FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA

*Inspiring Trust, Assuring Safe & Nutritious Food*

Ministry of Health and Family Welfare, Government of India

# Draft Guidance Document & Standard Operating Procedures for fixation of Maximum Residue Limits (MRLs) of pesticides in food commodities

**Food Safety & Standards Authority of India**

**(A Statutory Authority established under the Food Safety and Standards Act, 2006)**

**MINISTRY OF HEALTH & FAMILY WELFARE**

## TABLE OF CONTENTS

## Glossary

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| Ae | Acid equivalent |
| Ai | Active ingredient |
| ARfD | Acute Reference Dose |
| BMD | Bench Mark Dose |
| bw | body weight |
| CAC | Codex Alimentarius Commission |
| CAS | Chemical Abstracts Service |
| CCN | Codex classification number (for compounds or commodities) |
| CCPR | Codex Committee on Pesticide Residues |
| CEP | Critical End Point |
| CIB&RC | Central Insecticides Board and Registration Committee |
| cGAP | Critical GAP (25% more than the recommended pesticide dose) |
| CXL | Codex MRL |
| DAT | Days after Treatment |
| DM | Dry Matter |
| $DT_{50}$ | Time required for 50% dissipation of the initial concentration |
| EC | Emulsifiable Concentrate |
| ECD | Electron Capture Detector |
| EFSA | European Food Safety Authority |
| EMRL | Extraneous Maximum Residue Limit |
| EU | European Union |
| FAO | Food and Agriculture Organization of the United Nations |
| FSSAI | Food Safety and Standards Authority of India |
| GAP | Good Agricultural Practice (recommended pesticide dose) |
| GC | Gas Chromatography |
| GC | Grain cereals |
| GC-ECD | Gas Chromatography with Electron Capture Detection |
| GC/MS | Gas Chromatography/Mass Spectrometry |
| GC/MSD | Gas Chromatography/Mass Selective Detector |
| GC-NPD | Gas Chromatography coupled with Nitrogen-Phosphorus Detector |
| GEMS/Food | Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme |
| GFP | Good Feeding Practice (animals) |
| GVP | Good Veterinary Practice |
| GLC | Gas Liquid Chromatography |
| GLP | Good Laboratory Practice |
| HPLC | High Performance Liquid Chromatography |
| HR | Highest residue in the edible portion of a commodity found in trials used to estimate a maximum residue level in the commodity |
| HR-P | Highest residue in a processed commodity calculated by multiplying the HR of the raw commodity by the corresponding processing factor |
| IARI | Indian Agricultural Research Institute |
| ICAR | Indian Council of Agricultural Research |
| ICMR | Indian Council of Medical Research |
| IND GAP | Indian Good Agricultural Practice |

| | |
|---|---|
| IEDI | International Estimated Daily Intake |
| IESTI | International Estimate of Short-Term dietary Intake |
| ISO | International Organization for Standardization |
| IUPAC | International Union of Pure and Applied Chemistry |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| JMPR | Joint FAO/WHO Meeting on Pesticide Residues |
| LC | Liquid Chromatography |
| $LC_{50}$ | median lethal concentration |
| $LD_{50}$ | median lethal dose |
| LOAEL | Lowest-Observed-Adverse-Effect Level |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| MRL | Maximum Residue Limit |
| MRDB | Maximum Reasonably Balanced Diet |
| ND | non-detect - below limit of detection |
| NIN | National Institute of Nutrition |
| NSSO | National Sample Survey Organization |
| NOAEL | no-observed-adverse-effect level |
| OECD | Organization for Economic Co-operation and Development |
| PBI | plant back interval |
| Pf | Processing factor |
| PFA | Prevention of Food Adulteration Act |
| PHI | Pre-Harvest Interval |
| Ppm | Parts per million |
| RAC | Raw Agricultural Commodity |
| RP | Reference Point |
| RSD | Relative Standard Deviation |
| RWCF | Reasonable Worst Case Feed |
| SAU | State Agricultural Universities |
| SOP | Standard Operating Procedures / Protocols |
| SC | Suspension Concentrate |
| SL | Soluble Liquid |
| SD | Standard Deviation |
| STMR | Supervised Trials Median Residue |
| STMR-P | Supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor |
| TF | Transfer Factor |
| TLC | Thin-Layer Chromatography |
| TMDI | Theoretical Maximum Daily Intake |
| TRR | Total Radioactive Residues |
| USEPA | United States Environmental Protection Agency |
| US-FDA | USA – Food and Drug Administration |
| WHO | World Health Organization |
| WP | Wettable Powder |
| WDP | Water Dispersible Powder |
| WDG | Water Dispersible Granule |

## EXECUTIVE SUMMARY

Pesticides are important inputs for crop protection and sustaining production by managing the pests (insects, mites, nematodes, pathogenic diseases, weeds, vertebrate pests and the like) in crops during the cultivation and post harvest storage practices of food commodities.

The regulation including registration of pesticides for pest management in India are governed under a comprehensive statute called " The Insecticides Act,1968" by the Central Insecticide Board and Registration Committee (CIB & RC), under the administrative control of Ministry of Agriculture and Farmer's welfare. During the process of pesticide registration the scientific data on chemistry, mode of action, bioefficacy, toxicity to non-target organisms, phytotoxicity, pesticide residues on the crop, soil, water etc. are required to be evaluated by the Registration Committee of the Central Insecticide Board (CIB & RC). The pesticides which are efficacious against the target pests(insects, mites, nematodes, weeds and plant diseases); safe to human beings, animals and environment are registered for import, manufacture, transport, distribution, storage, sale and use in the country.

The presence of pesticide residues in food is a major concern for consumer safety and food trade. Many developed and developing countries fix their own Maximum Residue Limit (MRL) of pesticides on Raw Agricultural Commodities (RAC) based on their country specific Good Agricultural Practices (GAP) and dietary consumption pattern.

In India, the State Agricultural Universities (SAU's) / Indian Council of Agricultural Research (ICAR) generate the multi-location supervised field trial data for pesticide residues following GAP approved by CIB&RC on approved registered crops. The Food Safety and Standard Authority of India (FSSAI) under Ministry of Health and Family Welfare evaluate the supervised trial residue data based on the approved GAP , for fixation of MRL, keeping in view the dietary exposure and risk assessment only after approval of the same pesticide by the Registration Committee (RC).

At International level, Codex Alimentarius Commission (CAC) have been periodically prescribing guideline documents for fixation of MRL since 1970s, based on the recommendation of FAO/WHO Joint Meeting on Pesticide Residues(JMPR) and these are being updated periodically as per scientific development, fresh experience/lesson learnt and knowledge from different countries for implementation of these procedures. In India, such a document/guideline in the form of a Standard Operating Procedure (SOP) is not available, although under the erstwhile Prevention of Food Adulteration Act (PFA Act)some ad-hoc procedure was being adopted in line with FAO/WHO or United States Environmental Protection Agency (USEPA). A need was therefore realized to critically evaluate each and every step involved in the fixation of MRL such as planning of field trials, sampling, analysis of samples, data interpretation and risk assessment to improve the existing system of MRL fixation in India and compile a guidance document for scientists, risk assessors, risk managers, policy planners and other stake holders including the manufacturers of the pesticide.

Keeping in view the above objectives, the Scientific Panel on Residues of Pesticides and Antibiotics of FSSAI in its 36$^{th}$ meeting held on 21.05.2015 recommended constitution of a working group to document these guidelines. The constitution of the group was as under:

a) Dr. K. K. Sharma (Convener)
b) Dr. S. K. Handa (Member)

c) Dr. A. K. Dixit (Member)
d) Dr. Paresh G. Shah (Co-opted Member)
e) Dr. Cherukuri Sreenivasa Rao (Co-opted Member)

The terms of reference of the Working Group are as under:

1) Group will work on preparation of SOPs for setting of MRLs of pesticides in following commodities:

(a) Agricultural commodities as well as their processed products
(b) Feeds and Fodder
(c) Milk and Milk Products
(d) Meat and Meat Products

2) Group will consult literature relating to procedures being adopted internationally and in other regulatory systems.
3) Work assigned is required to be completed in six months from the date of first meeting,
4) Necessary infrastructure including secretarial facility may be provided by FSSAI to facilitate timely accomplishment of work.
5) Since the work assigned involves multidisciplinary approach, working group may co-opt any expert for the purpose. However, prior approval of FSSAI may be taken.

The Working Group met 8 times and submitted its report to FSSAI on 07.10.2016. This report was placed before the Scientific Panel on Pesticides and Antibiotic Residues in its 47$^{th}$ meeting held on 25.01.2016.The Panel constituted a group comprising 4 of members to review the report submitted by the Working Group.

