Introduction

Pesticides are toxic substances to be used in the agricultural products under recommended limit and residues need to be lower the safety limit. In fact, those residues at the excess standard is still detected in many kinds of food. Now a day several methods for pesticide residues analysis are available, but the invested cost is very high. They need a long analysis time with the complicated instruments, such as GC, HPLC and GCMS. Therefore the simple test kit for screening pesticides (Organophosphorous and/or carbamate and/or other toxic cholinesterase inhibitors) has developed with fast and reliable result.

Although this developed kit can not detected all kinds of toxic residues, but it is now only the best one method to be chosen for screening toxic residues in agricultural products, soil and water and to be a suitability tool for food safety control in Consumer Protection Programme, especially this technique is highly sensitive for toxic bio-degrade products under field conditions.

Target groups of Testing

- Organophosphate group
- Carbamate group, Organochlorine
- Synthetic pyrethroid Cholinesterase inhibitors

Target Sample

- Vegetables,
- Fruits,
- Cereal grains,
- Medicinal plants,
- Dry salted fish,
- Soil,
- Mud
- Water from/ near contaminated area,
- Consumed water.

Details of GT-Kit

Compose of 2 parts
- Modified Equipment : modified warm water bath 1, Thermometer 1, Test tube 18, Pasteur pipette 5, Rack 1, Sample bottle 5, Plastic pipette 12, Aquatic air pumps evaporated kit 1 and hand book 1
- GT- Reagents Kit :
  - 10 tests/ชุด
  - 30 tests/ชุด
  - 300 tests/ชุด

Procedure of Analysis : Sample Extraction

Before the analysis, the sampling procedure, the weight of the analytical sample and the sample preparation should be followed the Codex's Recommendation. (The details describe in the GT hand book)

Chop or blend sample

Weigh 5 g. of the homogenous sample in to the sample bottle

Add 5 ml. of solvent-1 in to the sample bottle , cap the bottle tidy and shake vigorously 1 min., leave for 10-15mins.

Pipette 1 ml. of the extract in to a test tube and add 1 ml. of Solvent -2 in to the same tube.

Take to evaporate in the modified warm water bath(about 32-36 degree celceus) by connecting the evaporated kit with the air pump and the pasteur pipette and then insert the end of the pasteur pipette in to the sample test tube, adjust the air releasing from the air pump in to the extract, then leave for evaporation until the solvent-1(lower layer) disappeared.

Detection Step :

Take 3, 4,...test tubes label for :
- Tube 1 : cut point or unsafe
- Tube 2 : control or not detected
- Tube 3 : sample extract (name)
(3 tube for 1 sample, if need more sample, add more tube for another sample)

Pipet each 0.5 ml of GT-1 (2 parts of plastic pipette) in to tube 1 and tube 2 and add 0.25 ml(1 part of plastic pipette) of each sample extract in to tube 3, 4...Then put them all in to the modified warm water bath 32 - 36°C

During the waiting time, mix GT-2 + GT-2.1 and GT-3 + GT-3.1

Then add 0.375 ml. (1 ½ part of plastic pipette) of the mixture GT-2 in to tube 1(cut point/ unsafe tube) and each 0.25 ml(1 part of plastic pipette) of each sample extract in to tube 3, 4...Then put them all in to the modified warm water bath 32 - 36°C

For this step, the leaving solution is now called “Sample Extract”
When the time ended, add each 1 ml (4 parts of plastic pipette) of the mixture GT-3 in to every tubes and swirl.

Then add each 0.5 ml (2 parts of plastic pipette) of GT-4 in to every tubes and swirl.

The last step, add each 0.5 ml (2 parts of plastic pipette) of GT-5 in to every tubes, swirl and evaluate the results.

