Analysis of Organophosphate, Pyrethroid, and Methoprene Residues in Wheat End Products and Milling Fractions by Immunoassay

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ABSTRACT

Wheat grain was spiked with five levels of three grain protectant mixtures, aged, then milled and further processed into a wide range of end products including seven types of bread and noodles. Enzyme-immunoassay methods for quantitation of residues of three organophosphate (fenitrothion, chlorpyrifos-methyl, and pirimphos-methyl), two synthetic pyrethroid (bioresmethrin, permethrin) grain protectants and an insect growth regulator (methoprene) were applied to the analysis of both the milling fractions and the end products. Three parameters were investigated: 1) potential matrix interferences obtained using a simple methanol extraction protocol; 2) a comparison of data obtained using the immunoassay and conventional instrumental methods (gas-liquid chromatography or high-performance liquid chromatography); and 3) changes in the residue levels during milling and processing. Where matrix interference did occur, it was typically manifested as a decrease in assay sensitivity in the presence of the extract of the sample under study. However, methanol extraction of residues gave relatively few matrix interferences in the case of organophosphates, and matrix effects were seen in only some of the pyrethroid assays. The simplest approach to obtaining accurate results, when matrix effects were present, was to prepare the assay standards in an extract of a pesticide-free sample of the matrix under study. Generally, there was a close relationship between residue levels as measured by immunoassay and by instrumental analysis. The extent of residues in different milling fractions and persistence in different products varied with the compound and the product. As the milling extraction rate increased, the levels of residue in the flour, relative to the application rate, were greater. Similarly, baked products prepared from high-extraction-rate flours contained higher levels of pesticide, while white noodles (low-extraction-rate flour) and yellow noodles (alkali treated) contained low levels. Although the application rates used are lower, a greater proportion of pyrethroids, especially permethrin, were retained after milling and subsequent processing, compared with that of the organophosphates.

Harvested wheat grain often requires protection during storage from damage by a range of insect species. Cereal grains can be treated with degradable pesticides, including organophosphates, carbamates, synthetic pyrethroids, and insect growth regulators to prevent insect infestation before processing and consumption (Bengston et al 1983, Snelson 1987, Arthur 1992). The identity and amount of pesticide residues in wheat products is important because: 1) small amounts of pesticide or pesticide metabolite may persist in the baked or cooked product; 2) pesticides used on some grains and in some countries may not be allowed in other situations; and 3) an increasing proportion of customers specify "residue-free" grain. Insecticide use brings with it the likelihood of residues appearing in foods derived from these grains. We have developed antibody-based tests for the major pesticides used on stored wheat grain (Hill et al 1991, 1992, 1993; Skerritt et al 1992a,b; Edward et al 1993), and in some cases performed limited assessment of these assays with milling fractions (Skerritt et al 1992b, Edward et al 1993). With wheat grain, good correlations have been obtained between immunoassay data and data obtained using conventional instrumental methods (Skerritt et al 1994).

In addition to grain handlers and traders, cereal processing companies and regulatory agencies are potential users of these methods. For processors, analysis of baked or cooked end products and intermediate products such as flours are also important. Therefore, we undertook a large performance trial of the antibody assays with these wheat end products. Three parameters were studied: 1) identification of sample matrix interference in the immunoassays using a simple methanol extraction protocol, without clean-up; 2) comparison of immunoassay results with data from gas-liquid chromatography (GC) or high-performance liquid chromatography (HPLC) analyses; and 3) assessment of the breakdown or loss of residues during processing.

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MATERIALS AND METHODS

Grain Treatments and Products

Hard wheat (11.5% protein) from Western Australia was treated with five treatment levels of three pesticide mixtures: fenitrothion and bioresmethrin; chlorpyrifos-methyl and methoprene; pirimiphos-methyl and permethrin, representative of the common mixtures used in commercial practice in Australia (Bengston et al 1983), and including several of the major grain protectants used singly in other countries (Snelson 1987). Treatment levels were: fenitrothion, chlorpyrifos-methyl, and pirimiphos-methyl (2.5, 5, 7.5, 10, 20 ppm, mg/kg), bioresmethrin, methoprene, and permethrin (0.25, 0.5, 0.75, 1, 2 ppm). The grain was stored in sealed containers at 20–25°C for two months before conditioning and milling. This period, together with losses on spiking, meant that residues in the grain at the time of testing were expected to be 40–80% of these original values (Ardley and Desmarchelier 1978; Desmarchelier 1978, 1980; Noble et al 1982; Bengston et al 1983; Papadouolou-Mourkidou and Tomazon 1991).

Milling fractions were prepared using a Buhler mill: flour (60, 75, 82% extraction rates), 90:10 flour (90 parts of wholemeal plus 10 parts of 75% extraction rate flour), pollard, and bran. Wholemeal was prepared by milling wheat in a Falling Number mill to pass a 0.8-mm screen.

Breads were made according to standard commercial practice (Quail et al 1993). Pan bread formula contained 3% yeast, 2% salt, 2% fat, and 0.5% improver (flour basis). White bread was prepared from 75% extraction rate flour, bran-enriched bread from 90:10 flour and wholemeal bread from wholemeal.