The constitution of the Review Group was as under:

a) Dr. M. S. Mithyantha, Convenor
b) Dr. S. NaseemaBeevi – Member
c) Dr. Kaushik Banerjee-Member
d) Dr. K. K. Sharma – External Expert

The terms of reference for the Review Group were:

a. Examine the report and recommendations on Standard Operating Procedures for fixation of MRL of Pesticides in Food Commodities
b. Prepare the comparative analysis of the MRL recommended by the Scientific panel vis-à-vis Codex
c. Examine NIN data on food consumption vis-à-vis NSSO data and recommend their utility.

In all, the Review Group held five meetings on 06-04-2017, 08-05-2017, 10-08-2017, 02-11-2017 and 27-02-2018. The Review group requested for a special expert view for finalisation of the SOP and hence, the following two experts were specially invited for the 4$^{th}$ and 5$^{th}$ meetings:

a) Dr. P. G. Shah, Gujarat Agricultural University.
b) Dr. Cherukuri Sreenivas Rao, National Centre for Plant Protection, Hyderabad

The Review Group submitted its report to Scientific Panel in its 51st Meeting held on 10thApril, 2018. The Working Group as well as the Review Group placed on record the special contribution made by Late Dr. S.K Handa in preparation of the document.

The comments of the Review Group were examined in detail by the Scientific Panel on Pesticide Residues in its various meetings. Based on the comments of the Review Group and suggestions and comments offered by the members of the panel, the "Standard Operating Procedures on fixation of Maximum Residue limits" have been prepared and enclosed.

The Guidance Document-SOP consists of ten Sections as brief below:-

In **Section 1**, Introduction to the document has been given with emphasis on vast development in the field of Science and Technology, brief lines describing about requirement for framing the Guidelines and its importance for the scientists, analysts, regulators, policy makers and stakeholders to understand the basic principles for data generation, evaluation, risk assessment, fixation of MRL and implementation thereof.

In **Section 2**, SOP related to the generation of residue data as per the GAP such as number of trials to be conducted, plot size, crop variety, locations and pesticide application have been described.

**Section 3** deals with the sampling and residue analysis as these are crucial steps for the residue estimation because the sample should be true representative of the experiment field/lot. It describes sampling principles and procedure for sample collection, sample handling, sample size, portion of sample to be analysed and sampling intervals as per the Codex guidelines. This section also includes pesticide residue definition for enforcement, risk assessment and guidelines for method validation.

**Section 4** deals with the importance of metabolism studies in MRL fixation and **Section 5** describes the guidelines for risk assessment keeping in view the latest international procedures for utilization of GAP and critical GAP (cGAP) data in (Organisation for Economic Co-operation and Development)(OECD) MRL calculator and the utilization of monitoring data of India for MRL fixation on spices. The review group has recommended the pre harvest interval (PHI) on vegetables and fruits as 3 and 15 days, respectively; the average body weight as per existing practice to be 50 kg based on Indian studies; adoption of average food consumption factor partly moderated from the latest National Institute of Nutrition (NIN) data. Certain refinements have been suggested by the Review group in the case where the Theoretical Maximum Daily Intake (TMDI) exceeds the Adequate Daily Intake(ADI) such as recommendation of longer PHI for risk assessment, adoption of national monitoring data for realistic exposure, actual food commodities consumed per day by an adult etc.

**Section 6** describes dietary exposure of pesticide residues to human beings based on consumption of various food commodities.

**Section 7** explains about hazard identification being the foremost component of risk assessment

**Section 8** informs about risk characterization.

**Section 9** describes in detail the commodities that are processed before consumption and extrapolation of MRL from the RAC to the processed products. This document also includes information on crop grouping of extrapolation for MRL.

**Section 10:** For fixation of MRL on animal feed, meat, milk, offals and eggs, exhaustive procedure

## SECTION 1

## INTRODUCTION

Use of pesticides has become an integral part of modern agriculture in order to reduce crop losses, both in the field and in storage, in addition to the utilization of these compounds for keeping public health pests under check. Pesticides are registered for pest management on crops and animals and also house-hold purposes as per provisions of the Insecticide Act 1968. In India, such registrations are regulated by Central Insecticide Board and Registration Committee (CIB & RC). As per the requirement, Maximum Residue Limits (MRLs) are fixed by FSSAI on the basis of data generated through supervised field trials and risk analysis parameters taking into account the health guidance value derived from toxicological data and dietary exposure. However, there is a need to have a defined document and harmonized procedure describing the guideline for conducting supervised field trials and risk assessment for finalization of MRLs of pesticides for food safety.

In line with the information furnished by the WHO/FAO JMPR, India started prescribing tolerance limits of pesticide residues since 1970s (which was later reckoned as MRL), as a result of the increased uses of many pesticides in agriculture. It was during the same period the registration process for pesticides along with the requirement including data on pesticide residues in crops, water and soil was standardized. Pesticide Tolerance Limits were decided and notified by the Central Committee for Food Standards (CCFS) under the Prevention of Food Adulteration Rules, 1955. The said Committee fixed the Tolerance Limits based on the available Indian data and the values taken from international standards.

In view of the vast development in the field of science and technology, there was a strong need for documenting the detailed procedure for fixation of MRL after the enactment of Food Safety and Standards Act in 2006. This is the guidance document for establishment of MRL of pesticides which has been almost harmonized with procedure adopted by the JMPR and some other countries Worldwide, the agricultural practices and food consumption pattern vary from country to country. Recognizing this fact, each country establishes its own national MRL based on their individual country specific Good agriculture practice (GAP) and dietary consumption pattern.

This document describes general guidelines based on the following requirements:

(1) Chemistry of the product
(2) Toxicological information, its evaluation and deriving ADI and Acute Reference Dose (ARfD)
(3) Metabolism in plants, soil, water, laboratory animals, livestock and poultry
(4) Supervised field trials based on the approved/critical GAP
(5) Dietary Exposure and Risk Assessment
(6) Fixation of MRL in RAC
(7) Fixation of MRL in processed foods
(8) Fixation of MRL in animal products (Milk and Milk products, Meat and Meat Products and Eggs)

This is very important for the scientists, analysts, and all the stake holders to understand basic fundamentals and principles to take up the studies in a systematic and scientific manner. The broad objectives of such trials are to know the persistence and dissipation of residues on the commodities

by following the proposed or established GAP; to conduct dietary risk assessment based on STMR/highest residues; and to workout MRLs.

In this regard the Scientific Committee of FSSAI on the recommendation of the Scientific Panel on Pesticides and Antibiotic Residues constituted a Group for Preparation of Standard Operating Procedures (SOPs) for fixing of MRLs in 1) Agricultural Commodities and their Processed Products 2) Feeds and Fodder 3) Milk and Milk products and 4) Meat and Meat products. In the guidance document, the detailed information on field trial requirements for conducting residue analysis, calculating MRLs using OECD calculator and Risk Assessment and fixing MRLs for Raw Agricultural Commodities (RACs) is given. The document gives only the general guidelines, and the reader needs to refer the other documents for further details depending on the need.

In addition to the crops grown in fields, these guidelines are also applicable for crops treated after harvest, e.g. stored grains. However, such guidelines for the crops cultivated under protected environment (polyhouse/greenhouse/net house) are required to be prepared separately considering ever increasing area under protected cultivation.

Present guidelines include the following topics:

- Good Agricultural Practices (GAPs)
- Field Trial Parameters
- Sampling Principles and Procedures
- Residue Definition
- Method Validation for Residue Analysis
- Metabolism
- Risk Assessment
- Case study for fixation of MRLs
- Crop Grouping
- Processed food, Animal feed and Animal Products

Fixation of MRL require following parameters also:

1. ADI derived from Toxicological studies.
2. ArfD derived from Toxicological data.
3. Toxicological properties of plant metabolites
4. Residues from Supervised Trial as per approved critical GAP- Supervised Trial Media Residue (STMR), Highest Residue (HR),MRL
5. Analytical methods-LOQ
6. Animal feeding study/Animal metabolism
7. Processed study-Processing factor
8. Fixation of MRL using OECD Calculator
9. Food consumption data
10. Dietary risk Assessment.

This document gives the guidance for establishing MRLs on agricultural and food commodities and is meant for the use of:

1. Industries dealing with agriculture includingagro-chemicals / pesticides, pesticide applicatormanufacturers, agricultural commodities and products, and environmental agencies.
2. Research Institutes that study the behavior of pesticide residues, by conducting supervised field trials and determining pesticide residues in crops and the environment.
3. Regulators such as: CIB/RC, FSSAI and its Panels
4. Traders in Agricultural commodities-both domestic and international.
5. Food toxicologists and environmentalists

Based on this SOP relevant Checklist/Template need to be designed for use by

1. Pesticide Industry- Need to know what are the data required for fixation of MRL- A check list is required
2. Research Institutes/Laboratories who shall generate data for fixation of MRL- Checklist/Template, procedures
3. CIB-Registration Committee Secretariat experts/members how to evaluate the data-check list/template
4. Secretariat of FSSAI-What data need to be submitted/What and how to scrutinise-Checklist/template
5. Members of FSSAI-Scientific Panel on pesticide residues.

