

# Residues Control by Using Rapid Bioassay of Pesticide Residues (RBPR) for Market Inspection and Farm Education

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## Abstract

Following the execution of residue control program using housefly bioassay technique during 1964-1987, Taiwan Agricultural Research Institute has renovated and implemented a rapid bioassay method since 1985. The established rapid bioassay of pesticide residues (RBPR) system is aiming at detecting the noxious organ phosphorus, carbamate insecticides as well as ethylene bisdithiocarbamate fungicides using housefly acetylcholinesterase and *Bacillus thuringiensis* as the probes. Standard assay procedures have been developed to detect the residues on fruits, vegetables, rice, tea, spices, etc., and are able to complete 50 shipments within 4-6 hours. In Taiwan, more than 300 RBPR stations inspect a half million agricultural products annually, and have constructed a residue control system that covers farmer association and farm cooperative in production areas; wholesale markets; food suppliers for military services, school and group lunching; supermarket chain stores; private enterprises, etc. RBPR has also been adopted by the Republic of Korea, Vietnam, Philippines, Panama and many Southeast Asian countries, and 11 international RBPR training workshops were held during 1993-2010. The institute also is responsible for supplying RBPR reagent kits and high quality of housefly acetylcholinesterases to local users and abroad.

**Keywords:** Insecticide, Fungicide, Residue, Acetylcholinesterase, RBPR.

## Introduction

Agriculture in Asia is usually different from America or Europe on farm scale and the marketing system. The pesticide residue monitoring system develop by the Environmental Protection Agency (EPA) of the United States usually is not functioned as a safety valve for consumer protection, but is mainly designed to conduct the general survey of residue situation and offer suggestions for long-term improvement. The immediate consumer protection action can not be derived from the EPA residue control practice. “You detect what you haven’t eaten” versus “you detect what you have already eaten“, clearly differentiates the residue preventing mechanism from the residue monitoring mechanism.

Unfortunately, pesticide residues are only monitored by the chemical analysis methods in developed countries such as United States and Japan, which may not be practical for regions such

as Taiwan and Southeast Asia countries. Chemical analysis is precise enough but lack of speed, hence is incapable of screening out the residue contaminated vegetable before been served and mostly only serves as investigation purpose. In Taiwan, the Food and Drug Administration of Department of Health conducts a general survey that analyzes 1,800 fruit and vegetable samples annually (Lee *et al.*, 2008; Kuo *et al.*, 2009), but is unable to stop the contaminated shipments before marketing, i.e., all vegetables confirmed with pesticide residue violation had been sold and consumed. Detailed residue monitoring is needed, but the government is also responsible for stopping the residue contaminated shipments for further trading, and that is the major purpose of RBPR (Rapid Bioassay of Pesticide Residues) to be involved in residue control.

Starting from the first RBPR station in central Taiwan in 1985 and increased to 38 stations within five years, the RBPR system supervised by Taiwan Agricultural Research Institute (TARI) grows steadily to more than 300 in 2010 and has established a strong residue control network island-wide. Current RBPR technology can deal with the most hazardous pesticides mentioned in the Food Quality Protection Act (FQPA) legislated in 1996 in the United States, i.e., organophosphorus (OP) and carbamate insecticides (Carb) (EPA, 2000 & 2002). In other word, the action we have been taken for the past years fits exactly the true spirit of the FQPA and has gained popularity and acceptance to whatever country it has been introduced.

The technology is simple, the detection speed is quick, the expenditure is low and the result is clear in toxicological sense. RBPR is designed particularly for its residue preventing function, and has been generally accepted in Taiwan, Korea, Vietnam and Panama because this rapid monitoring system profits the general public by stopping the pesticide contaminated shipments in time.

