PART II

APPLICATION OF ANALYTICAL METHODS TO REAL SAMPLES

CHAPTER 1

INTRODUCTION AND REVIEW

Throughout the world from historical times, developing countries are known to be the major producers of spices. Asian and African countries have geoclimatic conditions conducive to the cultivation of many important spices. India, since time immemorial, has been known as the land of spices. India is also the world's largest producer, consumer as well as exporter of spices[1]. Each state in India is home to one or more commercially prominent spices. However, one of the important issues faced by developing countries like India, especially in plantation crops like spices, is that a major share of cultivation happens in small and marginal farms. In such places, due to various constraints, adoption of good agricultural practices, and optimal and judicial application of pesticides, are not widespread. Chances of indiscriminate use of pesticides are high in such situations, and thus the chances of incidence of residues in the food produced, at levels above the regulatory limits, are comparatively high. Assessment of dietary risks due to pesticide residues in food thus becomes an important concern. This chapter reviews the process of fixing maximum residue limits for pesticides by national regulatory authorities in India and the common techniques used for dietary hazard characterization due to pesticide residues.

Risks due to pesticide residues

Agricultural production is beset by attacks from a lot of pests like insects, mites, fungi, weeds, vertebrate pests etc. Although pesticides are tremendously important from the point of view of ensuring productivity of agricultural commodities by controlling these pests, uncontrolled and indiscriminate use of pesticides produce residues in food which are harmful to non-target organisms also. Thus, pesticide residues pose food safety risks, and are regulated by legislation in most countries. To manage these issues, a set of authorized good agriculture practices (GAP) are usually adopted for different agricultural commodities. These practices lay down recommended safe dosages for approved pesticides, application conditions etc., which have been demonstrated in the field to have effective control of target pests without resulting in large amounts of pesticide residues. Many countries across the world have fixed maximum residue limits (MRLs) for pesticides in raw agriculture commodities, based on GAP practices and pattern of consumption of agricultural products.

In India, the use of chemicals for pest management in crops and animals is regulated by the Central Insecticide Bureau and Registration Committee (CIB&RC). Only pesticides which are registered by this body can be legally used in India. The MRLs for the registered pesticides in agriculture commodities are fixed, based on requirements, by the Food Safety and Standards Authority of India (FSSAI). These MRLs are derived using data from supervised field trials, taking into account toxicological information of the pesticides and dietary exposure data.

Fixing MRLs for raw commodities

As mentioned above, the usual practice of setting MRLs is derived based on statistical analysis of data from GAP based supervisory field trials for a commodity using the pesticides under consideration. However, supervisory field trials are cost- and labour-intensive. So, whenever there is a felt need, there is also the provision to fix MRLs based on monitoring data in commodities which do not have GAP trial data[2]. The MRL calculations are usually done using the MRL Calculator developed by the Organisation for Economic Cooperation and Development (OECD), United States Environmental Protection Agency[3]. This calculator is an Excel sheet which takes residue monitoring data from field trials as input and calculates the following three values as output: supervised trial median residues (STMR), highest residue, mean residue and the maximum residue limit.

In a typical multi-location supervised field trial, residue data is generated at different interval days after application of the pesticide as per GAP and a set pre-harvest interval (PHI), typically taken as 3 days. This data is loaded as input in the OECD MRL calculator, which will calculate the MRL value based on appropriate statistical approaches. A dataset of 10-15 results is considered to generate MRL values of acceptable uncertainty levels.

Dietary exposure and hazard estimation of residues

When pesticide residues are present in food commodities (raw or processed), a consumer of such food is exposed to these residues. The extent of such exposure from a food commodity is based on the dietary consumption pattern that commodity, which is expressed as g person^{-1} day^{-1}. Consumption pattern for long term effects is used for MRL calculations, and consumption pattern for short term effects, based on 95th percentile, is used for short term risk assessment. For spices[2], the consumption pattern for long term effects used for MRL calculations ranges from 2 - 3 g person^{-1} day^{-1}, which is much lower than that for staple foods. A comparison is shown in Table 2.1 below.