## SECTION 2

## 2. FIELD TRIALS

### 2.1 GOOD ARGICULTURAL PRACTICES (GAP):

Good Agricultural Practicesin the use of pesticides include the nationally authorised and recommended safe dosage use of pesticides using recommended quantity of water through recommended pesticide applicator equipments under actual farm conditions, where effective pest control is expected to be achieved. Optimum farm field dosage of a given pesticide is arrived at from the response curve of the laboratory experiments for computing the median lethal dosage. It encompasses a range of pesticide dosage applications up to the maximum authorised and recommended use, applied in a manner which leaves a residue in the most minimum quantity . Authorised safe uses of pesticides in crops is determined at the national level and include nationally registered or recommended uses which takes into account the public and occupational health and environmental safety considerations (FAO training Manual on fixation of MRL, 2016; FAO, 2020).

In India, the GAP is approved by the statuary authority, CIB&RC. The data needs to be generated based on the approved protocol/ guidelines for this purpose. Pesticide evaluation for MRL fixation has unique terminology where words and phrases have their own meaning arising out of a long history of debates and discussion about pesticide residues. Registered and approved use of a pesticide may vary considerably from country to country and the use patterns are often very different, especially in regions with vast differences in climate and pestilence in crops. Also, growing conditions and naturally, types of crops may also cause differences in the use pattern. According to the definition of GAP, a pesticide should be applied in such a way as to leave a residue which is the most minimum. Residue levels exceeding the smallest amount practicable, due to unnecessarily high application rates ("overdose") or unnecessarily short pre-harvest intervals (PHIs), are contrary to the concept of GAP. The pesticides in GAP include nationally recommended dosages against target pest on specific crop, time of application and method of application under actual conditions necessary for effective pest control and to observe the pre-harvest interval (PHI).

Supervised field trials are conducted to determine pesticide residue levels in or on RAC, including Feed commodities, and should be designed to reflect pesticide use patterns that lead to the highest possible residues according to GAP. On the basis of the supervised field trials, the Supervised Trials Median Residue (STMR), Highest Residue (HR) and the Maximum Residue Limit (MRL) are estimated. STMR and HR estimates are used in dietary risk assessment, while the MRL is used to compare the data/information derived from monitoring of pesticide residues in agri-commodities and to ascertain whether the pesticide is used in compliance with the label (i.e. with GAP) recommendation. MRL is also used for enforcement purposes. The selection of supervised trials, which correspond to the critical GAP (*c*GAP) and suitable for estimation of MRL, STMR and HR values is essential. The *c*GAP refers to the worst-case scenario where there is a possibility of deviation from the recommended GAP, for example, application of pesticides at higher doses based on the geographical situations, crop canopy and pest incidence. In case of residue studies, it is desired to take up the studies at both recommended and at 25% higher than doses recommended in order to accommodate over use of dosage than the recommended one. As per the present guidelines,

the CIB & RC requires data on supervised field trials using recommended dose and double the recommended dose.

As a general pre-condition, for reliable estimation of MRLs an adequate number of independent trials are required at different agro-climatic zones in the country.

## 2.1 FIELD TRIAL PARAMETERS:

**The user of this manual is advised to refer OECD document 509[1] for the purposes of planning field trials for MRL fixation.**

### 2.2.1 Number of trials:

Prior to planning of the field trials for residue studies, the required number of field trials and number of locations are decided to obtain sufficient data for requisite statistical analysis. The number of field trials and samples is dependent on the variability of pesticide use conditions, geographical distribution, the consequent variation of the residue data, and importance of the commodity in terms of production, trade and dietary consumption.

Field trials should be conducted in agro-climatic zones, where the crops are predominantly grown commercially and should reflect the main types of crop production and husbandry practices, which can significantly impact residues, for example crop variety, fertilizer, dosage of pesticides, irrigation, crop specific pruning etc. Similarly, in case of soil applied pesticides, since soil type influences the pesticide dynamics, the field trials should include field sites with different soil types.

To obtain statistically robust data set to estimate MRLs, HR and STMR, the number of trials should be minimum 8 and samples are dependent on the variability of conditions in use, the consequent scatter of the residue data and the importance of the commodity in terms of production, trade and dietary consumption. It is emphasized that the above number of trials reflect the absolute minimum of supervised field trials needed for estimating maximum residue levels and a higher number of trials (a minimum of 8 and ideally at least 15 for major crops) are recommended as maximum residue level estimates become increasingly unreliable as the number of data points decrease *(Ref:FAO Manual no. 225; 2016[2]).*

For stored products (e.g., potatoes, grains, seeds, fruits), post-harvest treatment should be carried out in a number of storage locations with variable conditions such as temperature, humidity, storage method (stacks/boxes) etc.

### 2.2.2 Plot Size:

The plot size may vary from crop to crop, but it should be large enough to allow application of test pesticide simulating use by farmers in general and provide sufficient representative sample. Each plot size should be minimum 20 square metrefor row crops, four trees for orchard crops and eight vines for vineyard crops. Control plot should be in the immediate vicinity of the treated plots and utmost care should be taken to avoid contamination / spray drift. For this purpose adequate buffer zones should be left in between adjacent plots. The number of plots (replications) for each treatment

[1] https://www.oecd.org/env/test-no-509-crop-field-trial-9789264076457-en.htm Accessed on 10 November, 2020

[2] FAO Manual on the Submission and Evaluation of Pesticide Residues Data

in each location has to be as per the requirement for statistical analysis, i.e. the degree of freedom for error component should be at least 12.

### 2.2.3 Crop Variety:

The pesticide behavior of the same crop varies from variety to variety, based on various morphological and physiological characters such as hairiness, smoothness, surface texture, crop canopy, erectness, early ripening, late maturity etc., which may have impact on uptake and degradation of pesticide and their metabolites. The pesticide behavior on the same crop changes in different seasons. It is not possible to conduct trials on all varieties/hybrids of the same crop, however, most popularly cultivated, highly preferred and consumed crop variety during high production season should be considered for residue trials.

### 2.2.4 Location:

The field trials should be conducted in a region where the particular crop is predominantly grown commercially and should reflect the recommended package of practices by the authorised institution of the state**.**

### 2.2.5 Other field operations:

While conducting the field trial, the general agricultural practices such as inter-cultivation, irrigation, fertilizer application, weeding, pruning *etc.* shall be followed as per the most common and recommended practices to ensure the best crop growth condition, so that pesticide residue dynamics will not alter, and such operations shall be performed in the same manner in all plots and trials.

### 2.2.6 Pesticide applications:

The application of pesticides using appropriate applicator equipment ensures that the spray solution is equally distributed across the crop in the plot and simulates the commercial field application in a farmer's field. The pesticides, in few cases, are available in different formulations, and in such cases, the application and selection of formulation should be based on the recommendations. The most common formulation types which are diluted in water prior to application include Emusifiable concentrate (EC), Wettable Powder(WP), water dispersible granules (WDG), water dispersible powder (WDP), suspension concentrates (SC) (also called flowable concentrates) and soluble liquids (SL) etc.

The spray solution should be prepared fresh as per the proposed recommendations. Sufficient volume of spray solution should be used to cover the entire field so as to reach the target site/pest, and necessary care must be taken by the person, who is spraying to ensure safety. The pesticide application should not be made in strong wind, during rain or when rainfall is expected shortly after application.

The trials should be conducted with three treatments, *viz*. control, proposed dose, *c*GAP dose (25% higher than proposed dose) and each treatment should be replicated as per the statistical requirement.

The numbers of pesticide sprays are dependent on the target pest and crop, and also based on the frequency of the pest occurrence and are derived from the bio-efficacy data. Maximum number of sprays and minimum intervals between each spray should be given, when pesticide is proposed for re-use in the event of re-occurrence of pest in the field situations, should reflect the reality and simulate the actual practices by the farmers.

For all pre-harvest applications, the application rate should be expressed in terms of amount of product and/or active ingredient per unit area, e.g., *g.a.i.* per hectare, and information of the amount of water to be used for dilution to achieve the desired concentration at the target in liters/hectare.

