses on bulk extracts (six samples at each level for canary seeds and mealworms, four samples at each level for linseed, grass, and sorrel seeds). The mean of five determinations of the ^{14}C content of the added liquid paraffin carbon source (used as a lower ^{14}C carbon source than tributyrin) was subtracted from the total activity; the mean was 0.017 mBq and the standard deviation 0.0067 mBq. Some of the blank samples gave relatively high ^{14}C measurements that raised some mean blanks above the means of some of the low level spiked samples. The results indicate that AMS may be used over a concentration range of ~3 orders of magnitude.

The measurements on similar samples by GC-MS (four replicates for canary seeds and mealworms; three replicates for linseed, grass, and sorrel seeds) in **Table 8** show that measurements can be made down to a level of ~0.1 ng of hexaconazole per sample (i.e., 0.1 mg/kg on a 1 mg sample as shown in **Figure 3**; 0.004 mg/kg on a 25 mg sample). The highest levels were diluted to avoid contamination of the GC-MS instrument, but residues may be measured over at least 3 orders of magnitude.

Table 7. Results from AMS Analysis of Spiked Bulk Extracts of Seeds and Mealworms

sample	hexaconazole added (ng)	activity added (mBq)	activity found (mBq)	coefficient of variation (%)	hexaconazole found (ng)
canary seed	0.000	0.000000	<0.025[a]		<11.1[a]
mean 7.0 mg	0.75	0.00168	<0.025[a]		<11.1[a]
(6 replicates	0.075	0.000168	<0.025[a]		<11.1[a]
at each level)	7.5	0.0168	<0.025[a]		<11.1[a]
	75	0.168	0.268	7	119.8
	750	1.68	2.715	6	1212
mealworm	0.0000	0.000000	<0.216[a]		<96.4[a]
mean 30 mg	0.0375	0.000084	<0.216[a]		<96.4[a]
(6 replicates	0.375	0.00084	<0.216[a]		<96.4[a]
at each level)	3.75	0.0084	<0.216[a]		<96.4[a]
	37.5	0.084	<0.216[a]		<96.4[a]
	375	0.84	1.4781	3	659.9
linseed seed	0.000	0.0000	<0.165[a]		<0.737[a]
mean 8.67 mg	0.086	0.0192	<0.165[a]		<0.737[a]
(4 replicates	0.859	0.192	0.188	8	0.841
at each level)	8.59	1.92	2.00	3	8.96
	85.9	19.2	18.5	5	82.9
	859	192	too high		
grass seed	0.000	0.0000	<0.020[a]		<0.088[a]
mean 1.75 mg	0.018	0.0040	<0.020[a]		<0.088[a]
(4 replicates	0.180	0.040	0.043	7	0.192
at each level)	1.80	0.402	0.413	5	1.85
	18.0	4.02	4.88	5	21.8
	180	40.2	too high		
sorrel seed	0.000	0.0000	<0.022[a]		<0.097[a]
mean 1.04 mg	0.011	0.0024	<0.022[a]		<0.097[a]
(4 replicates	0.105	0.0235	0.0298	31	0.133
at each level)	1.05	0.235	0.279	6	1.25
	10.5	2.35	2.63	6	11.8
	105	23.5	too high		

[a] Less than 2 standard deviations above mean of blanks.

Comparison of the Two Techniques, AMS and Large Volume Injection GC-MS. The results from the two techniques are compared in **Figure 5**. The use of large volume injection

Table 8. Results from GC Analysis of Spiked Bulk Extracts of Seeds and Mealworms

sample	hexaconazole added (ng)	hexaconazole found (ng)	standard deviation	coefficient of variation (%)
canary seed	0.000	<0.112[a]		
mean 7 mg	0.075	0.138	0.041	30
(4 replicates	0.75	0.936	0.059	6
at each level)	7.5	7.28	0.932	13
	75	70.8	1.83	3
	750	743	45.0	6
mealworm	0.0000	<0.013[a]		
mean 30 mg	0.0375	0.041	0.003	7
(4 replicates	0.375	0.452	0.011	2
at each level)	3.75	4.15	0.203	5
	37.5	36.4	2.81	8
	375	388	6.15	2
linseed seed	0.0000	<0.047[a]		
mean 8.67 mg	0.0860	0.164	0.035	22
(3 replicates	0.860	0.981	0.118	12
at each level)	8.60	9.19	0.635	7
	86.0	94.9	3.41	4
	860	1040	6.74	1
grass seed	0.0000	<0.009[a]		
mean 1.75 mg	0.0180	0.065	0.038	58
(3 replicates	0.180	0.226	0.022	10
at each level)	1.80	2.31	0.160	7
	18.0	20.7	1.42	7
sorrel seed	0.0000	<0.072[a]		
mean 1.04 mg	0.0105	0.079	0.042	54
(3 replicates	0.105	0.118	0.018	15
at each level)	1.05	0.96	0.129	12
	10.5	12.9	0.778	6
	105	132	7.49	6
	180	243	6.74	4

[a] Less than two standard deviations above mean of blanks.

with GC-MS enables residues down to 0.01 mg/kg to be measured in samples as small as 10 mg in mass. Radioactive labeling is not required, so this technique is most suitable for assessing pesticide exposure in field studies. However, the technique will measure residues of only expected compounds (e.g., the parent pesticide but not unidentified degradation products). The concentrations at which the AMS can be used depend on the specific activity of the applied pesticide (in this study, the specific activity was far lower than in conventional radiolabeling experiments). The technique measures the total radioactivity of the sample, so it will include the natural radioactivity of the carbon content of the sample matrix unless the pesticide is extracted. Degradation products will also be included if the sample is analyzed whole; otherwise, they may or may not be included depending on the extraction and cleanup procedure. This technique can be adapted therefore to suit laboratory studies on metabolism and degradation of pesticides in small samples.

ACKNOWLEDGMENT

We thank M. Clook of Pesticides Safety Directorate and P. J. Edwards and J. Evans of Syngenta PLC for their advice during the planning of this study. We are grateful to Zeneca (now Syngenta) PLC for donating samples of radiolabeled hexaconazole and technical active substance used in this study. We thank Chris Connors for treatment of some grain samples, Daniel Leong for the AMS analysis, and Andrew Calam and Sally Gregory for technical assistance.

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Received for review January 5, 2004. Accepted March 24, 2004. This work was supported by MAFF (now the U.K. Department for Environment, Food and Rural Affairs) under Project PN0915. CBAMS Ltd. also received financial support from Pfizer U.K., Novartis, GlaxoSmithKline, Johnson and Johnson, and the University of York.

JF040012U