Quantification of Nonpersistent Pesticides in Human Samples by Isotope Dilution Mass Spectrometry: Applications of New Analytical Techniques.

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The widespread use of pesticides in agriculture has led to a ubiquitous presence of these compounds in the environment. The health effects of non-persistent pesticides in exposed and general human populations is not yet known. The extensive use of these compounds has created a great interest in developing laboratory and epidemiology studies on possible health risks. We have recently developed methods for a pilot study to analyze blood samples collected from farmers and their families in two states. These samples were collected before, during, and after pesticide application. A target group of non-persistent insecticides, herbicides, and fungicides have been identified and isotope dilution mass spectrometric methods have been developed.

The analysis of these pesticides in serum requires an efficient sample preparation procedure followed by high resolution chromatography and high resolution mass spectrometry so that interferences do not hinder the analysis. The method developed for the quantification of this complex mixture of non-persistent pesticides includes a solid phase extraction of serum that has been treated with an equal amount of saturated ammonium sulfate (Figure 1). This procedure yields an extract which is analyzed by GC/MS. A DB-1701 column is employed for the GC separations and the pesticides are recorded on a high resolution magnetic sector mass spectrometer (VG 70-4SE). Specificity and sensitivity are obtained by ionization with low energy electron impact (30 eV) and selective ion monitoring at a resolution of 10,000. Employing this procedure with isotope dilution mass spectrometry allows for an accurate and sensitive method of quantification. Parts-per-trillion (ppt) levels of many of the non-persistent pesticides were found in the serum pools from the general population . An example of the data from a farm in Iowa is shown in Figures 2 and 3. This farm sprayed a mixture of atrizine and metolachlor. As can be seen, exposure patterns for these two herbicides are very similar. Serum levels were in the low ppt for pre-application season. The applicator's serum levels then rose to high ppt levels on the pre-application day. During application, pesticide levels rose to parts-per-billion in applicators and were then eliminated from the body in subsequent days. Spouse's levels also rose

during the pesticide application. The pesticide levels in two farms in North Carolina were also examined. The first farm sprayed the common herbicide alachlor. As can be seen in Figure 4, the serum levels were low in the applicator and spouse in the sample obtained in the pre-application season. A small increase in the serum alachlor was seen on application day for the spouse. The applicators serum alachlor level rose to 2.3 ppb on application day and then was cleared from the serum on the next two days. The second farm in North Carolina sprayed the insecticide carbaryl. Carbaryl is metabolized very quickly in the blood to form α-naphthol. The Naphthol level was in the mid ppt range for the applicator in the pre-application season (Figure 5). During application the serum naphthol levels rose to 510 ppb, and then showed a large decrease the following two days (1.8 ppb and 563 ppt respectively). The spouse's levels were below detection limits during the pre-application season and increased to 100 ppt on application day. The serum level then dropped to 32 ppt the following day and was below detection limits in the second day after application. These results show that exposure patterns and levels can be analyzed in the serum of farm workers and their spouses.

Methods to analyze urinary metabolites of several nonpersistent pesticides were also developed. Dicamba, 2,4-D, and α-naphthol (the metabolite of carbaryl) were of particular interest. This analytical method employed isotope dilution MS/MS analysis. The sample cleanup procedure was an enzymatic digestion with glucuronidase and sulfatase followed by a phase transfer derivatization to form chloropropyl ethers and esters, organic solvent extraction, and silica gel solid phase extraction. The extracts were then analyzed by negative ion chemical ionization GC/MS/MS. The GC analysis employed a DB-5 column. Chemical ionization was done with isobutane as the reagent gas and the MS/MS transformation were accomplished by multiple low energy collisions with xenon. Good exposure patterns were seen for farm that sprayed dicamba and 2,4-D. Also, there was a good correlation between serum and urine data for α-naphthol.

In addition to the current analytical methods, new approaches such as ultra-sensitive, fast, multidimensional GC/high resolution MS (2DGC/MS) are currently being developed. An example of a 2DGC chromatogram of a pesticide mixture is shown in Figure 6.