In case of seed treatment with formulations of pesticides, the proposed package of practices for a crop remains the same as those for foliar applied products except that the pesticide is treated with the seed prior to sowing. Application rates for seed treatments are normally expressed as the amount of active ingredient per unit of seed weight, i.e., *g a.i.*/ kg seed, and seeding rate, i.e., kg seed/hectare.

## SECTION 3

# 3. SAMPLING AND ANALYSIS

## 3.1 SAMPLING PRINCIPLES AND PROCEDURES

The best information about the residue behavior of the pesticide under study would be obtained by the analysis of the entire yield of a plot. Since this is not practicable, representative samples have to be taken. The samples are the representatives of the whole of experimental plot, and in residue studies, sampling plays very important role as the crop growth, foliage vary from plant to plant and place to place within the plot. The more the number of spots / plant samples in the experimental plot, the true and representative sample is obtained. However, practicability in collection, handling and analysis and also economics in drawing more number of samples has to be kept in mind in preparing the sampling plan. Hence, to obtain reliable results from few representative samples, selection of sampling points, methods, handling (packing, labeling, shipping and storage) of samples needs to be well defined without affecting the quality of results.

In selecting sampling points and the sampling methods, all factors that control the residue distribution over the entire experimental plot must be considered. The best approach for any given plot can only be determined by a sufficiently trained person who is capable of recognizing the importance and usefulness of the residue data sought, and who can interpret the results.

### 3.1.1 Sampling spots and methods:

Generally, the selection of the portions that make up the field sample should be made depending on the circumstances: (i) randomly, i.e.by the use of random numbers; (ii) systematically, i.e, in the case of field crops on a diagonal ("X" or an "S" course); and (iii) stratified random sampling from predetermined sampling-positions, e.g., in the case of tree fruits inner part and outer part of the canopy, i.e., fruits, directly exposed to spray and those covered by foliage, proportionally to the abundance of fruits in each strata; within one strata each fruit has an equal chance to be included in the sample.

While sampling, it is recommended to avoid taking samples at the beginning or at the extreme ends of plots, take the required weight or number of samples from the field.

Check (Control) samples from untreated experimental plots are very important, and quality of control samples should be similar to that of the test samples, e.g., maturity of fruit, type of foliage, etc. Sampling should proceed from the control to the lowest treatment and so on to the highest treatment.

In supervised field trials, the whole Raw Agricultural Commodity (RAC) should be sampled as it moves in commerce. For some crops, there may be more than one RAC. For example, the RACs for field corn include the grain (seed) and forage. One sample from each RAC should normally be taken from treated plots at each sampling interval. Some crops may be shipped without having been stripped, trimmed or washed; therefore these procedures should only be applicable for residue samples to the extent that these are commercial practices prior to shipment. Of course, the pesticide residue data on trimmed or washed samples may be generated optionally for use in refinement of risk assessments if needed.

### 3.1.2 Sample handling:

The personnel who collects the samples from plots should take care not to remove surface residues during extremely careful handling, packing or preparation and avoiding any damage or deterioration of the sample which might affect residue levels. Adhering soil may have to be removed from some crops, such as root crops to provide representative RAC, which can be done by brushing and, if necessary, gentle rinsing with cold running water.

It is vital to avoid any contamination with the pesticide under study or with other chemicals during sampling, transportation or subsequent operations. Samples should be collected in clean polythene bags of suitable size and adequate strength (preferably minimum of 40 microns) and ensure that the bag material shall not interfere with the analysis. Avoid contamination of the sample by hands and clothes, and do not allow contact of the samples with storage containers and vehicles. In case the sample has to be transported for analysis, it has to be packed in dry ice and subjected to analysis with minimum time lag and earliest opportunity. The transporter as well as the analyst should ensure the absolute maintenance of the cold chain between field and analysis.

### 3.1.3 Sample size:

Under normal circumstances, one sample per plot (one composite sample from many sub-samples collected from throughout plot as per para 3.1.1 above is sufficient. Additional samples may be taken and in case if sample is lost or destroyed during transportation. Sample integrity should be maintained throughout the procedure.

Detailed sampling procedure as per Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLs CAC/GL 33-1999.

### 3.1.4 Sampling intervals:

For all dissipation studies with foliar spray, the samples should be collected from zero day (2 hours after spray) onwards till the residues reach the limit of quantification (LoQ) or the cropat the harvest stage, whichever is earlier. The interval of sampling should be at day 1, 3, 5, 7, 10, 15, 20, 25, 30.In case residue is above LoQ on day 30,samplingshall be continued at 5 day interval, till the residues reach atLoQ or stage of harvest, whichever is earlier. (e.g., if the residue on day 3 is at LoQ the residue may not be estimated further).In the case of grain crops and those crops which are harvested only at maturity, samples for residue analysis are taken at the time of harvest. In case of fruits and vegetables which are harvested more than once, sampling is done at different intervals after the spray.

It is to be ensured that collection of samples, processing and analysis to be made concurrently for the respective samples. When multiple applications are involved, a sampling point immediately prior to the final application is desirable to determine the contribution of earlier applications and the effect on residual half-life. Sample preparation:

MRL need to be fixed both for RAC and the edible portion which are traded separately. The portion of the commodity to be analyzed is the portion of the RAC/ edible portion which is to be prepared as the analytical sample for the determination of pesticide residues.

The details on the portion of the commodity to be analyzed for pesticide residues are as per "Portion of Commodities to which Codex Maximum Residue Limits Apply and which is analyzed CAC/GL 41-1993, Page 1-9"

### 3.2 Residue Definition

The sample analysis for pesticide residues is an important stepand should be performed with highest accuracy and reliability. Prior to analysis, the concept of residue definition for RL should be understood clearly. The residue definition of the target parent pesticide compound, and all the compounds (parent and its metabolites) in the definition to be analysed following approved analytical protocols and methods. The next important step is to understand the principles and practices of method validation to evaluate the suitability of methods for analysis of all the compounds in the definition at the desired level (usually lowest possible level) for regulation. It is recommended to understand the concept of residue definition for the pesticide under test, and perform the method validation including recovery and estimation of LOQ prior to planning the supervised field trials, to ensure that the suitable method is on hand for ready analysis of field samples. After choosing the most suitable method through method validation, the field samples are analyzed. The residue data are presented in the prescribed format for further use in risk analysis and fixing MRLs and PHIs, as required.

By definition, the term "Pesticide residue" pertains to the concentrations of the specified pesticide compound / compounds in the food, such as raw agricultural commodities, meat or animal feed, processed food etc. resulting from the application the given pesticide compound or a number of them in accordance with approved package of practices to protect crops from pests as well as agricultural commodities in storage. For all practical and legal purposes, the term includes the parent compound, its metabolites, derivatives, reaction products and impurities which are considered to be of toxicological significance to humans and non-target organisms. In order to arrive at the legal "residue" value of a pesticide, one has to study the metabolism, metabolic products and their individual toxicities, in animals, plant and soil.The information/data on the metabolite spectrum of the pesticide compound becomes a prerequisite for establishing the residue definition. Information on metabolism of most registered (and used) pesticides are available in the published literature.In the case of new pesticide compounds, fresh research studies should be carried out for establishing metabolic pathways and the toxicities of all known metabolites. Although there are no clear guidelines to select the metabolites to consider as toxic, generally a limit of acute oral toxicity up to 5000 mg/kg for any metabolite is considered toxic and this shall be taken into account for deciding the residue analytical method. Legally the term "residue" includes the sum of concentration of the parent pesticide compound and its toxic metabolites. During analysis of the raw agriculture commodities and stored commodities for pesticide residues, either the parent compound and the identified metabolites can be assayed separately, or one can use an analytical procedure by which the sum of the parent pesticide compound and its toxic metabolites is estimated together and expressed as parent compound.

The following are the case studies for understanding the concept of residue definition and expression is presented hereunder:

#### 3.2.1 Expression of the residue in terms of the parent compound:

It is preferable to express the quantity of pesticide residue in terms of the parent compound. Even if the residue consists mainly of a metabolite, the residue shall be expressed in terms of the parent pesticide after molecular weight adjustment. If the parent compound can exist as an acid or its salts or a base or its salts, the residue is preferably expressed as the free acid (e.g., RCOOH) or free base

(e.g., $RNH_2$). For example, sum of the herbicide 2,4 D, its salts and esters expressed as 2,4 D, and sum of methiocarb, its sulphoxide and its sulphone, expressed as methiocarb.

**3.2.2 Expression of the residue in terms of the parent compound without weight adjustment:**

No allowance is made for molecular weights in the definitions of pesticide residues of some older compounds. Because such definitions are widely accepted, any change in the existing norm should be carefully considered. The best time for the reconsideration of an existing residue definition is during a periodic review/re-evaluation. For example, the DDT is expressed as sum of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and *p,p'*-TDE (DDD) ;sum of heptachlor and heptachlor epoxide is expressed as heptachlor and sum of imidacloprid and its metabolites containing the 6-chloropyridinyl moiety, expressed as imidacloprid.