Result Evaluation: compare color in the tubes

<table>
<thead>
<tr>
<th>Color in the tube</th>
<th>the result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample tube ≤ tube 2 (control/not detected)</td>
<td>Not detected</td>
</tr>
<tr>
<td>Sample tube &gt; tube 2 but &lt; tube 1 (cut point/unsafe)</td>
<td>There are some toxic residues expected safe* for consumption</td>
</tr>
<tr>
<td>Sample tube &gt; tube 2 and ≥ tube 1 (cut point/unsafe)</td>
<td>There are some toxic residues expected unsafe** for consumption</td>
</tr>
</tbody>
</table>

Note: * safe for consumption = there are some toxic residues inhibited the cholinesterase enzyme at less than 50%, these toxic residue amounts can be washed out by the consumer

** unsafe for consumption = there are some toxic residues inhibited the cholinesterase enzyme at 50% or more than, these toxic residue amounts can not be washed out by the consumer, they are only decreased by washing.

Caution

- The targets in using the solvent-1 are to dissolve the toxic residues from the sample and to destroy the interfering enzyme deposited in the sample. Therefore the solvent-1 is toxic for human health, avoid breathing and leave it evaporated in the ventilated place or hood.

- When the chemical reagents contact your skin, wash out with the clean water.

- Should wear glove during analysis.

- Place testing kit out of children reach.

- During the analysis, be aware the contamination among GT-reagents.

Storage of GT-Reagents

- Keep a set of GT-reagents kit in a refrigerator/cool place, except GT-1 & GT-2 in the freezer, the shelf life about 1 year, but in the case of limited space, keep only GT-1 & GT-2 in freezer and keep the other reagents in the air condition room.

- The mixture of GT-2 + 2.1 and GT-3 + 3.1 (after used) need to be kept in the refrigerator and the GT-2 mixture can be used within 10 days, the GT-3 mixture can be used within 3-4 days.

GT- Pesticide Residual Test Kit

Analysis of Toxic Residues in Food and Related Samples (Organophosphate and/or carbamate Pesticides and/or Other Toxic Cholinesterase Inhibitors)

The 3rd award on “Inventor’s Day 1997”
From The National Research Board of Thailand

Patent No. 8446

Asianmedic Co., Ltd. Tel: 662.6918348
Hotline: 090.898.5188
www.Asianmedic.com foodtest@asianmedic.com
**GT Pesticides Detection Kit (Code: AP001)**

Pesticides are toxic substances to be used in agricultural products residues need to be lower than the safety limit; there are still many kinds of food in the market that still has residues that exceed the safety limit. Several methods can be applied to detect pesticides residue in food samples, such as GC, HPLC and GCMS. Those methods require a long analysis time as well as complicated procedures and instruments. This Sensitive pesticides Detection kit provides a fast, Easy, Simple and reliable test solutions to detect pesticides residues in food samples.

**Pesticides Test targeted groups**
- Organophosphate group
- Carbamate group
- Cholinesterase Inhibitors

**Target samples:**
Vegetables, Fruits, cereal grains, medicinal plants, dry salted fish, soil, mud and water from/near contaminate area and drinking water

**Details:**
This kit is composed of 2 parts
1. Test kit (reagents only) 10 test and 30 test/Kit
2. Modified equipment (you do not need this if you have access to laboratory equipment)
   A. 1 unit of modified warm water bath
   B. 1 unit of Thermometer
   C. 18 test tubes
   D. 5 Pasture pipettes
   E. 12 plastic pipettes
   F. Aquatic air pump
   G. 1 unit of evaporated kit


Extraction Procedure
1. Chop or blend sample
2. Weight 5 g of the homogenous sample in to the sample bottle
3. Add 5mL of Solvent-1 into the sample bottle, closed tight and shake vigorously for approximately 1 minute. Let is stand for 10-15 minutes
4. Pipette out 1mL of Solvent -2
5. Now, prepare the modified warm water bath (32-36C) by connecting the evaporated kit with the air pump and the pasture pipette and then inset the end of the pasture from the air pump in to the extract, and then incubate until the Solvent-1 (lower layer) is evaporated now the solution is called “Sample Extract”