Flat (Arabic) bread was prepared using 82% extraction rate flour, with 1% yeast and 1% salt (on a flour basis); baked at 500°C for 30 sec. Chinese steamed bread was prepared from 75% extraction rate flour, with 2% yeast; steamed for 20 min. White (salted) noodles were prepared from 60% extraction rate flour with 3% salt; boiled for 6 min. Yellow (alkaline) noodles were prepared from 75% extraction rate flour with 1% sodium carbonate, and boiled for 7.5 min. Noodle doughs contained 32% water before boiling. Each of these products was prepared from 300 g of flour.

Immunoassays

Details of the immunoassay methods used have been described elsewhere (Hill et al 1991, 1992, 1993; Skerritt et al 1992a,b; Edward et al 1993; Skerritt et al 1994). Products were analyzed without drying. Samples (10–40 g) were extracted 48 hr in methanol using either 2.5 ml of solvent per gram (wheat, wholemeal, flours, noodles), 4 ml/g (breads), or 10 ml/g (pollard, bran) with intermittent shaking. The breads and noodles were first homogenized for 2 min in the methanol with a probe homogenizer (Ystral, Dottingen, Germany). They were each extracted and analyzed in two separate assays; immunoassay data presented are the means of these analyses.

Step 1. Microwell plates were precoated with appropriate antibodies by incubating overnight with 1 µg of antibody per 100 µl of 50 m*M* sodium carbonate buffer, pH 9.6, in each microwell. The fenitrothion and methoprene microwells were coated commercially by Millipore (ImmunoSystems Inc., Scarborough, ME; now EnSys Inc., Durham, NC).

Step 2. Methanol extracts of the grain products were allowed to settle for 1 hr after blending, then were diluted 1:5 to 1:50 in 50 m*M* sodium phosphate, 0.9% NaCl, pH 7.2, 0.05% Tween, 1% bovine serum albumin. For permethrin, 0.005% Tween was used, as the antibody binding is affected by higher concentrations (Hill et al 1991). Predilution of extracts in methanol was performed such that extracts had a final methanol concentration of 5%, except for fenitrothion and bioresmethrin (10%).

Step 3. Diluted extracts or pesticide standards (100 µl) then pesticide-peroxidase conjugates (100 µl) diluted in the same diluent used for the grain products were added to each well and incubated for 1 hr at 20°C.

Step 4. The plate was washed, 160 µl peroxidase substrate, 3,3',5,5'–tetramethylbenzidine chromogen was added (Hill et al 1991) and incubated 30 min at 20°C.

Step 5. Stopping reagent (40 µl of 1.25*M* sulfuric acid) was added, and absorbance was measured at 450 nm.

Immunoassay results were calculated from standard response curves, prepared by spiking pesticide standard into methanol or methanol extracts of pesticide-free grain, milling fractions, and baked products. They were then diluted as for the samples.

Instrumental Methods

Grain, milling fractions, and end products were stored at –20°C between immunoassay and instrumental analyses to prevent further degradation of pesticide residues. The organophosphate analyses of the milling fractions and all the permethrin and methoprene analyses were performed by the authors, while the bioresmethrin analyses and organophosphate analyses of the end products were performed by a contract laboratory (Academy of Grain Technology, Werribee, Victoria, Australia). In the case of organophosphate analyses, which were performed by both laboratories, the same wheat grain samples were analyzed in both laboratories and data cross-checked for consistency before proceeding to the end products. Grain products were stored by the contract laboratory at 4°C until analysis. Methanol extracts of grain fractions containing organophosphates were analyzed by GC with a thermionic detector, after fractionation on 6% SE30: 4% SP2401 on Chromosorb W, 100-120 mesh (Alltech, Deerfield, IL). Permethrin was extracted using methanol, and determined by RP-HPLC (Novapak C-18 column, Waters, Milford, MA) using elution with 50% acetonitrile in water. Bioresmethrin was determined from hexane extracts using normal-phase HPLC (Micro Porasil column, Waters), eluted at 1 ml/min with a mobile phase of 0.3% propanol in hexane; detection at 225 nm. Methoprene was determined in methanol extracts of grain, milling fractions, and wheat products. Aliquots (1 ml) of each extract were evaporated to dryness under nitrogen and redissolved in 2% tetrahydrofuran (THF) in hexane. Analysis was also performed on a MicroPorasil column, but with elution at 2 ml/min in 2% THF in hexane; detection at 254 nm. Instrumental data not presented for the pyrethroids in end products.

Moisture Contents

Analytical data for both instrumental and immunoassay analyses were not corrected for moisture contents. Baked products were analyzed immediately after preparation; noodles were air-dried at room temperature for 24 hr after boiling. To interpret residue data on a dry weight basis and to identify the possible role that moisture content may have in extraction efficiency and matrix effects, moisture contents were determined in each milling fraction using a Brabender moisture oven (Brabender, Duisberg, Germany) and in each end product using a modified two-stage air-oven method (AACC 1983).