**3.2.3 Quantitative conversion from parent into another chemical entity:**

If the parent pesticide compound is quantitatively converted to another chemical entity during adoption of the analytical method, the residue is preferably expressed as the parent. For example, residue definition of aluminum phosphide is expressed as phosphine (hydrogen phosphide, IUPAC: phosphane).

**3.2.4 Conversion of metabolites and parent compound into a single compound in the analytical method:**

If metabolites are known to be present in significant amounts but the analytical method measures the total residue as a single compound, the residue is expressed as the parent compound. The metabolites included in the residue should be listed, if feasible. For example, for the quantification of fenthion residues, parent compound, its oxygen analogue and their sulphoxides and sulphones are all oxidized to a single compound (fenthion oxygen analogue sulphone). Hence, residue definition of fenthion is sum of fenthion, its oxygen analogue and their sulphoxides and sulphones, expressed as fenthion.

**3.2.5 Lack of specific methods for the residue definition for enforcement purpose:**

Ideally it should be possible to measure the residue as defined, with a LOQ adequate for proposed MRLs, with a high degree of specificity by a multi-residue analytical method. Although circumstances may warrant exceptions, the definition of a residue should not normally depend on a particular method of analysis. However, in the case of dithiocarbamate it is necessary to describe the residue as ".... determined and expressed as ..." to produce a practical definition for residues. For example, all the dithiocarbamates fungicides (such as mancozeb, thiram, maneb, zineb, ferbam, ziram, metiram, propineb) are subjected to acid treatment to evolve carbon disulphide ($CS_2$), which is used for expression of residues in / on the food. In such cases, one is not sure that which the particular pesticide present on the commodity, is responsible for $CS_2$ evolved. Therefore, for example, residue definition of mancozeb for compliance with MRLs can only be expressed as total dithiocarbamates, determined as $CS_2$ and expressed as mg $CS_2$/kg.

**3.3 Limit of Quantification / determination**

Limit of Quantification (LoQ) is the smallest concentration of the analyte that can be quantified. It is commonly defined as the minimum concentration of analyte in the specific test sample that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test.

*Explanatory note:* 'Limit of quantification' and 'limit of quantitation' are used synonymously and are abbreviated to LoQ. The estimation of the LoQ of an analytical method for residues in specified substrates being the lowest level where satisfactory recoveries are achieved. Previously LoD (limit of determination) was being used with the same meaning as LoQ.

The analytical method should be adequately sensitive to provide sufficient reliability in accuracy (70-120%) and precision [Relative Standard Deviation (RSD) less than 20%] of results at or below the MRL. In general, an analytical method is expected to have an LOQ of 0.01 mg/kg or lower. If it is difficult to achieve this LOQ of 0.01 mg/kg for any specific pesticide-commodity combination, the LOQ should be optimized at a level as close as 0.01 mg/kg which should be as low as reasonably achievable (ALARA).

### 3.4 Analytical Methods

#### 3.4.1 Requirements of analytical methods

As part of the evaluation process it is necessary to assess the validity of the analytical methods used in the residue analysis.

Each method is examined, based on its validation data and performance characteristics (including efficiency of extraction), for its overall suitability for the purpose intended, the compounds determined by the method and the substrates that may be analysed. Particularly important are the data for analytical recoveries. Method validation is needed on matrices representative of those in the trials and studies. The LOQ for the method is the lowest residue concentration where reliable recoveries (usually 70–120%) and relative standard deviation of replicate analyses (usually $\leq$ 20%) are achieved. The limit of detection provides an indication of presence of low level resides in various matrices, but as they do not provide quantitative data, they are not taken into account in estimation of residue levels. It is however, recognized that over time the LOQ may vary or change compared to the value estimated during method validation.

Analytical methods are used to generate the data for estimating dietary exposure, to establish Maximum Residue Limits (MRLs), and to determine processing factors. Analytical methods are also used in enforcement of any MRLs that may be established. It is important to note that the methods should be able to determine all analytes included in the residue definition for the particular pesticide. The major residue components should be determined individually as far as technically possible. The use of non-specific methods is generally discouraged. For some analytes, specific residue analytical methods might be unavailable or difficult to perform. In these cases, conversion to a common moiety is valid when all components containing that moiety are considered toxicologically important and when no single component is an adequate marker of residue concentration. Under these circumstances, a "common moiety method" may be used.

For enforcement methods surveillance laboratories prefer multi-residue methods, which could include a large number of analytes, as the laboratories generally do not have sufficient capacity to apply individual methods for all compounds present. This fact is clearly demonstrated by the published results of national monitoring studies which indicate that compounds recoverable with multi-residue procedures are much more frequently analysed than those requiring individual methods. When the analyte is not amenable to the multi-residue method techniques, a single residue method may be provided.

In practice, data may have to be generated in such a way as to provide the flexibility to establish two separate residue definitions where appropriate, one for dietary risk assessment and a second for MRL compliance monitoring. In such cases, where possible, one should either separately analyse for the individual components of the expected residue definition, rather than carrying out a common moiety method; or first carry out analyses according to a common moiety approach and a second series of analyses of the field trial samples for a suitable indicator molecule in parallel, if the common moiety methodology is unsuitable for practical routine monitoring and enforcement of the MRL at reasonable cost. The availability of appropriate methods for monitoring purposes should be considered.

The method(s) should:

- have the ability to determine all of the likely analytes that may be included in the residue definition (both for dietary risk assessment and enforcement) in the presence of the sample matrix;
- distinguish between individual isomers/analogues when necessary for the conduct of dietary risk assessments;
- be sufficiently selective so that interfering substances never exceed 30% of the limit of analytical quantification (LOQ);
- demonstrate acceptable recovery and repeatability;
- cover all crops, including those used as feed, animal tissues, milk and eggs as appropriate, and by-products used as feed;
- cover all edible animal commodities if animals are likely to consume treated crops;
- include processing fractions if detectable residues occur.

In general, residue analytical methods applied in various studies should be validated for all matrices to demonstrate that they fit for the purpose. The extent of validation depends on the information already available and reported. Full validation data should be provided only for new methods or when existing methods are significantly changed (e.g. change of solvent systems or quantitation techniques). Such changes may be required when adapting methods to different commodities.

In the case of studies involving plant material, the number of commodities to be tested is dependent on the use of the product. Validation data should be submitted for all sample matrices to be analyzed and should be carried out for all components of the expected residue definition for enforcement and dietary risk assessment. Full validation experiments should be performed predominantly on one raw agricultural commodity (RAC) from each of the representative commodity categories.

If animals are likely to consume treated crops and if feeding studies are required / submitted, methods for determination of residues in products of animal origin should be validated in the following matrices: milk, eggs, and all edible tissues. The tissues normally include cattle muscle, fat, liver, and kidney as well as poultry muscle, fat, and liver. In most cases, the recovery data for cattle commodities are valid for products of goats, hogs, horses, sheep, and poultry.

Details of method validation procedures, including testing the efficiency of extraction and confirmation, the criteria for acceptable performance parameters and format for reporting the method are given in several internationally accepted guidance documents.

The minimum requirements of the full validation scheme are:

- six recovery experiments conducted on at least 2 levels (LOQ and 10× LOQ);

- analysis of two control samples;
- A minimum of 5-point calibration covering the analytical range of the method

When an existing fully validated method, is adopted for other "comparable" commodities within a category usually reduced or limited validation sets are sufficient.

During the analysis of the samples the performance of the methods should be verified with appropriate quality control tests.

The minimum general performance criteria of the acceptable methods are:

- the concentration- response relationship should be linear in the calibrated range (both pure solvents and/or matrix-matched calibration);
- the analyte concentration does not change during whole analysis procedure in the extracts and calibration solutions;
- the average recovery is within the limits of 70 to 110 percent.

Analytical methods provided should include:

- specialised methods used in the supervised trials and environmental fate studies which were submitted for evaluation, and
- enforcement methods.

The methods should be summarized including a clear outline of the compounds determined and the commodities for which the method is recommended. In addition, the specificity, repeatability of the method, the limit of quantification and the range of residue levels for which the method has been validated, the mean recovery and the relative standard deviation of recoveries at each fortification level, including the limit of quantification, etc. should be given.