Figure 1

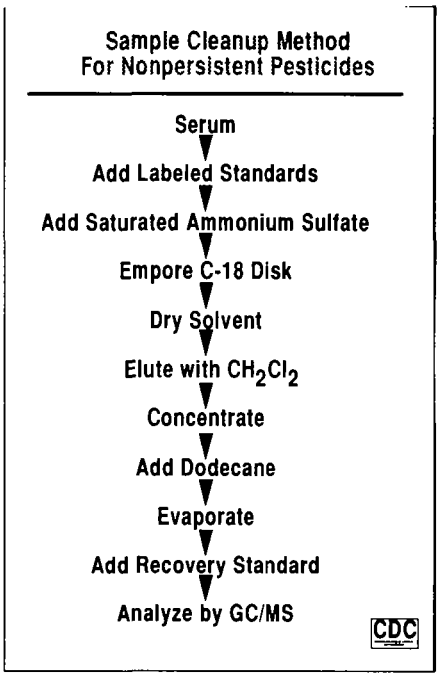

Figure 2

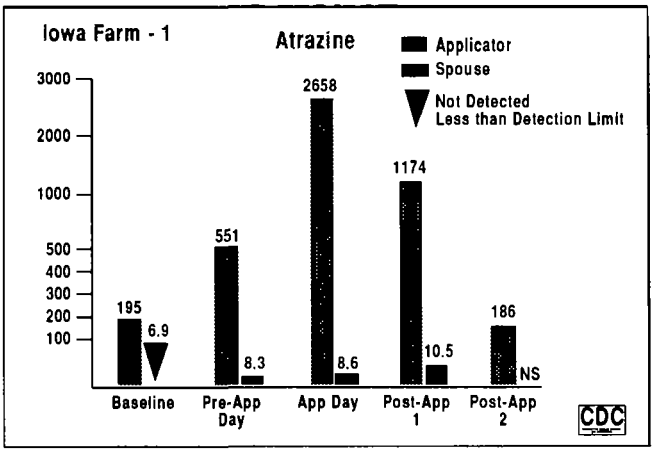

Figure 3

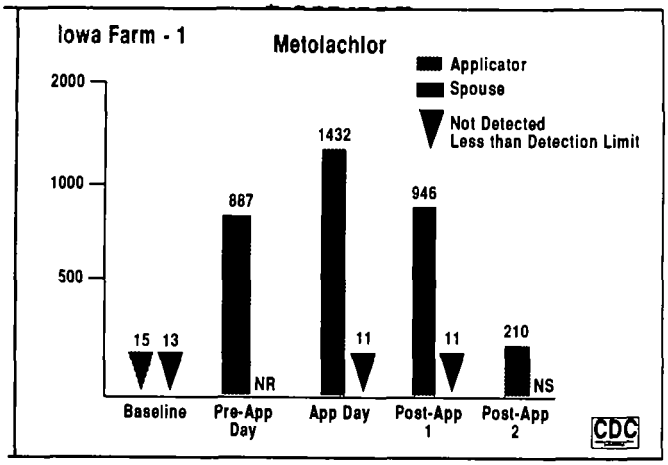

Figure 4

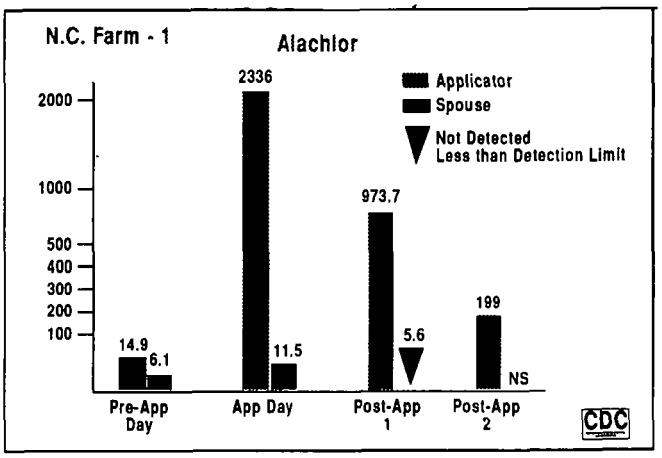

Figure 5

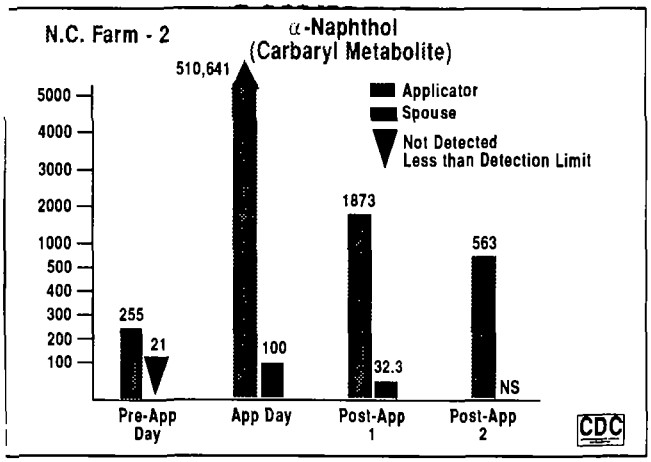

Figure 6