Testing Procedure
Prepare and label at least 3 test tubes for:
• Tube 1: Cut point or unsafe
• Tube 2: Control or not detected
• Tube 3: Sample extract (sample name)

3 test tubes for 1 sample. Please add more test tubes if you are testing more samples. Run each samples with two control tubes (Tube 1 and 2)
1. Add 0.25mL of Solvent-2 into Tube 1and 2
2. Add 0.25mL of each Sample Extract from the extraction procedure to Tube 3 or your sample tubes
3. Put all the tubes into the modified warm water bath at temperature (32-36C)
4. Pipette each 0.5nL of Marin -1 solution into every tubes and incubate for 5-10 minutes
5. During incubation mix Main-2 solution: Main-2+Main 2.1 and Main-3 solution: Main -3+Main 3.1
6. Add 0.375mL of the mixed Main-2 solutions from step 5 into Tube-1 (cut point of unsafe control tube).
Add0.25mL of the Main-2 solution in step 5 into Tube 2 (safe control tube) and Tube
7. (sample tube) incubate for 30 minutes
8. Add 1 mL of main -4 solutions from step 5 into every tube and swirl
9. Add 0.5mL of Main 5 solution into every tube and swirl
10. Evaluate the results

Color in the tube
Sample tube <tube 2
Sample tube > tube 2 but< tube 1
Sample tube > tube 2 and > tube 1

Result Interpretation
Not detected
There are some toxic residues expected safe for consumption
There are some toxic residues expected unsafe *** for consumption

Note:
Safe for consumption = there are some toxic residues inhibited the cholinesterase enzyme at less than 50% these toxic residues amounts can be washed out by the consumer
Unsafe for consumption = there are some toxic residues inhibited the cholinesterase enzyme at 50% or more. This amount of toxic residue cannot be washed out by the consumer, they are only decreased by washing

CAUTION
• The reason in using the Solvent-1 are to dissolve the toxic residues from the sample and to destroy the interfering enzyme deposited in the sample. Therefore Solvent -1 is toxic for human health, avoid breathing and leave it in open, as it will evaporate
• Wash out with clean water and soap when chemical reagents in contact with skin
• Wear gloves
Keep away from children
Do not use reagents from different are chemical
This not food DO NOT eat or drink

Storage
Keep all reagents in a refrigerator/Cool place Except for Main-GT1 and Main GT2 should be in the freezer
1 year shelf life if stored properly
Once Main GT2, Main-GT2.1, Main-GT3.1 are mixed, store in the refrigerator
Main-GT2 mixture can be used within 10 days after mixing
Main-GT3 mixture can be used within 3-4 days after mixing

Asianmedic Co., Ltd  foodtest@asianmedic.com www.asianmedic.com
## STANDARD METHODS COMPARATION

<table>
<thead>
<tr>
<th>Samples</th>
<th>GT-Test Kit</th>
<th>Standard Methods (GC &amp; HPLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not detected</td>
<td>Safe</td>
</tr>
<tr>
<td>Vegetables 528 samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>(25.4%)</td>
<td>(25.4%)</td>
</tr>
</tbody>
</table>

From the above table, 44 unsafe samples by GT test kit were compared to the standard method.

### GT Test Kit

<table>
<thead>
<tr>
<th>Sample</th>
<th>Not Detected</th>
<th>Detected</th>
<th>Safe</th>
<th>Unsafe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables in the pack of the food, ready to cook 50 samples</td>
<td>23 (46.0%)</td>
<td>22 (44.0%)</td>
<td>5 (48.0%)</td>
<td>26 samples detected (52.0%) but no codex MRLs in ready to cook food. Therefore can’t decide to point that those samples are safe or unsafe for consumption</td>
</tr>
<tr>
<td>Cereal beverage 9 samples</td>
<td>7</td>
<td>-</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

May contain some toxic cholinesterase inhibitors or any pesticides that can’t be analyzed.

![Image of the table](image-url)

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