### 3.4.2 Method Validation and Residue Analysis:

The definition of validation is “Confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled". Methods for pesticide residue analysis generally comprise of series of steps from sampling to the instrument-based analysis for inferencing the quantity in the given RAC. The residue analysis involves important steps such as extraction, cleanup and analysis by using instruments. At every step, there is possibility of losing the target pesticide compound, and hence under the existing standard sample preparation and analytical conditions, the arising errors / uncertainties need to be measured. These parameters and the corrections thereon shall be used during sample data interpretation. This is intended for evaluation, verification and suitability of the method following day-to-day activities within the rigidity of the prescribed method. This shall be a continuous process of the laboratory, since any deviations / changes in the processes and instrument parameters lead to deviations in the uncertainties. For example, a minor alteration of the existing method, introduction of the new method, changes in instrument consumables which can affect the sensitivity etc. can adversely affect the residue analysis.

## SECTION 4

# 4. METABOLISM

## 4.1 REQUIREMENTS:

The physical and chemical properties of the active ingredient, the metabolism and degradation of the compound in animals, plants, soil and water are studied to determine the composition and distribution of residues. The fate of residues in the environment is evaluated to assess the possibility of uptake of residue by the crop, e.g., from a soil treatment from multiple applications in successive years, by following crops, and the contamination of the environment by persistent residues likely to lead to residues in food or feed commodities. Based on this information and taking into account the available analytical methodology as well as the toxicological significance of metabolites and degradation products, the definitions of residues for enforcement purposes and for dietary intake calculations, is recommended.

The analytical methods with accompanying chromatograms and information on stability of residues during sample storage are evaluated to assess the reliability of trial data and to estimate Limits of Quantification of residues which can be realistically achieved in regulatory laboratories.

It is emphasized that residues derived from supervised field trials can only be used for estimating maximum residue levels if the trial conditions can be matched with relevant national GAPs supported by approved labels. The estimated maximum residue level is based on already approved maximum national uses (critical or maximum GAP) which normally lead to the highest residue concentration in the portion of commodities to which MRLs apply.

The estimated maximum residue levels for residues in commodities of animal origin are mainly based on the results of farm animal feeding studies and residues occurring in feed items and, to a lesser extent, the information obtained from animal metabolism studies. MRLs for animal commodities may also relate to the residues arising from direct animal treatments.

The fates of residues during processing and cooking, as well as residues in the edible portion are taken into consideration in the estimation of dietary intake.

The results of national monitoring programmes provide useful information, on residues occurring under practical use conditions, which are used for the estimation of extraneous residue levels (EMRLs) and as a special case MRLs in spices.

### 4.1.1 BASIC PROPERTIES OF THE COMPOUND:

#### 4.1.1.1 Identity and physical chemical properties

ISO common name
Chemical name
(IUPAC)
(Chemical Abstract)
CAS Registry. No.
CIPAC No.
Synonyms
Structural formula
Molecular formula
Molecular weight

#### 4.1.1.2Physical and chemical properties

Provide a detailed physical and chemical characterization for new and periodic review compounds as guidance for the interpretation of available test data.

#### 4.1.1.3 Pure active ingredient

Appearance
Vapour pressure (in mPa at stated temperature)
Octanol-water partition coefficient (at stated pH and temperature)
Solubility (Water and organic solvents at stated temperatures)
Specific gravity (... g/cm3 at ...stated temperature)
Hydrolysis in sterile water in the dark (at stated pH and temperature)
Photolysis in sterile water
Dissociation constant
Thermal stability

#### 4.1.1.4 Technical material

Minimum purity (in %)
Melting range
Stability
Reference to FAO specifications for TC or TK (TC, technical material; TK, technical
concentrate).

#### 4.1.1.5 Formulations

Provide a list of commercially available formulations.
Reference to FAO specifications for formulations

Data submitted on physical and chemical properties of pure active ingredient are evaluated in order to recognize the influence of these properties on the behaviour of the pesticide during and after its application on crops or animals. Data on physical and chemical properties are also needed for an understanding of analytical methods.

The volatility of the compound and its stability in water and after radiation from ultraviolet light may considerably affect the fate and behaviour of residues on treated crops after application.

The solubility of the pesticide is of particular interest, as the ability of the compound to penetrate plant and animal tissues is dependent on its solubility in water and organic materials, as is its behaviour during processing.

### 4.2 Metabolism and environmental fate

Chemical degradation and metabolism are major mechanisms of disappearance of pesticides after application to plants, animals or soil. The rates of degradation and metabolism are dependent on the chemistry of the compounds and factors such as temperature, humidity, light, surface of the crops, pH of crop liquid and composition of soils. Metabolism studies provide fundamental information on the fate of the compound, provide a qualitative or semi quantitative picture of the composition of the residues, suggest probable residue behavior and indicate the distribution of residues within various tissues. The site and level of residues may also depend on whether the compound is absorbed by the leaves or roots of crops, whether it is mobile in the plant, and its persistence and

mobility in soil. In addition to the chemical characteristics of the pesticide, the metabolism in animals depends on the species and the conditions of the dosing.

The research Data on pesticide metabolism are used in evaluating both the toxicological and residue profiles of pesticides. The metabolism in experimental animals is examined and compared it with that in farm animals and in crop plant species on which the pesticide is used. This is required to decide upon the relevance of the toxicological studies to humans, and to define the residues in plants and farm animal products. If there are plant or farm animal metabolites which have not been identified as mammalian metabolites in experimental animals, the toxicological end points of those metabolites needs to be explored. Separate dosing studies with these metabolites may be necessary for assessment of their toxicological properties if significant residues occur in food items.

The information on the composition of the terminal residue obtained from metabolism studies is used to assess the suitability of the pesticide residue analytical methods for the development of pesticide residue data from supervised trials and to decide on the definition of residues.

Information about the given pesticide is required on:

- Plant metabolism
- Rotational crop studies
- Animal metabolism
- Environmental fate in soil, and water-sediment systems

These studies provide data and inference on the approximate level of total residues, identify the major components of the total terminal residue, indicate the route of distribution of residues and its mobility (uptake from soil, absorption by plants or surface residue, excretion in animals, soil degradation) and show the efficiency of extraction procedures for various metabolite components of the pesticide found in the residue.

In addition, data derived from *in vitro* methods are useful to show if the pesticide is likely to undergo hydrolysis (acid, base, or enzymatic), oxidation or reduction, photolysis, or other chemical changes; e.g. during processing of RACs.

Metabolism studies are conducted to determine the qualitative metabolic fate of the active ingredient (A.i) and elucidate its metabolic pathway. Many pesticides undergo chemical change during and after application to crop plants, that falling on soil, that which move into the water and livestock. The composition of the terminal residue must, therefore, be determined before the laboratory residue analytical methodology is finalized and pesticide residues quantified.

Radio-labelled active pesticide ingredients are required to undertake quantification of the total, extractable and unextracted radiolabel residues. The active ingredient (A.i.) should be labelled so that the degradation pathway can be traced as far as possible. The radiolabel should be positioned in the molecule so that all significant moieties or degradation products can be tracked. If multiple ring chemical structures or significant side chains are present, separate studies reflecting labelling of each ring or side chain will normally be required if it is anticipated that cleavage between these moieties may occur. A scientifically based rationale may be required in lieu of conducting studies with multiple radiolabels if no cleavage is anticipated.

In choosing the position to be labelled in the pesticide molecule, assurance is needed that a stable position is selected. The preferred isotope is $^{14}C$, although $^{32}P$, $^{35}S$, or other radioisotopes may be more appropriate if no carbon or only labile carbon side chains exist in the molecule. The use of tritium ($^{3}H$) as a label is strongly discouraged due to the possibility of hydrogen exchange with

endogenous materials. If a potentially labile side chain or tritium labelling is chosen, a metabolism study be associated with the chemicalA.i, and not related to loss of the label from the basic structure of the A.i.molecule.

The specific activity of the radio-labelled active ingredient should be adequate to meet the general data requirements of the metabolism study (quantification of 0.01 mg/kg total radioactive residue in edible tissues, milk, eggs or crop matrices). Studies with targeted (1X) application rates are generally necessary to assess whether threshold levels are exceeded or not. However, dosing with an exaggerated rate, e.g., 5X, is recommended when it is anticipated that residue levels from 1X treatment will be too low to define the metabolic pathways.

The desired goal of a metabolism study is the identification and characterization of at least 90% of the total radioactive residue in the pesticide applied edible tissues of food and / or forage crops, milkand eggsas the case may be. In many cases it may not be possible to identify significant portions of the total radioactive residue especially when low total amounts of residue are present, when incorporated into biomolecules, or when the active ingredient is extensively metabolised to numerous low level break-down chemical components. In the latter case it is important to demonstrate clearly the presence and levels of such components, and if possible, attempt to characterize them. Studies should utilize state-of-the-art techniques and include citations of such techniques when used.