## **Prospects of Rapid Monitoring and Control of Pesticide Residues**

Vegetables are essential parts of the human diet. They provide carbohydrates, proteins, lipids, vitamins and mineral elements. The production of vegetables nowadays depends heavily on new technologies, and pesticide usage is almost unavoidable especially in the subtropical and tropical regions where vegetable production confronts with serious insect and disease problems. Although new safety-concerned pesticides have been developed in the past two decades, many pest problems still remain unsolved and rely heavily on hazardous pesticides, particularly, the neurotoxic OP and Carb insecticides (Cheng *et al.*, 1988; Fukuto, 1978; Kao and Cheng, 2001; O'Brien, 1967). Fungicides are generally less toxic to animals, but one major group, ethylene bisdithiocarbamates (EBDC's), has a problem in ethylenethiourea (ETU), an impurity as well as carcinogenic metabolite.

In early 1960's, when synthetic pesticides were only very few, residue monitoring and prevention were simple and easy. Later, the number of chemical pesticides increased tremendously, and monitoring samples for unknown residues has become an enormous task in the laboratory. Not until every pesticide been examined, to ensure that vegetable free from residue is not possible. In

developed countries, large farm scale makes residue control easier, particularly when the cold storage and transportation allow extra time for recall. On the other hand, many developing countries have only small farms and the farmers are mixing and spraying pesticides independently, which makes the residue control an impossible mission. If the pesticide residue prevention still remained as a country's public health policy, an alternative rapid residue screening process based on the toxicological technology can help to stop the agricultural products with signs of contamination.

In reality, pesticide residue monitoring does not have any practical value for consumer protection if the system could not prevent the contaminated vegetables from been consumed. Many residue-related problems can also be corrected simply by installing an economic, rapid, sensitive and accurate screening process before the laborious chemical analysis is applied. Therefore, constant monitoring of fruits and vegetables for these dangerous pesticide residues is feasible, and to carry out the control action is absolutely needed for consumer protection.

### **Housefly Bioassay in Taiwan (1959-1987)**

After the first OP insecticide, parathion, was introduced to Taiwan for agricultural pest control in 1952, more and more residue problems arose and had initiated official pesticide residue survey and research programs since 1959. The housefly (*Musca domestica* L.) bioassay (HB) method was developed by TARI during 1959-1962, and had put into practice during 1964-1987 by 18 bioassay stations in major wholesale markets.

In 1980s, the number of registered synthetic insecticides increased rapidly and the introduction of synthetic pyrethroids (SP) imposed another problem for HB because the HB could not distinguish the much safer SP from the more dangerous Carb and OP insecticides. Questions also came from the consumers as the general public were not satisfied with the pre-harvest warning, and demanded for effective residue control on harvested vegetables. Unfortunately, the duration between the arrival of agricultural products at market to auction was only three and half hours in the wholesale markets, and the 6-hour HB method could not fulfill this request, hence the main task was to speed up the test.

### **Development of RBPR in TARI (1980-)**

In order to develop a rapid residue screening method, TARI had analyzed the *pro* and *con* of several residue analysis methods. The chemical analysis is detailed, sensitive and accurate because advanced analytic instruments such as gas chromatography (GC), high performance liquid chromatography (HPLC) or even GC-MS and LC-MS are used in some cases. One of the common features of those instruments is the sample should be clean and free from undesirable impurities, hence the time-consuming and laborious cleaning process is unavoidable in sample preparation.

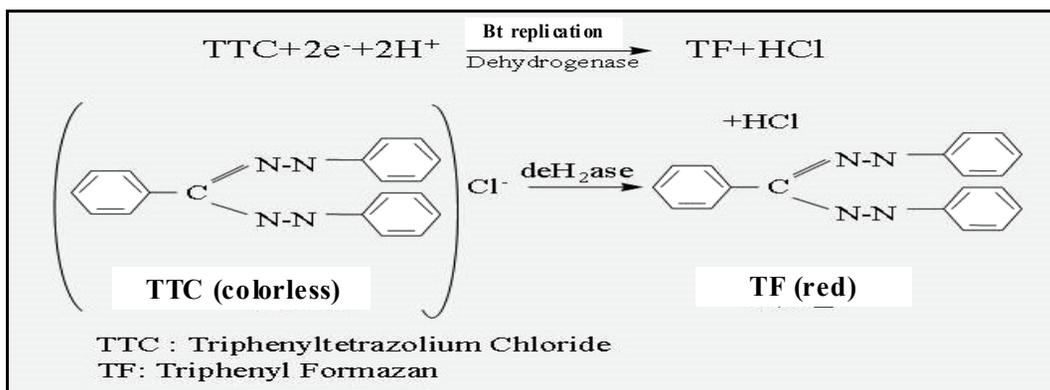
When looking for the potential alternatives, we found that the common and unique feature of



