

**DETECTION OF ATRAZINE AND 2,4-D IN RAW MILK**

<b>MATRIX</b>	Raw Milk
<b>ANALYTES</b>	Atrazine, 2,4-D
<b>RANGE OF DETECTION</b>	Atrazine: 5 to 250 ppb 2,4-D: 0.05 to 2.5 ppm
<b>MATERIALS</b>	<p>RaPID Assay, Kit and Sample Diluent for Atrazine and 2,4-D</p> <p><i>Reagents:</i> methanol (pesticide grade), glacial acetic acid, distilled or deionized water, diatomaceous earth (Celite 545).</p> <p><i>Equipment:</i> 15 mL polypropylene centrifuge tubes (graduated and conical) with screw caps, 20 mL scintillation vials, 10 mL disposable plastic syringe, 25 mm syringe filter holder (Gelman), 90 mm shark skin filter paper (Schleicher &amp; Shuell), scissors.</p>
<b>PREPARATION OF APPARATUS</b>	<p>Cut out a 25 mm circle of shark skin filter paper with the scissors and place the filter disc on top of the stainless steel support screen of the filter holder (outlet portion). Position the O-ring on top of the filter disc. Holding the outlet portion of the filter holder level, connect the inlet portion and hand tighten securely to insure a proper seal.</p> <p>Remove the plunger from the syringe and attach the syringe barrel to the inlet side of the filter holder. Position the outlet of the filter apparatus over the 20 mL vial.</p>
<b>EXTRACTION PROCEDURE</b>	<p>Prepare 100 mL of 75% methanol / 2% acetic acid in distilled or deionized water (75 mL methanol + 2 mL glacial acetic acid + 23 mL water).</p> <p>Pipet 1 mL (1 g) of milk into the 15 mL centrifuge tube. Add 1 mL of the methanol/acetic acid solution. Vortex the tube for 30 seconds.</p> <p>Dilute the sample to the 10 mL volume mark with distilled or deionized water, cap the tube, and vortex for 30 seconds.</p> <p>Add 150 mg (1/8 tsp) of Celite 545 to the sample, cap the tube, and vortex for 30 seconds.</p>
<b>FILTRATION</b>	<p>Briefly vortex the sample to resuspend the milk solids if they have settled. Immediately pour the sample into the syringe barrel. <u>Wait 30 seconds before filtering.</u> This allows the solids to settle against the filter disc and promotes better filtration. Insert the plunger into the top of the syringe barrel and apply slight pressure by hand to create a steady dropwise flow of filtrate into the vial.</p>
<b>ANALYSIS</b>	<p>Vortex the filtrate in the glass vial for 15 seconds. Remove 0.2 mL of the filtrate and mix it with 0.8 mL of the kit Sample Diluent.</p> <p>Analyze the diluted filtrate according to the package insert for the Atrazine or 2,4-D RaPID Assay.</p>

## INTERPRETATION

Calculate the pesticide concentration in the raw milk by multiplying the assay result by the appropriate factors introduced by the procedure:

$$\text{assay result} \times \frac{\text{vol. extraction mixture(mL)}}{\text{vol. milk(mL)}} \times \frac{\text{vol. filtrate(mL)} + \text{vol. diluent(mL)}}{\text{vol. filtrate(mL)}}$$

For the procedure shown above:

$$\text{assay result(ppb)} \times \frac{10}{1} \times \frac{0.2 + 0.8}{0.2} =$$

assay result(ppb) x 50 = concentration of pesticide in raw milk (ppb)

NOTE: If the assay result is indicated "nd" (non-detectable) or if the result is less than the concentration of STANDARD 1, do not multiply the result by 50. Report the result for this milk sample as not detectable.

## PROCEDURAL NOTES

The extraction and filtration procedures may require some practice in order to develop a consistent technique. To gain familiarity with the techniques, "practice samples" should be processed before final results are generated on milk samples submitted for atrazine or 2,4-D analysis.

If the seal around the filter disc is broken or not secure during filtration, the filtrate will have a milky appearance. If this is the case, the sample should be filtered again.

The filter apparatus can be disassembled and reused after rinsing the syringe and filter holder parts with methanol. Do not reuse the sharkskin filter.

## EXPECTED RESULTS

The EPA tolerance for atrazine in milk is 20 ppb (CFR 40, Part 180 (1990)).

The EPA tolerance for 2,4-D in milk is 100 ppb (CFR 40, Part 180 (1990)).

When STANDARD 1 is used as the "cut off" concentration as described above, the range of detection of the assays includes its EPA tolerance limit.

Due to the magnitude of the correction factor used, the accuracy of the final result will depend on the care taken in making dilutions.

When raw milk was fortified with atrazine or 2,4-D and extracted using the procedure shown above, average recoveries of fortified pesticides ranged from 85 to 115%.

## TECHNICAL ASSISTANCE

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