During the conduct of the pesticide metabolism research studies, it may be helpful to retain radio-labelled samples for future analyses by the subsequently developed analytical methods (for enforcement, data collection or dietary risk assessment) in order to assess the extraction efficiency of these methods (sometimes referred to as "radio validation" of methods). Samples retained should include representative portions of crops, muscle, liver , milk and eggs. If specific metabolites accumulate in specific organs, samples of these organs should also be retained. However, if the analytical methods mirror those used in the radio labelled studies, such data would generally not be necessary. The radio validation of the extraction process of analytical methods should be as apart of the research study report on the analytical method, or it may stand by itself as a report, or given in the metabolism report itself.

The information provided for evaluation should include documentation on the proposed metabolic pathway, including a table with associated chemical structures and names (CAS and IUPAC, as available), the quantities of the metabolites in the different parts of the plants (surface, leaves, stems and edible root), in different animal tissues (fat, muscles, kidneys, liver, eggs and milk) and in different soil types. Any postulated intermediates/metabolites should also be indicated in the pathway. The rate of the formation and disappearance of metabolites in plants, animals and soil must also be investigated.

The capability of the analytical methods utilized in the metabolism study to determine the components of the residue, whether free, conjugated, or unextracted, should be clearly specified. In case of metabolism studies, the stability tests should show that the basic profile of radiolabelled residues has not changed throughout the duration of the study. If instability of the active ingredient is suspected or observed, based on other information, steps should be taken to safeguard the integrity of the study. In those cases, where a metabolism study cannot be completed within six months of sample collection, evidence should be provided that the identity of pesticide residues did not change during the period between sample collection and final analysis. This can be done by

analyses of representative substrates early in the study and at its completion. The substrate should be the item-wise stored securely, i.e., if the matrix extract is used throughout the study and the matrix is not extracted later in the study, the stability of the extract should be determined shown.
If changes are observed, e.g., disappearance of a particular HPLC peak or TLC spot, additional analyses or another metabolism study with a shorter collection interval may be necessary.
The metabolism studies on farm animals and crops should provide the basic evidence to support proposed pesticide residue definition(s) for food commodities, and provide evidence as to whether or not the pesticide and its metabolite residue could be classified as fat soluble.

### 4.3 Plant metabolism:

Plant metabolism research studies of pesticide chemistry should be designed in such a way as to represent the composition of the residues when the pesticide is applied on the crop under maximum GAP conditions. When low pesticide residue levels in crops are expected from the maximum dosage application rate, experiments at increased and exaggerated dosage rates may be needed to aid in metabolite identification. The crop should be treated with radiolabelled active ingredient, preferably containing formulation ingredients typical of an end-use product as applied in the field.
A metabolism study is necessary for each type of crop group for which the pesticide use is proposed. Crops can be considered to belong to one of five categories for crop metabolism studies:

- root crops (root and tuber vegetables, bulb vegetables)
- leafy crops (Brassica vegetables, leafy vegetables, stem vegetables, hops)
- fruits (citrus fruit, pome fruit, stone fruit small fruits, berries, grapes, banana, tree nuts, fruiting vegetables, persimmon)
- pulses and oilseeds (legume vegetables, pulses, oilseeds, peanuts, legume fodder crops, cacao beans, coffee beans)
- cereals (cereals, grass and forage crops).

Pesticide metabolism studies on one crop from a category will cover the entire group for purposes of metabolism in those crops within the group. In order to extrapolate metabolism of a pesticide to all crop groupings, metabolism studies on a minimum of three representative crops (from the five different crop categories) should be conducted. If the results of these three studies indicate a comparable metabolic route, then additional research studies will not be needed on crops in the other two categories.

The studies should reflect the intended use pattern of the pesticide A.i. such as foliar, soil/seed, or post-harvest treatments. If, for instance, three research studies have been conducted using foliar application and at a later date the authorisedpesticide uses also include soil application, e.g., seed treatment, granular, or soil drench, then an additional study reflecting soil application should be carried out.

On the other hand, if different metabolic routes are observed among the representative crops from studies conducted in a similar manner, e.g., foliar spray of pesticide with similar pre-harvest interval (PHI) and growth stages, further studies should be conducted for uses on crops in the remaining categories for which MRLs are being requested. Differences in the quantities of

metabolites belonging to the same pathway will not trigger the need for additional research studies.

There are situations where an *authorised use is unique*, in terms of the crop and/or its growing conditions, for which a metabolism study would be necessary, in addition to the three representative crops. For example, if a use exists on rice grain, a metabolism study data should be submitted for rice grain, regardless of other available metabolism studies.

Genetically modified (GM) and non-GM crops may metabolize the pesticide differently. Full and detailed information will be required for GM crop with metabolism differences from the non-GM crop. For genetically modified crops that do not involve the insertion of alien gene(s)to offer pest (insects / plant diseases) conveying resistance through gene expression metabolism, no additional pesticide metabolism studies are needed. However, the rationale for concluding that the gene does not alter pesticide metabolism should be studied in detail. When an alien gene is inserted that conveys active ingredient resistance due to pesticide metabolism, then a crop metabolism study should be conducted for each crop grouping to which the GM crops belong. If one such study shows a similar pesticide metabolism innon-GM crops, however, no additional studies would be needed. In case a different metabolic route is noticed for the pesticide, then two additional studies should be required including for different varieties of the same crop species.

**4.3.1: characterisation and identification of residues**

In crop metabolism studies, samples of all RACs should be obtained for characterization and/or identification of residues. In commodities with inedible peel such as oranges, melons, and bananas, the distribution of the residue between peel and pulp should be determined. For crops that are sometimes consumed at an immature stage, such as baby corn or leafy salads, samples should also be taken of such commodities for analysis. Where mature inedible crop parts, e.g., apple leaves, potato foliage, are used to help identify residues, the edible parts must also be sampled and analysed to demonstrate the similarity of metabolic profiles. If more than one use pattern is involved, extra samples need to be taken to reflect, for example, the different PHIs.

Metabolism and residue studies conducted in rotational crops (sometimes referred to as follow-up, following or succeeding crops) are typically required for uses of pesticides where it is reasonable to expect that a food or livestock feed crop may be planted as the succeeding crop after the harvest of a pesticide treated crop (or in some cases replanting of crops after failure of the pesticide treated crop).Requirement of metabolism studies are indicated in all soil applied pesticides and herbicides.

*Metabolism in rotational crops* studies are conducted to determine the nature and amount of pesticide residue uptake in rotational crops that are used as human food or as livestock feed. Such studies are generally not required for uses of pesticides on permanent or semi-permanent crops including, banana, berries, citrus fruits, coconut, grapes, guava, mango, mushrooms, papaya, top fruits, pineapple, plantain etc. However, in most field conditions crop rotations with cereals and pulses; cereals with cotton; different vegetables etc are common in India and the metabolism in such rotated crops is always desired.

**4.3.2: identification of terminal residue components**

- Identification of the major components of the terminal residue in the various RACs, thus indicating the components to be analysed for in residue quantification studies, i.e., the residue definition(s) for both risk assessment and enforcement.
- Elucidation the degradation pathway of the active ingredient in rotated crops.
- Provide information on rotational crop restrictions based on residue uptake levels.
- Other consideration for rotational crop studies should include soil type, dosages used and the sequence of crops in the rotation (which should be the normal practice in the region )

The study may also be performed either in a greenhouse or in an outdoor plot or container or a combination of the two, e.g., rotated crops can be grown under greenhouse conditions in soils that were treated and aged under outdoor or field conditions.

The residues in rotational crops are usually composed of various metabolites in low concentrations and the compounds included in the residue definition are generally below the LOQ and do not require any further action. Rotational crop studies are normally not required for pesticide uses in permanent crops, e.g., various tree and vine crops, or semi-permanent crops, such as asparagus, where rotations are not part of the normal agricultural practices.

In cases where the Total Radioactive Residues (TRRs) exceed the trigger value (0.01 mg/kg) in a RAC from crops in the confined rotational crop metabolism studies, then the nature of the residues in those test crops having a TRR greater than 0.01 mg/kg will normally need to be determined.

### 4.4 Farm Animal Metabolism:

These studies are required whenever a pesticide is applied directly to livestock, to animal premises or housing, or where significant residues remain in crops or commodities used in animal feed, in forage crops, or in any plant parts that could be used in animal feeds.

Separate animal feeding studies (farm animal feeding studies) are required for ruminants and poultry. Except in special cases, it is not necessary to carry out metabolism studies with pigs since information on metabolism in a monogastric animal is available from studies with rats. If metabolism in the rat is different from that in the cow, goat and chicken, pig metabolism studies may be necessary. Such differences may include (but are not limited to) the following:

- differences in the extent of the metabolism
- differences in the nature of the observed residue
- the appearance of metabolites with sub-structures, which are of known potential toxicological concern.