Fortunately, the obstacle was later overcome by combining the bioassay with the simple and rapid thin layer chromatography (TLC) technique. Once the pesticide was identified by the rapid qualitative analysis, the residue concentration could be estimated from the correlation of concentration and toxicity of that particular insecticide. To process large quantities of vegetable samples, the AChE test was modified by using the microplate reader in the enzyme kinetic mode, which has been adopted in major markets since 1993.

### Fungicide residue assay using *Bacillus thuringiensis*

The second test of RBPR is specially designed for the EBDC's not only because it once consisted 70% of the fungicide market, but also due to its carcinogenic impurity and metabolite, ETU (Fishbein, 1977). In this regard, a number of microbes including *Bacillus cereus*, *Saccharomyces cerevesiae*, *Ustilago maydis*, and *Bacillus thuringiensis* (Bt) were tested, and Bt was finally chosen for its safety, sensitivity, availability and reliability (Chiu *et al.*, 1991; Hammerschla and Sisler, 1973; Salama *et al.*, 1981). By incorporating the triphenyl tetrazolium chloride (TTC) as described by Bitton and Dutka (1986) to detect the dehydrogenase generated during Bt replication (Fig. 2), this assay method has been proven to be practical, economical, rapid and even more sensitive than the CS<sub>2</sub> test. In addition to the EBDC's, Bt test is also very sensitive for the detection of antibiotics, chlorothalonil, TPTA, etc. Bt test has been incorporated into RBPR system in 1989 and can detect hundred of samples within hours. By adopting Bt test, not only the analysis time is shortened, tremendous saving in manpower and resources in fungicide analysis can also be achieved.



**Figure 2.** Using TTC to detect the dehydrogenase generated by the growth of Bt.

## Features of RBPR

In Taiwan, the Food Safety Law defines the standard procedure for pesticide residue analysis, which is the same for either fresh vegetables or storable products. For rice, the residue analysis may be in time for the residue clearance because rice can be stored for a period of time. As to the

fast marketing fruits and vegetables, the same safety regulation became incompetent because when the lengthy residue analysis was completed, the contaminated vegetable had already been sold and consumed.

Many efforts have been made to correlate the sensitivity of RBPR to that of chemical analysis. These efforts are worthwhile but not essential since the practicability of RBPR depends on the actual situation of pesticide usage in that region. If Carb, OP and EBDC's are used, it is suitable to apply RBPR; but when SP, insect growth regulators and other category of insecticides are used, RBPR is less fit (Fukuto, 1978). In other words, the practicability of RBPR is dynamic or pesticide-dependent.

### **Detect the cumulative toxicity**

The tolerance of pesticide is estimated at the chronic toxicity, and usually at sub-ppm level; however, high levels of residues are more hazardous than the sub-ppm problem. RBPR can detect the total toxicity of different pesticides within specific group i.e., the higher residue, the easier to detect, hence can screen out the major residual problem. For a pesticide, if RBPR is sensitive enough to reach its chronic tolerance, it is perfect; but when RBPR is not sensitive enough to reach the tolerance, it still can prevent the dangerous acute or sub-acute poisoning. Particularly when the farmers tend to use many pesticides in the tank mixture, chances to violate the regulation is higher.

### **Efficient residue control**

Since RBPR is able to detect the residues of OP and Carb in 10 minutes, if the sample sent to the station was for pre-harvest check, delay the harvest also can be attained. But when agricultural products are harvested and delivered to market or food processing center, the rapid screening process will act as safety valve, and products with higher risk can be withheld for further trading or processing in time.

Simply follow the standard assay procedures, two technicians can detect insecticide and fungicide residues of 50 samples within 4-6 hours and screen out high residue risk shipments. For massive samples assay in the wholesale markets, major suppliers or supermarket chains, incorporate the microplate reader can shorten the assay time and expand the control efficiency.