RESULTS

Assay Performance

Each immunoassay was specific for its target pesticide and did not detect other grain protectants or likely breakdown products (Hill et al 1991, 1992, 1993; Skerritt et al 1992a, 1994; Edward et al 1994). An exception was the immunoassay for permethrin which also detects 1R-phenothrin (Skerritt et al 1992b). Assay sensitivity in whole grain was sufficient to detect the common reporting limits for instrumental analyses of each compound. Assay limits of detection, determined as 10% inhibition of color development (in wheat grain) were as follows (Skerritt et al 1994): chlorpyrifos-methyl, 0.02 ppm; pirimiphos-methyl, 0.03 ppm; bioresmethrin, 0.05 ppm; fenitrothion and permethrin, 0.08 ppm. However, because the accuracy and precision of analysis are low near these limits, values are reported as <0.1 ppm rather than attributed a specific value. The mid-points of the standard curves ranged from 0.5 ppm (in grain) for permethrin to 3.5 ppm for fenitrothion (Skerritt et al 1994).

Moisture Contents

Methanol was used as extractant for the immunoassay because it is an effective extractant of each of the pesticides from various grain matrices (Sharp et al 1988). The moisture content of the products was sufficient to enable methanol extraction without addition of water (Sharp et al 1988). Moisture contents for the different products were: wholemeal: 12.3%, 90/10 meal: 12.4%, 82% extraction rate flour: 11.9%, 75% extraction rate flour, 12.1% and 60% extraction rate flour, 12.8%. Wholemeal bread: 37%, 90/10 bread: 37%, bread from 75% extraction rate flour: 36%, steamed bread: 40%, flat bread: 29%, uncooked white salted noodles: 32% and uncooked yellow alkaline noodles: 33%

Matrix Interference

The use of selective GC detectors enabled the organophosphate residues to be determined instrumentally in most products without matrix interference (Snelson 1987, Sharp et al 1988). For bioresmethrin, where detection is by ultraviolet absorption, methanol was not used as the extractant by the contract analysis laboratory because of significant matrix interference. Therefore, hexane was used, even though as an immiscible solvent it is a poor extractant of bioresmethrin from high-moisture products such as breads.

The presence of potential matrix interference in milling fractions and products was studied by spiking pesticide at four or five different concentrations into a methanol extract of the particular fraction or product, and comparing the concentrations measured to

