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Determination of Atrazine Residues in Food by Enzyme Immunoassay

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Atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine), a triazine herbicide, is used to control annual grasses and broad-leaf weeds in corn, pineapple, sugarcane and macadamia orchards. This herbicide is used extensively worldwide. In the United States atrazine is the second most applied pesticide; approximately 79 million pounds is used annually (Anonymous). Although it is allowed on only four agricultural commodities for human consumption, many more crops are exposed to atrazine from soil carry over in rotational programs. Because of its extensive use and limited toxicological data (Wilson et al. 1987), there is a need for monitoring food for atrazine residues.

Analysis of atrazine residues in food is presently performed either by gas or liquid chromatography. Gas chromatographic procedures use primarily capillary columns and either a nitrogen-phosphorous, an electron-capture or a Hall electrolytic conductivity detector (Roseboom and Herbold 1980; Bailey and LeBel 1978). Liquid chromatographic methods employ reversed-phase columns with ultraviolet detection at 223 nm (Anonymous 1988; Majors 1980). Chromatographic techniques require very expensive equipment. Furthermore analyses are costly. Recently new technology (immunochemical) has been applied to the analysis of pesticides (Newsome 1986). Immunoassay methods have the advantages of being less expensive and usually more sensitive while their disadvantages are accuracy and specificity.

This paper describes an immunoassay for analyzing atrazine in food. The method is an excellent screening procedure and because of the antibody's cross-reactivity, it could be used to screen or confirm other triazine herbicides.

MATERIALS AND METHODS

Atrazine and all other triazine pesticides were obtained from the EPA and Ciba-Geigy. Preparation of pesticide solutions was the same as previously described (Bushway et al. 1988).

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Description of the preparation of antiserum, antibody tubes and reagents for the kit are given in Bushway et al. (1988).

Analysis of liquid foods by immunoassay (such as milk, soft drinks and fruit juices) was performed by adding 100-uL of a pH adjusted sample (if pH is below 6.0, adjust to 7.0 with sodium hydroxide) to an immunoassay tube followed by 160-uL of atrazine "enzyme conjugate". The mixture was allowed to incubate 5 min at room temperature before rinsing (4 times) with distilled water to remove unreacted sample and enzyme conjugate. Finally, 160-uL each, of substrate (hydrogen peroxide) and chromogen (tetramethylbenzidine) were added. The colored reaction product was allowed to develop for 5 min before the reaction was "stopped" with 1 drop of 2.5 N sulfuric acid. During the substrate-chromogen incubation period a blue color develops. Then after the addition of sulfuric acid the blue color is replaced by yellow. The intensity of yellow was quantified by measuring the difference in optical density (ΔOD) between the control and each standard or sample at 450 nm with a hand-held battery-powered differential photometer from Artel, Inc. As many as 8 samples plus a control can be run at once without losing accuracy. A control sample (no atrazine present) must be run with each set of tubes because its OD is used to calculate the ΔOD/OD values (where ΔOD is the difference in OD of the samples or standards from the control divided by the OD of the control read against water) of the standards and samples. Control samples can be obtained from organic farmers and verified using classical techniques.

Immunoassay analysis of solid food was identical to liquid food except for an addition of an extraction step. Five grams of food was weighed into a 50-mL Erylenmeyer flask to which was added 10 mL of a 90/10 mixture of acetonitrile:water. Samples were blended for 2 min at medium speed followed by filtration through a 0.45-um syringe filter. The filtrate was either diluted 1:10 or evaporated to dryness under reduced pressure depending upon the detection limit needed. Finally like liquid foods a 100-uL sample was removed and run through the immunoassay procedure.

HPLC conditions were identical as described by Bushway et al. (1988) except that the injection volume was 10-uL and the mobile phase was 200 mL methanol:200 mL water:100 mL acetonitrile:5 mL tetrahydrofuran.

RESULTS AND DISCUSSION

The immunoassay showed a linear relationship (Figure 1) from 0.5 to 10 ng/mL (50 to 1000 pg/tube) which was observed between the logarithm of the atrazine concentration and the ΔOD/OD at 450 nm. For samples having greater than 10 ng/mL, a dilution must be made.

A ΔOD of 0.92 or larger indicates a sample concentration of more than 10 ng/mL which means the sample should be diluted and the analysis rerun.