### **Cost saving**

Chemical analysis is originally designed for individual pesticide detection. The residue analysts gathered different protocols, summarized them, abstracted the common features, reorganized the procedures with compromise, and adjustments were made to conduct multiple residue analysis. Until now, none of any multiple analytical methods can cover all pesticides. For any unknown samples, several methods, instruments as well as clean-up procedures are needed to

complete the analysis. In Taiwan, analysis of the residues of 200 pesticides in one sample costs 150 to 300 US dollars, and average 3-7 days is needed. Without a screening process, all those efforts might just prove to be a 90% waste in the end.

RBPR can reduce both the labor and cost of analytical materials; meanwhile minimize the polluted organic wastes in the chemical analysis. TARI produces the housefly AChE and supplies RBPR reagents to local users and abroad, and the reagent cost of one assay is 0.5 US dollar. When conduct massive samples using the microplate reader, the cost is even lower because more diluted reagent is used.

## **Management of RBPR**

Ever since the establishment of the first RBPR station in 1985, the number of stations increases gradually and the testing items also are expanded from fruit and vegetable to tea, rice, spices, etc. Currently, more than 300 stations have been established in Taiwan, and users include farmer associations and farm cooperatives in crop production areas; wholesale and retailed markets; military food supply system; school and group lunching programs; supermarket chains and private companies; public health system and education purpose.

In practice, three fronts of RBPR are involved in the residue control in Taiwan. The first front is the ready-to-harvest stage in producing areas that facing the growers directly; most of the stations are established by local farmer association or farm cooperative. The second front is the delivery stage; wholesale markets in production districts or major cities are responsible for the safety check. The third front is closer to the consumers; suppliers for military food, group meal and school lunching as well as supermarket chain stores are the final check points. Every year, more than half million of agricultural products are screened by RBPR, and nearly 10,000 shipments are rejected due to toxicity risk. Effective actions taken by the RBPR stations include delay harvest, thorough wash and recheck, warning but accept the shipment, reject the shipment, destroy the products, etc.

Several factors contributes to the success of RBPR include feasible technology, sound training and audit programs, high quality reagents, efficient feedback mechanism, widely acceptance by the users and consumers, etc.

### **Intensive training program and audit system**

In Taiwan, all the technicians work for RBPR need to be trained by TARI in a two-day training sessions. Lectures and practicum on basic principles, application status, and technical details on sampling, extraction, assay procedures, reagent preparation, instrument setting, etc. will be given. A certificate validates for two years will be issued after the station hand in the monthly report steadily. Renewal of certificate will be made for the technicians and stations that passed the

annual evaluation; any station did not send in the report for 6 months will be rejected from the RBPR system.

### **Production of high quality housefly AChE**

The core of TARI's residue control program is the susceptible housefly colony established at TARI since 1958. Mass rearing of this housefly colony has been ongoing for more than half century and produces 200,000 flies weekly. After sophisticated purification processes, high quality AChE can be obtained from housefly brain and provided needed enzyme material for RPBR application. Compared to the commercial AChE products of electric eel, horse, etc., TARI's AChE is extraordinarily stable and highly sensitive to the neurotoxic agent, and has gained great popularity by local biotechnology companies and users from abroad.

### **Efficient feedback system**

Residue problems may arise occasionally due to improper pesticide application, disobey pesticide labeling, etc. Based on the working reports provided by the RBPR stations, TARI gathers the latest information on residue situation island-wide and releases RBPR Newsletter every month. Whenever there is unusual signs or risk of residue in particular area or crop, a case study will be initiated through massive sampling from the markets or production areas; qualitative and quantitative analysis of residual pesticides will be conducted; suggestion or warning will be made to related parties to ensure the safety of agricultural products. Many stations are actively involved in such kind of study and have provided useful information regarding production status.