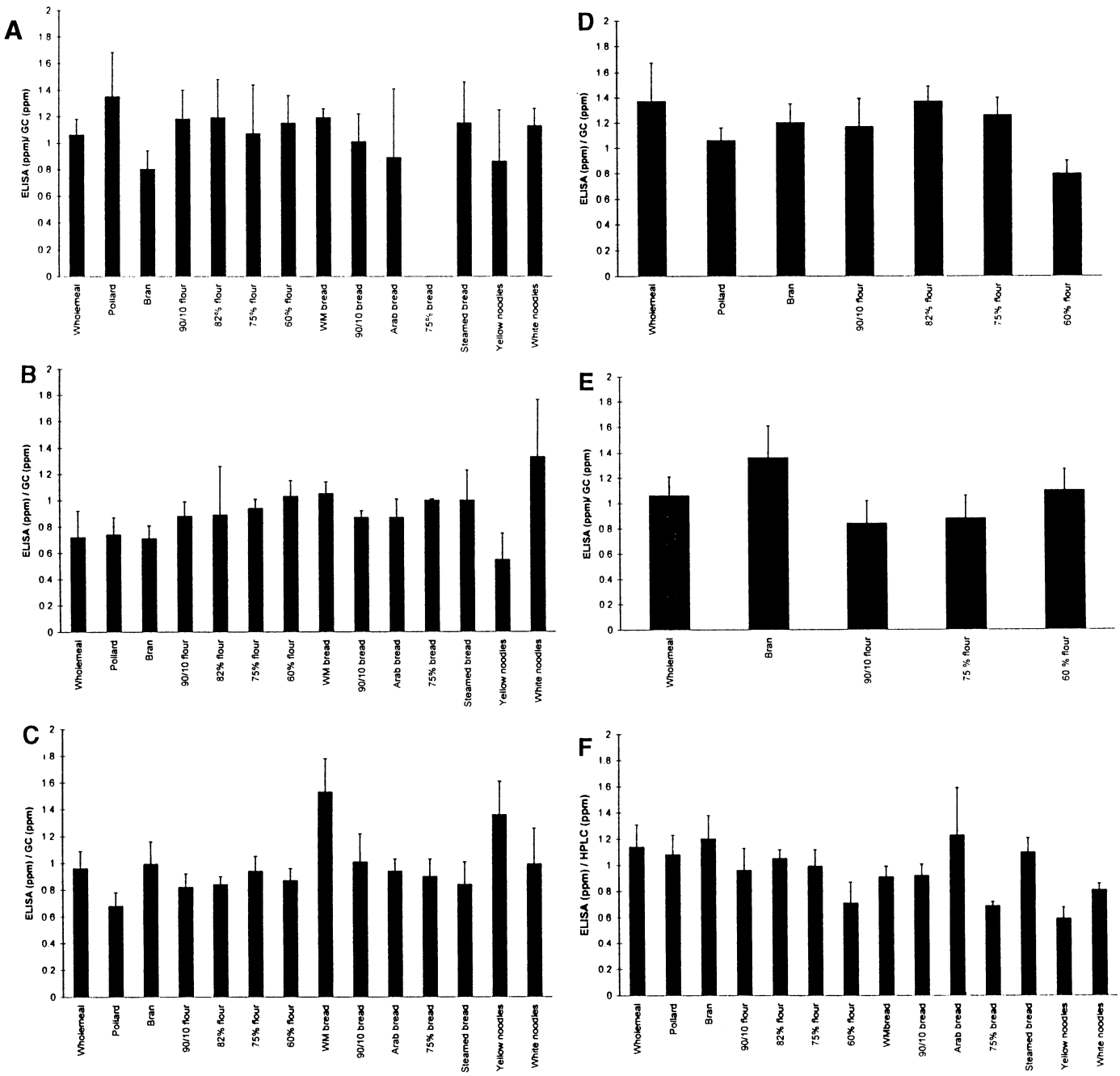

Fig. 1. Relationship between enzyme-linked immunosorbent assay (ELISA) and gas chromatography GC (A–C) or high-performance liquid chromatography (HPLC) (D,E) instrumental data for: **A,** fenitrothion; **B,** chlorpyrifos-methyl; **C,** pirimiphos-methyl; **D,** bioresmethrin; **E,** permethrin; **F,** methoprene. Milling fractions: wholemeal, 90/10 flour, 82 % extraction rate flour, 60 % extraction rate flour, pollard, and bran. Data for fenitrothion in 75% extraction rate bread were not available. Error bars indicate standard deviations of the mean.

that of standards prepared at the same concentrations in an extract of whole grain. In addition, the color development produced in the immunoassay using an unspiked and untreated grain extract was compared with that produced by methanol (solvent) alone.

These procedures were performed to determine whether the particular matrix gave rise to inhibition of the antibody-antigen reaction, which, if unaccounted for, would either create false positive or negative results. Skerritt et al (1992b) demonstrated that milling fractions usually caused little interference in the organophosphate assays. Similarly, in this study, only some of the baked or cooked products caused matrix interference with the organophosphate immunoassays. Where matrix effects occurred, there was usually a decrease in assay sensitivity rather than inhibition of the antibody-antigen reaction (decrease in assay absorbance values). Significant matrix interference was defined as a consistent detection of either a 20% or greater change in assay absorbance in the presence of unspiked extracts of pesticide-free matrix, or a 30% change in assay sensitivity relative to that in extracts of wholemeal. Data for the milling fractions and end products that demonstrated matrix interferences and for which analyses relative to standards prepared in an extract of a pesticide-free sample of the matrix are indicated by dashed lines in the figures.

For fenitrothion, extracts of bran, 90/10 (bran-rich) flour and wholemeal bread altered assay absorbance, while the sensitivity of the assay was decreased only by pollard extracts. None of the