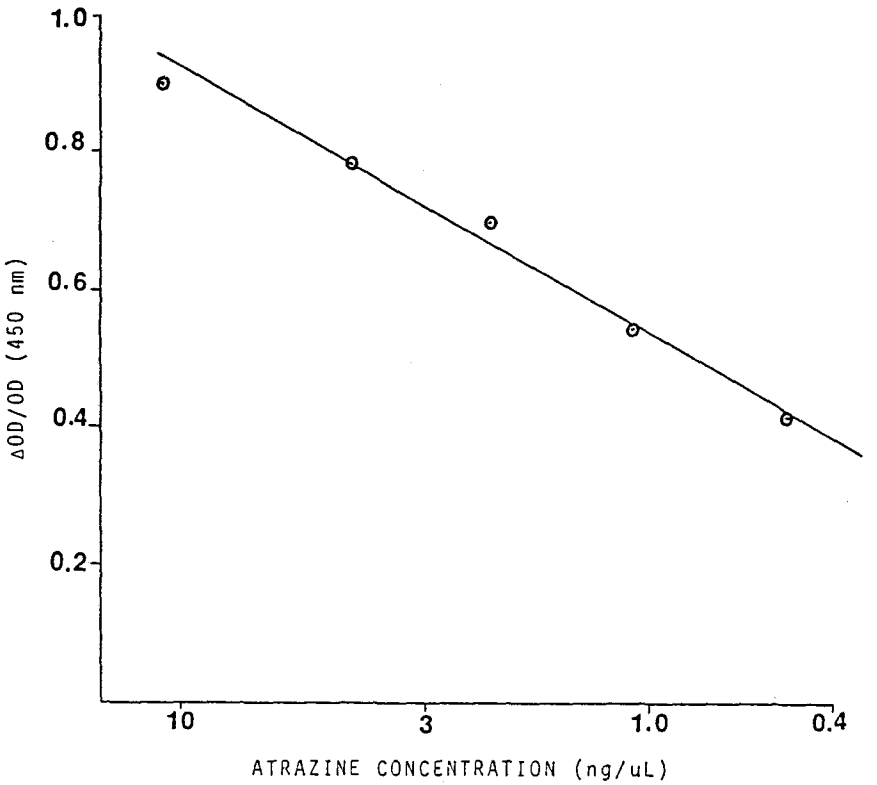

Figure 1. Standard curve for the determination of atrazine concentration.

For quantification of atrazine in food by immunoassay, one should derive the standard curve by adding atrazine to a non-atrazine food extract in place of the normal buffer solution. It appears that food has a substance or substances that compete and interfere with the enzyme conjugate antibody reaction. This phenemonon was observed during a food spiking study when the samples were analyzed by both HPLC and immunoassay. A comparison of the results showed nonagreement. Percent recovery was not the problem because the HPLC data showed 95 to 100% recoveries for the extraction which was the same for both methods. However, when a standard curve generated from a non-atrazine food extract was used for the immunoassay calculations, good agreement was seen between both methods.

As with any analytical procedure, consistency and accuracy from day to day is important. Reproducibility results of the atrazine immunoassay for food can be seen in Tables 1, 2 and 3. Tables 1 and 2 show the consistency data obtained from analyzing fortified solid food, different days for different foods over a period of 2 months. Percent coefficients of variation (% CV) ranged from 13.7 to 2.4. This range is considered excellent for a residue method.

It is especially good for the immunoassay technique as it is just a screening technique. As mentioned above the % CV represented in these tables are comprised of data from 5 different solid foods analyzed over several weeks. Thus, these % CV indicate that the method is rugged because they were obtained from different sample matrices and run on different days. As one would expect, the % CV are lower as the optical density values increase.

Table 1. Reproducibility of the atrazine immunoassay for atrazine-fortified solid food

	Amount added in ppb					
	2.2	4.4	11.0	22.0	110	110[a]
Number of Samples Analyzed	26	26	26	26	26	26
% Coefficient of Variation	12.2	8.9	5.4	4.2	2.4	5.3

Solid Food = fresh sweet corn, fresh pineapple, canned corn, canned pineapple and fresh macadamia nuts.
[a] Extract analyzed after 1:5 dilution.

Table 2. Reproducibility of the atrazine immunoassay for atrazine-fortified solid food.

	Amount added in ppm				
	0.05	0.10	0.25	0.50	1.0
Number of Samples Analyzed	36	44	35	42	52
% Coefficient of Variation	11.1	11.8	12.1	13.7	13.5

Solid Food = fresh corn, fresh pineapple, canned corn, canned pineapple and macadamia nuts.

Table 3 represents the reproducibility of atrazine determination in liquid foods. Again samples were analyzed on different days but over a period of 1 week. The results are very similar to the ones obtained with solid foods making the immunoassay also a good screening method for atrazine in foods such as milk, juices and soft drinks.

Once the accuracy and reproducibility of the immunoassay was defined, then the method was used to analyze numerous foods. The list is given in Table 4. Of all foods analyzed for atrazine, which included several brands of each type, only 6 samples showed any detectable amounts of atrazine. They were 2 fresh corn samples and 4 liquids from canned corn. According to the

Table 3. Reproducibility of the atrazine immunoassay for atrazine-fortified liquid foods

	Amount added in ppb					
	0.5	2.5	5.0	10.0	50.0	50.0[a]
Number of Samples Analyzed	7	7	7	7	7	7
% Coefficient of Variation	10.9	8.4	4.2	3.8	4.8	6.7

Liquid Food = fruit juices (apple-pineapple, pineapple-orange, pineapple, pineapple-orange-kiwi), soft drinks (non diet and diet cola) and milk (whole).
[a] Extract analyzed after 1:5 dilution.

immunoassay, the level present in these samples was between 1 to 2 ppb. It was possible to confirm by HPLC that the corn liquids did contain 1 to 2 ppb atrazine. All the other food tested showed no detectable amounts of atrazine or any other detectable triazine. Other triazines could be detected because of cross-reactivity (Bushway et al. 1988). Therefore our results from Table 4 indicate that residues of atrazine are not a problem in food.

The enzyme immunoassay offers an excellent screening or confirmation method for atrazine in foods that is reproducible and inexpensive compared to other methodologies. Furthermore, because of the cross-reactivity of the atrazine antibody, the immunoassay has the potential to be a broad spectrum triazine test. However, in no way can this immunoassay method be used as the only test.

Table 4. List of foods that were analyzed by atrazine immunoassay.

Whole Milk	Fresh Pineapple
Fruit Juices	Canned Pineapple
Molasses from corn	Fresh sweet corn
Dark corn syrup	Canned corn
Light corn syrup	Frozen corn
Brown sugar	Raw Macadamia nuts
White sugar	Canned corn liquid
Soft drinks	Corn chips
Corn meal	Potato
Corn oil	

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