Estimation of dietary hazard (or risk) due to pesticide residues require two steps, *viz.* hazard identification and hazard characterization. For these steps, data from toxicological studies as well as human epidemiological studies are used. The two important values for hazard identification are acceptable daily intake (ADI) and acute reference dose (ARfD). To calculate these values, first the no-observed-adverse-effect-level (NOAEL) is estimated toxicologically, which is defined as the largest concentration of a substance that causes no detectable adverse effect on a target organism. Typically, NOAEL is divided by a safety factor of 100 to arrive at ADI or ARfD in units of mg kilogram-body-weight-^{1} day^{-1}. ADI is usually expressed as a range while ARfD is expressed as a value. The safety factor may vary depending on the test organism and the compound being studied.

Table 2.1: Comparison of dietary consumption of cereals and spices[2]

Commodity	**Dietary consumption ($g\ person^{-1}\ day^{-1}$)***
Cereals	
Bajra	113
Barley	59
Maize	100
Ragi	56
Rice (Milled and parboiled)	257
Sorghum (Jowar)	163
Wheat (whole flour)	192
Other cereals / millets	45
Spices	
Cardamom	2
Black pepper	2
Coriander	3
Cumin	2
Fenugreek	2
Ginger	3
Chillies (dried	3
Garlic	2
Other spices	2

*FSSAI recommended dietary consumption for long-term effects (for MRL calculation).

To perform hazard characterization due to residues, the national estimated daily intake (NEDI) values are used. NEDI is calculated as follows:

$$NEDI = \sum STMR \times \frac{F_i}{bw}$$

Where STMR is the supervisory trial median residue ($mg\ kg^{-1}$), F_i the per capita food consumption ($kg\ person^{-1}\ day^{-1}$) and bw the average body weight (kg). Next, the ADI is converted to per person per day by multiplying ADI by 60, where 60 kg is taken as the reference body weight for dietary risk assessment in Indian context[2]. Dietary hazard is then characterized by comparing NEDI with ADI. If this comparison indicates that the residues generated by field trials will not generate dietary intakes exceeding the ADI, then the calculated MRL value based on the field trial is adopted as the actual MRL under the GAP conditions. Ideally, NEDI ≤ 80% of ADI means that there is minimum hazard due to pesticide dietary exposure.

Fixing MRLs for processed commodities

The process described above summarized the fixing of MRLs in raw agriculture commodities. However, such commodities undergo processing before reaching the consumer, thus fixing MRLs of processed foods also requires consideration[2,4]. The steps of processing can affect the pesticides in the raw commodity, and based on the physicochemical properties of the residue, the final product can contain a residue concentration which is different from the raw commodity.

Processing can involve several widely different operations, ranging from simple to complex. Washing, peeling, grinding, cooking, oil extraction etc can all considered as processing steps. Some of these can result in reduction of residues (e.g., washing) while soe others can result in magnification of residues (e.g., oil extraction, preparation of spice oleoresins). Other steps like cooking can convert a residue into a metabolite which can be more toxic than the original residue. All these factors contribute to the complexity of fixing MRLs for processed foods. To arrive at MRL for a processed food, the processing factor is calculated:

$$P_f = \frac{R_p}{R_r}$$

where R_p and R_r are the residue concentrations (mg kg^{-1}) in the processed and raw commodity respectively.

Except in cases where residue gets concentrated on processing, processing factor need not be applied if $R_r \leq$ LOQ. A value of $P_f > 1$ indicates residue enrichment, while P_f

< 1 indicates residue reduction. The processing factors are calculated through a rigorous and involved procedure involving multiple independent trials and processing steps. In cases where P_f is available, compliance evaluation is done after multiplying the MRL by this factor.

Dehydration factors in spices

A specifical case of processing factor which is applicable to spices involve the case were processing involves only loss of moisture. Spices are typically dry commodities with moisture content reduced to 8-10%. This results in residue concentration, though the risk associated with the absolute quantity of pesticide present as residue does not change. Thus, a dehydration factor needs to be applied to the MRL before performing compliance evaluation in spices. This is calculated as follows:

$$DF = \frac{1}{(1 - \frac{W}{100})}$$

where W is the water content (%) in the spice.