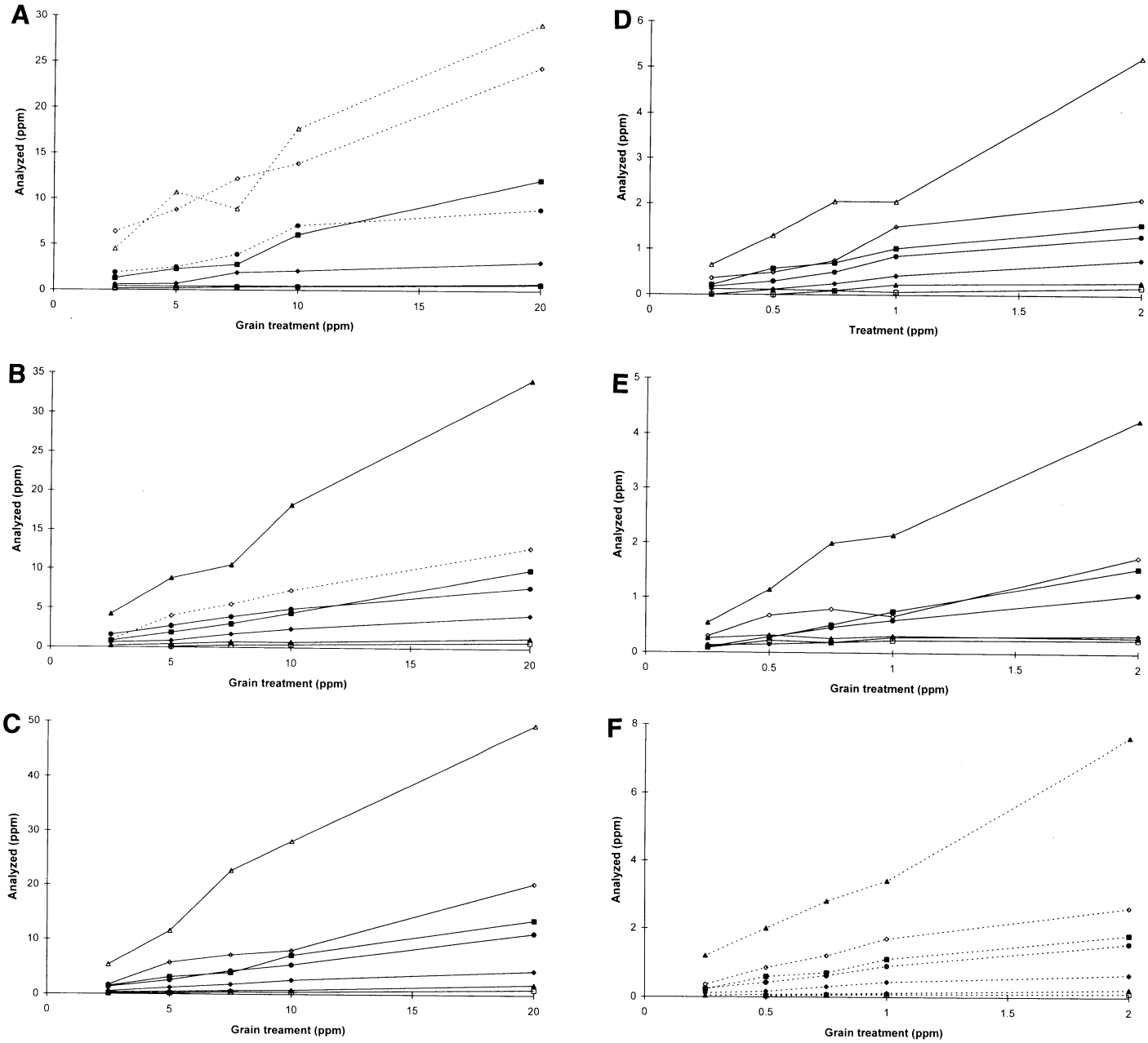

Fig. 2. Persistence of grain protectants into milling fractions, as assessed by immunoassay. **A,** fenitrothion; **B,** chlorpyrifos-methyl; **C,** pirimiphos-methyl; **D,** bioresmethrin; **E,** permethrin; **F,** methoprene. Solid lines indicate levels determined relative to a standard curve prepared in methanol, dashed lines indicate levels determined relative to a standard curve prepared in methanol extracts of pesticide-free matrix. Milling fractions: wholemeal (■), 90/10 flour (●), 82 % extraction rate flour (♦), 75 % extraction rate flour (▲), 60 % extraction rate flour (□), pollard (◇), and bran (Δ). Data shown are the means of two determinations in triplicate or quadruplicate, which typically did not differ by more than 20%.

samples altered the absorbance of the chlorpyrifos-methyl assay, while the sensitivity of this assay was altered consistently by extract of pollard, yellow noodles and 60% extraction rate flour. The pirimiphos-methyl assay appeared to be free of matrix interference. The standard curves for the pyrethroid assays prepared in milling fraction and product extracts did not differ from those prepared in wholemeal extract. Only extracts of yellow noodles gave decreased assay absorbance (apparent false positives) in the bioresmethrin assay, also causing decreased assay sensitivity.

Relationship Between Instrumental and Immunoassay Data

For brevity, the results shown in Figure 1A–F are expressed as the means and standard deviations of the ratios between the values obtained by immunoassay to those obtained by instrumental analysis for three to five samples. Ratios were only calculated where both values reported were ≥0.1 ppm. Again, where matrix effects had been detected, the immunoassay results are calculated with respect to a standard curve prepared in matrix extract. Incomplete instrumental data were obtained for bioresmethrin (Fig. 1D) and permethrin (Fig. 1E) due to matrix interference in product analyses.

There was generally a good agreement between immunoassay and instrumental data, especially given that the immunoassay and instrumental analyses were performed in separate laboratories. The mean ratios between the two values were significantly different from unity in only a few cases. There was not a systematic bias of either immunoassay or instrumental data, with the possible exceptions of chlorpyrifos-methyl and bioresmethrin, where several matrices gave slight underestimates and overestimates, respectively, in the immunoassay relative to the instrumental analyses. Immunoassay data for the various flours were usually quite