### **Modification of assay techniques**

When more and more RBPR stations are established, we need to handle various agricultural products other than fruits and vegetables, especially when containing some natural inhibitors. For example, modification of sampling and assay procedures have been made for rice, tea, citrus, strawberry, potato, onion, ginger, garlic, corn, and some spices, Chinese medicines, various mushrooms, etc., and put into practice by needed stations (Cheng, *et al.*, 2000; Kao *et al.*, 2003).

RBPR stations in major wholesale markets and supermarket chain stores usually inspect more than one hundred shipments per day, and have installed microplate reader to speed up the testing processes; while the stations in regional production area use spectrophotometer to examine 10-50 samples daily. For the tea and rice production areas, the use the microplate reader is also recommended because massive samples may come in within very short period. Standard testing procedures of microplate reader and spectrophotometer are available and incorporated into training upon request.

## **RBPR International**

Sponsored by the Food and Fertilizer Technology Center (FFTC) and Council of Agriculture, 11 RBPR training workshops have been held since 1994 in Taiwan, Philippines, Thailand, Republic of Korea, Vietnam, etc. (FFTC, 1994 & 2009). Asian Vegetable Research and Development Center also sent the trainees from Vietnam, Lao and Cambodia to TARI to learn for RBPR techniques.

A similar program in Korea was initiated by Dr. Chan-Hoi Choi, who introduced RBPR to the National Agricultural Cooperative Federation (NACF) in 1996. After continuously testing, NACF sponsored more than 200 local farmer associates to establish RBPR stations within two years and are responsible for the follow-up training programs. The largest wholesale market in Seoul, Garak Wholesale Market, implements RBPR technology since 1998 and has combined RBPR with chemical analysis to execute a residue control program that is efficient enough to protect the consumers. Korea Food and Drug Administration has been using RBPR as rapid screening tool since 1998. Hyundai and Shinsegae Department Stores, Samsung Everland and other supermarket chains also apply RBPR to safeguard the quality of fruits and vegetables they supply.

Another example is in Vietnam. The Sub-Institute of Agriculture Engineering and Post-Harvest Technology in the Ho Chi Min City has adopted RBPR in 1997 and conducted residues survey programs since then. By applying RBPR technology, Mr. Tran Van An and his colleagues won the National Safety Prize in 2003 for their dedication in solving pesticide residue problems.

After the lectures on RBPR technology given by Dr. Edward Y. Cheng in mainland China in 1998, similar rapid bioassay method has been developed by several institutions. The national standard method was announced in August 2003 by the Department of Agriculture of People of Republic China, and has established a detection network.

In 2004, Mr. Miguel Zheng of the Taiwan Mission of ICDF (International Cooperation and Development Fund) presented the RBPR technology to the government of Panama, and started to conduct a three-year cooperative project between Panama and Taiwan government. The counterparts of this project include Ministry of Agriculture, Ministry of Health, Panama Municipal and Panama Agricultural Research Institute and have established national residues monitoring system and 3 bioassay laboratories including one pilot laboratory in Central Agricultural Market in Panama City. The pilot laboratory executes the annual investigation to evaluate the level of pesticide contamination and illustrate the high risk production areas. Collaboration with dealers and producers as well as communication with the consumers has been ongoing. RBPR technology is well accepted by major dealers in central market and supermarket chain stores such as RS, REY, SUPER 99, Machetazo, etc. In October 2008, a WTO committee praised the Taiwan ICDF for the RBPR work in Panama that targeting specific areas to be improved on a relatively small budget.

All of these indicate that people appreciate the real action rather than the annual report on residue survey. Due to the availability and easiness, RBPR can immediately stop the residue to poison people, and is a no boundary program that can be used in any region. The cost to implement RBPR is low, while the achievement has already been proven to be significant. The general public appreciates RBPR technology even though it is unable to detect all the pesticides. The consumer parties showed their understanding and patience as more research is needed for some pesticides that still can not be detected by current method. It has formed a unique working network on pesticide residue prevention.

We believe that through the application of this technology, pesticide residue working groups will win the heart of the consumer who usually supervises the residue control agencies of government closely. We would like to share these concepts and experiences with the people who have been troubled by the residual problems. In the future, we may work together to ensure safer agricultural products and better tomorrow.

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