The European Spice Association (ESA) has published a study in which dehydration factors ranging from 5 to 10 were calculated for various spices and herbs[5]. FSSAI has adopted a dehydration factor of 10 for dried chillies, thus the MRL of dried chillies is ten times the MRL of fresh chillies[6].

Assessing compliance with MRLs

In general, the result of an analysis for a pesticide in a commodity can be directly compared against the corresponding MRL. A result which is at or above MRL indicates non-compliance (‘fail’). A result which is below the MRL indicates compliance (‘pass’). Statistically, however, the measurement uncertainty associated with the result also has to be considered while evaluating MRL compliance. In this situation, there are four possibilities to be considered, which shown schematically in Figure 2.1. The mode of reporting of results will thus depend on the testing laboratory selecting the appropriate strategy for assessing compliance, which is stated formally as the 'decision rule'.

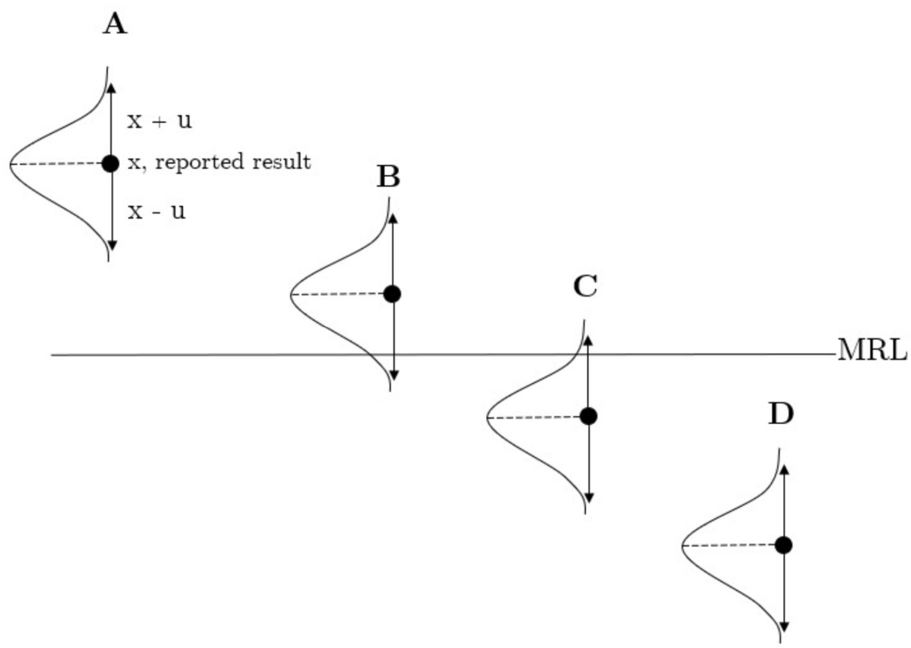

Figure 2.1 Using measurement uncertainty in assessing MRL compliance

There is no ambiguity in the cases A and D in Figure 2.1, as in both these cases the sample passes or fails even when uncertainty values are accounted for. However, in cases B and C, applying uncertainty will affect the decision on compliance. In case B, even though the absolute value of the result is above MRL, there is a possibility of the sample passing if negative uncertainty is considered. In case C, even though the absolute value of the result is below MRL, there is a possibility of sample failing when positive uncertainty is considered. In cases like pesticide residues where there is a potential hazard to the consumer of the food, decision rule C is usually applied.

Scope and objectives of the present study

In the present study, the sample preparation and instrumentation methods developed in Part 1 will be applied to real samples of spices collected from markets, in order to establish the capability of these methods for regulatory compliance evaluation against FSSAI MRLs. The residues detected in the samples will be characterized to gain insights into the levels and types of residue contaminations that can occur in different spices. These results will be used to perform an estimate of food safety hazard associated with presence of pesticide residues in spices.