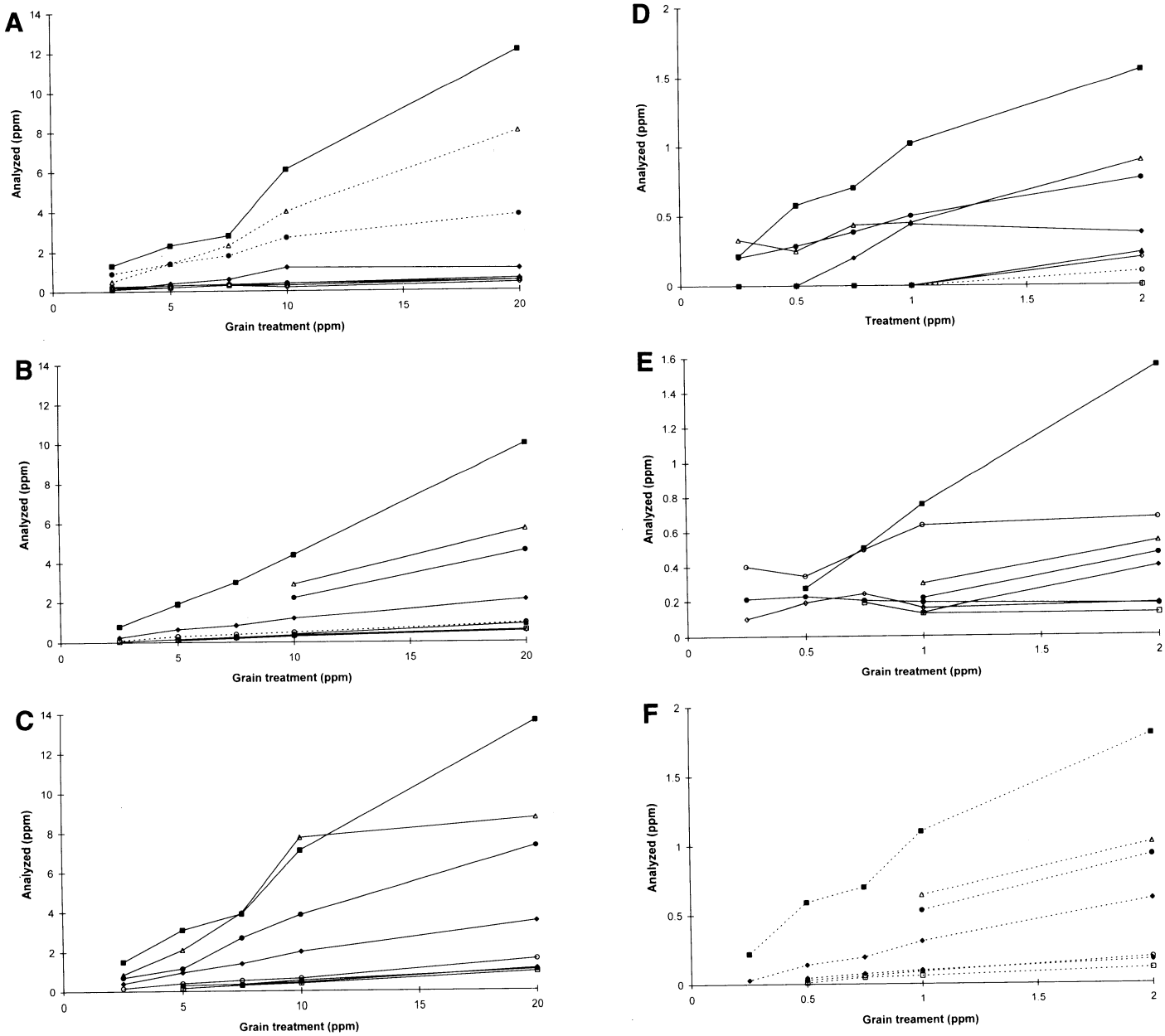

Fig. 3. Persistence of grain protectants into wheat products: **A,** fenitrothion; **B,** chlorpyrifos-methyl; **C,** pirimiphos-methyl; **D,** bioresmethrin; **E,** permethrin; **F,** methoprene. Data for bioresmethrin in white noodles are not available. End products: wholemeal (■), bread from 90/10 flour (●), Arabic bread (♦), bread from 75 % extraction rate flour (▲), steamed bread (◇), wholemeal bread (Δ), white noodles (□) and yellow noodles (○). Data shown are the means of two determinations in triplicate or quadruplicate, which typically did not differ by more than 20%.

closely related to the instrumental data. The errors for pollard and bran were higher, probably reflecting greater variability in extraction and lower precision in the analyses (the analyses were optimized for detection at the lower levels found in grain, flours, and products). Of the products, yellow noodles gave the poorest relationship between residue levels determined using instrumental and immunoassay methods for several pesticides.

Relationship Between Grain Treatments and Residues in Milling Fractions and Products

The relationships between the levels of grain protectant applied and residues in milling fractions and wheat products, as determined by immunoassay, are shown in Figure 2A–F and Figure 3A–F respectively. Data shown are the means of two extraction and immunoassay experiments, performed on separate days. Where a shift in the standard curve due to matrix interference had been detected in initial experiments for a particular pesticide-matrix combination, samples were analyzed with respect to standard curves prepared in an extract of matrix (shown as dashed lines in Figs. 2 and 3).

In Figure 4A and B, the residue level in the sample (determined by immunoassay) was calculated with respect to the level of the corresponding compound in wholemeal, arising from each application rate. The data in the figures represent the mean and standard deviation of this ratio across each of up to five treatment levels, in which residues were detectable >0.1 ppm. Each sample was analyzed twice, and the means and standard deviations of the average result of these analyses was calculated. In general, there was a linear relationship between the level of grain protectant applied and that recovered in each fraction. As noted previously for instrumental analyses, the levels on wholemeal were somewhat below those originally applied. For the organophosphates,

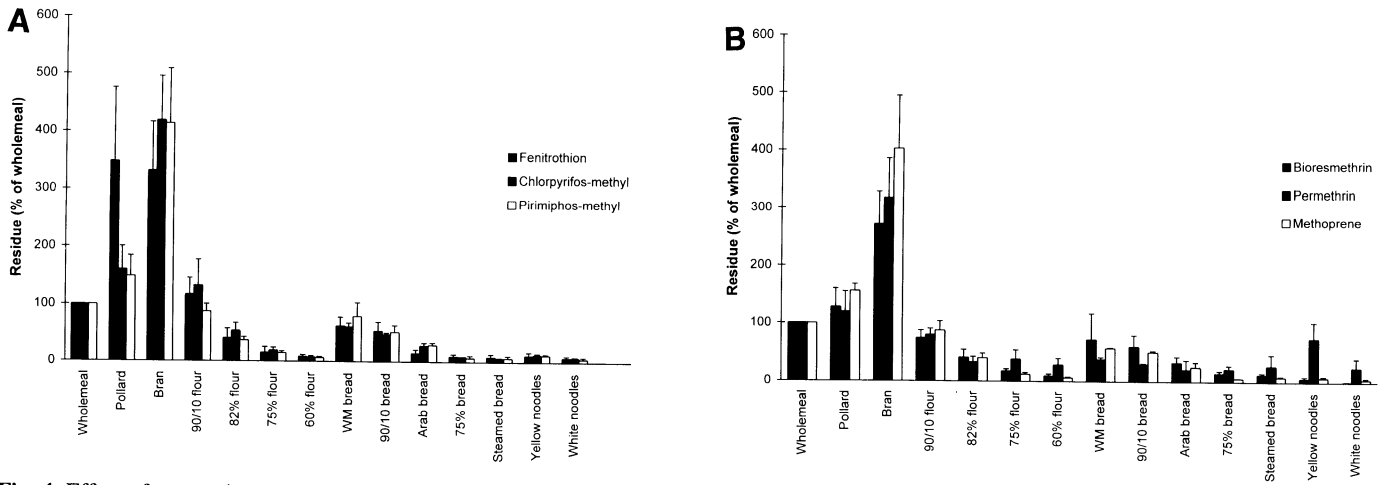

Fig. 4. Effect of processing on organophosphate residue levels (**A**) and methoprene and pyrethroid levels (**B**) in milling fractions and wheat products (relative to wholemeal = 100%, for each application level), determined by immunoassay. Error bars indicate standard deviations of the mean.

this range was 35–50% (chlorpyrifos-methyl), to 40–60% (fenitrothion), and 60–70% (pirimiphos-methyl). The difference between the treatment level and that analyzed after two months of storage has been attributed to both losses onto the spiking vessel and residue breakdown. In keeping with the latter effect, chlorpyrifos-methyl, the organophosphate with the lowest hydrolytic stability, showed the largest decrease from the original treatment level, while pirimiphos-methyl, the pesticide with the greatest stability, was present at levels closer to the applied levels (Singh and Chalwa 1980, Noble et al 1982). The wholemeal levels of the other compounds relative to the grain levels were 60–80% for permethrin and methoprene and 75–100% for bioresmethrin.

The pattern of distribution of residues into each milling fraction was similar for each of the three organophosphates (Fig. 2A–C). Because the applied chemical does not evenly distribute within the grain, milling fractions that contain larger amounts of material from the outer layers of the grain have higher residue levels than those from endosperm fractions. Therefore, we found that bran contained 320–410% the level of protectant found in the wholemeal (Figs. 2A–C, 4A), in keeping with the result of Webley (1994). Similar trends were seen with the pyrethroids and methoprene (Fig. 2D–F), with methoprene giving the highest bran levels. The pollard fraction, which contains some endosperm and some germ, contained 120–150% the level in wheat, except for fenitrothion, where the levels were somewhat higher (Figs. 2A and 4A).

There was a close relationship between the extraction rate and the residue levels in the flours. With the organophosphates, the 90/10 flour had similar levels to the wholemeal, the 82% extraction rate flour about half, the straight run (75% extraction rate) flour about 25% and the 60% extraction rate flour (used for white noodles) contained about 10% the residue level of the wheat (Fig. 2A–C). Similar trends were seen for the pyrethroids and methoprene, although somewhat greater levels of permethrin were seen in the lower extraction rate flours, suggesting that it penetrated further into the grain (Fig. 2D–E). The Codex Maximum Residue Limits (MRL) for the three organophosphates are 10 ppm for the grain, 2 ppm for any flour, and 20 ppm for wheat bran. The data in Figure 2 shows that each of these parameters is exceeded at the highest application rate (20 ppm), and that the 90/10 flour prepared from grain treated at the 10 ppm level sometimes exceeded the 2 ppm residue specification. However, where these pesticides are still used, they are often applied at lower rates such as 4–8 ppm, so that in practice, MRL should not be exceeded. Corresponding MRL values for bioresmethrin, permethrin, and methoprene are: wheat, 1 ppm (bioresmethrin), 2 ppm (pirimiphos-methyl), and 5 ppm (methoprene); bran, 5 ppm (bioresmethrin, permethrin, and methoprene); and flour, 1 ppm (bioresmethrin), 5 ppm (pirimiphos-methyl), and 2 ppm (methoprene).

At the highest application rate used in our study, none of these values were exceeded for permethrin, but each of them was exceeded at the highest bioresmethrin application of 2 ppm. The 2-ppm application produced residues above MRL only for bran.

The levels of each organophosphate in the bread products was closely related to the extraction rate of the flour from which the bread was prepared (Fig. 3A–C). Therefore, the wholemeal and 90/10 breads had significantly higher levels than did the white bread, prepared from 75% extraction rate flour. The decreases in pesticide content between flour and bread were ≈50% between flour and bread. Given that the water content of the breads was 36–37% (compared with 12–15% for the flours), and that fat and yeast were added in processing, the actual total loss of residues in baking is only ≈15–30%. The organophosphate residue levels in the noodles were similar to those in the bread products.

The trends with the pyrethroids (Fig. 3D–E) and methoprene (Fig. 3F) were similar, and in each case there was relatively little loss of residue from the parent flour during baking. In keeping with permethrin's greater penetration into the grain, levels were proportionally higher in the 60% extraction rate and 75% extraction rate flours and products derived from these, and lower in the 82% extraction rate flour and flat (Arabic) bread.

The levels of permethrin were significantly higher than bioresmethrin or methoprene in the noodle products. Although permethrin is an efficacious stored-product pyrethroid, the proportionally high persistence of this compound during grain processing has led to a reluctance by several countries to routinely use it as a grain protectant. In contrast to permethrin, the levels of the other compounds in the yellow and white noodles were quite low, reflecting hydrolysis by the alkaline treatment used in yellow noodle manufacture and the low extraction rate flour used for the white noodles.

DISCUSSION

This study has demonstrated that it is feasible to analyze grain protectant residues in milling fractions using immunoassay methods. Use of a single extractant for all compounds and end products, enables a single extract to be used in the analysis of a number of compounds. Moreover, while instrumental analysis of the six compounds under study requires a number of chromatography and detection methods to be used, the method employed for each immunoassay is virtually identical, as only the antibody and enzyme conjugate reagents differ. This can make the adoption of the immunoanalysis of a new compound by analytical laboratories straightforward. There was relatively little interference by extracts

of milling fractions and products in immunoassays, and where an extract was shown to affect assay sensitivity, accurate data could be generated by analyzing the samples with respect to standards prepared in a known pesticide-free extract of the product.

Matrix interference was more common in the baked products than in the milling fractions, possibly because of the presence of nongrain ingredients. Yellow noodles, which are alkali-treated, exhibited greater matrix effects than white noodles.

Several articles on the chemical breakdown of grain protectant residues during processing into milling fractions and baked-cooked fractions have been published. However, much data is in the form of either registration data or submissions to the Codex Alimentarius Commission for the purpose of establishing product MRL; this data has recently been summarized by Webley (1994). In several cases, the data is not as extensive as that described here for two reasons. First, residues resulting from only one to three application levels of chemical were studied, and (especially in earlier studies) the treatment levels used represented either the upper levels of commercial practice or exceeded current levels. Second, the earlier studies tended to focus on pan bread (produced from white flour) only, whereas in this study, we have also analyzed breads of several extraction rates, Arab flat breads, Chinese steamed breads and two types of noodles. Results were in agreement with earlier work, where it had been performed for a particular pesticide-product combination (reviewed in Snelson 1987 and Webley 1994). Webley (1994) reported data on the same group of end products investigated, following two (relatively high) application rates and storage for two intervals after application. The trends reported in that study are similar to ours, in which we have investigated five application levels, several of which more closely reflect current commercial practice. The percentage of residue lost by a particular processing step appeared to be similar at both high and low application rates.

In general, the greater the penetration of particular compounds into the grain (higher for pyrethroids) and the thermal and hydrolytic stability of the compounds (higher for pirimiphos-methyl and permethrin) the greater their persistence into end products. This trend was greater for permethrin than pirimiphos-methyl, and in our work we found that a slightly lower proportion of pirimiphos-methyl persisted into straight-run flour than had been reported previously (Mensah et al 1979). In addition, the reduction in pyrethroid levels on baking was minor, compared with the organphosphates, as noted previously in studies that utilized only one or two application rates of each compound (Wilkin and Fishwick 1981, Bengston et al 1983).

Although the present study used a Buhler mill rather than a commercial mill, and different milling treatments can produce different distribution of residues in milling fractions (Jermannaud and Pochon 1994), the results obtained were in general agreement with more limited earlier studies based on GC or HPLC (Snelson 1987, Webley 1994). There was an enrichment of residue in the outer and lipid-rich layers of the grain, namely bran and pollard. Webley (1994) found that, on average, 3.4–3.9 times the residue in wheat is found in bran, with no difference between the organophosphates and synthetic pyrethroids. Our results were similar, with chlorpyrifos-methyl and pirimiphos-methyl treatments leaving slightly higher residues in bran than the other treatments. In contrast, the residue levels in pollard were similar to those in the wholemeal except for fenitrothion, where levels were three times higher. The residue levels in the flours increased in proportion to the extraction rates. Most significant was the difference between the straight-run flour of 75% extraction rate and the 82% extraction rate flour used for the flat bread and one of the pan breads; the corresponding flours contained three to four times as much of each of the organophosphates. The difference in the pyrethroid residues in the two flours was not as great.

The results of this study have demonstrated that residue immunoassays are suitable for analysis of end products as well as grain. Since methanol was an effective extractant of the pesticides from the grain matrices (Sharp et al 1988), it provided an extraction process that is both simple and efficient. The ability to use a single extractant for detection by immunoassay facilitated multiresidue analysis. Many matrix-pesticide combinations (especially organophosphates) were free of assay matrix interference. Even where these effects do manifest, they do not prevent direct analysis of extracts by immunoassay. Quantitative data are readily obtained by comparison of analytical data with standards prepared in a methanol extract of a residue-free sample of the particular matrix. Under these conditions, quite close relationships were observed between instrumental and immunoassay data for each pesticide and matrix.

Losses of pesticide during processing varied considerably with the product and the pesticide. Although baking and boiling decreased residue levels from that in the initial grain or flour, residues were often readily detectable in end products after treatments of grain at commercial levels. Detections of residues were especially noted in wholemeal or bran-rich bread, an important consideration given the increasing tendency towards consumption of these goods due to increased dietary fiber content.

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