Usually the most important metabolism studies are those involving ruminants and poultry. Lactating goats or cows and in the case of poultry, chickens are the preferred animals.

For each set of experimental conditions for pesticides (dermal vs. oral application or for each radio-labelled position), the following number of animals should be as follows).A ruminant metabolism study can be carried out on a single animal. For poultry, the use of ten birds per experiments (or dose) is recommended. Additional animals may be included if it is scientifically required. It is not necessary to include control animals in livestock metabolism studies. The minimum dosage used in livestock oral metabolism studies should approximate the level of exposure expected from the feeding of treated crops with the highest observed residues. However, for oral studies, livestock should be dosed at least at a level of 10 mg/kg

in the diet. In the case of dermal application the minimum dose should be the maximum concentration from the label. Exaggerated dosages are usually needed to obtain sufficient residue in the tissues for characterization and/or identification. Ruminants and swine should be dosed daily for at least five days, and poultry for at least seven days.

If the metabolism study is intended to be used in place of a separate livestock feeding study with unlabelled compound, inclusion of a second animal (or group of birds in the case of poultry) treated with a realistic dose and extended dosing period is strongly recommended, if it is suspected that a plateau is not likely to be reached. Such a study may allow the authorities to propose maximum residue levels for animal tissues in the absence of livestock feeding studies. Use of a metabolism study in place of a feeding study would require fully adequate scientific reasoning, especially if a plateau has not been reached in milk or eggs in the metabolism study.

All estimates of relative dose used in animal metabolism studies should be based on a feed dry weight basis. It should be noted that the use of percent crop treated information and median residue values are not acceptable to determine the dose level in these experiments.

In livestock metabolism studies excreta, milk and eggs should be collected twice daily (if applicable). Tissues to be collected should include at least muscle (loin and flank muscles in ruminant and leg and breast muscle in poultry), liver (whole organ for the goat and poultry and representative parts of the different lobes of the liver if cattle or swine are used), kidney (ruminants only), and fat (renal, omental and subcutaneous). The TRR should be quantified for all tissues, excreta, milk, and eggs. For milk the fat fraction should be separated from the aqueous portion by physical means and the TRR in each fraction quantified.

### 4.5 Environmental fate in soil, water and water-sediment systems:

Research study data on environmental fate of pesticide chemistry are needed for evaluation of environmental fate relevant to the potential for uptake of residues by food and feed crops from those agro-ecologies where the pesticide is expected to be used.

These studies are normally required for all pesticides except those pesticide with a legally specific use only for seed treatment crops and post-harvest application in storage. The availability of relevant studies is essential for the assessment of the potential for residues in food and feeds.

## SECTION 5

## 5. RISK ASSESSMENT

### 5.1 Calculation and Fixation of Maximum Residue Limit

For pesticides approved in agriculture, the MRL is derived from supervised field trials conducted by adopting GAP.

The field residue data for all agricultural pesticides shall be usedin order to calculate and fix MRL. However, monitoring data shall be used for certain specific spices wherever GAP does not exist.

The MRL is derived from statistical analysis of the residue data from the field trial. For this purpose, OECD calculator, a well devised statistically designed programme is being used. The field residue data are processed using the OECD) calculator with which following information are derived:

(1) Values for supervised trial median residues (STMR)
(2) Highest Residue (HR)
(3) Mean Residue
(4) Maximum Residue Limit (MRL) unrounded
(5) Maximum Residue Limit (MRL) rounded

This calculation gives MRL based on Mean + 4 times SD

The statistical analysis to derive MRL residue data vary based on evaluation of existing methods. MRL calculator is recommended for the calculation of MRL from residue data. The data is subjected to OECD MRL calculator, to get HR, MRL and STMR.

For the analysis of data from supervised field trials, a statistical calculator has been developed by OECD for determination of MRLs from valid field residue data. The calculation process is based on "mean + 4 SD" methodology. The OECD MRL Calculator is statistically based, scientifically defensible and internationally harmonized. For each given data set, the calculator will calculate MRLs through multiple approaches (EU I, EU II, 95/99, Mean+3SD) and all the values will be listed in the output table. The OECD MRL calculator affords the best approach for the calculation, depending on the sample size and the distribution of the residue data. **(Refer to OECD MRL Calculator user guide and OECD MRL Calculator-Statistical White Paper)**

The OECD MRL calculator is an MS Excel spread sheet for calculation of MRL using single dataset and multiple datasets. In single dataset spreadsheet, if the data sets are smaller (4-6), estimate of MRL is of high uncertainty and if the data sets are larger (10-15), it becomes more reliable.

#### 5.1.1 Case Study: 1

**Calculation of MRL for Cypermethrin on Cauliflower with data sets using OECD MRL calculator**

The ICAR-All India Network Project (AINP) on Pesticide Residues conducted multi-location supervised field trials on cauliflower to study the persistence and dissipation of cypermethrin applied as per GAP. The samples were collected and analyzed at regular intervals starting from 0 day for deposits (2 hours after final application) till the residues are below the LOQ.

The residue data collected at 3 days after application from 2X (double the recommended) (at least 40% data should be from *c*GAP) and X dose (recommended) is taken into consideration for calculation of MRLs using OECD MRL calculator.

| | |
|---|---|
| Pesticide | : Cypermethrin |
| Crop | : Cauliflower |
| Number of locations | : 10 |
| Recommended dose | : 50 grams active ingredient / hectare (GAP) |
| 2X dose | : 100 grams active ingredient / hectare (*c*GAP) |
| Number of sprays | : 2 |
| First spray | : Curd formation stage |
| Second spray | : 10 days after first spray |

**Case Study: 1: The abstract of the data collected from multi-location supervised field trials is given below:**

| Sampling Interval Days after application | Residues in mg/kg | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PAU, Ludhiana | CCS, HAU, Hisar | Dr. YSPUHF, Solan | | IARI, New Delhi | | MPKV, Rahuri | AAU, Anand | IIHR, Bangalore | | KAU, Vellayani | CSAUAT, Kanpur | | PJTSAU, Hyderabad |
| | | | X | 2X | X | 2X | | | X | 2X | | X | 2X | |
| 0 | 0.40 | 0.40 | 0.53 | 0.78 | 0.12 | 0.24 | 0.34 | 0.34 | 0.60 | 1.50 | 0.66 | 0.63 | 0.94 | 0.38 |
| 1 | 0.26 | 0.34 | 0.36 | 0.53 | 0.08 | 0.20 | 0.25 | 0.19 | 0.53 | 1.33 | 0.43 | 0.42 | 0.60 | 0.31 |
| **3** | **0.17** | **0.20** | **0.13** | **0.20** | **0.05** | **0.09** | **0.16** | **0.16** | **0.44** | **0.99** | **0.20** | **0.31** | **0.47** | **0.24** |
| 5 | 0.06 | 0.15 | 0.08 | 0.09 | BDL | 0.05 | 0.09 | BDL | 0.34 | 0.88 | 0.15 | 0.20 | 0.32 | 0.19 |
| 7 | BDL | 0.09 | BDL | 0.07 | BDL | BDL | 0.06 | BDL | 0.29 | 0.64 | 0.09 | 0.09 | 0.19 | 0.10 |
| 10 | BDL | 0.05 | - | BDL | BDL | BDL | BDL | BDL | 0.15 | 0.46 | 0.07 | 0.03 | 0.10 | BDL |
| 15 | BDL | BDL | - | | BDL | BDL | BDL | BDL | 0.11 | 0.30 | BDL | BDL | 0.05 | |
| 20 | - | BDL | - | | - | | - | - | 0.05 | 0.21 | - | - | | - |
| 25 | - | - | - | | - | | - | - | BDL | 0.09 | - | - | | - |
| 30 | - | - | - | | - | | - | - | BDL | BDL | - | - | | - |

The residue values presented in each column are mean values of three replications.BDL = Below determination level (<0.05 mg/kg)

The residue data sets (mean values of three replications) at 3 days after last application from various locations is fed into OECD MRL calculator and the rounded MRL obtained is taken into consideration for further risk assessment to finalize the MRL based on risk assessment studies.
Note: Replication values for each data set also can be used for calculations of MRLs, by which more accurate proposed MRL can be calculated using OECD MRL calculator.

**The following is the output using the above input data.**

| CYPERMETHRIN | |
|---|---|
| CAULIFLOWER (3 days) | |
| INDIA | |
| GAP | |
| Total number of data (n) | 14 |
| Percentage of censored data | 0% |
| Number of non-censored data | 14 |
| Lowest residue | 0.050 |
| Highest residue | 0.990 |
| Median residue | 0.200 |
| Mean | 0.272 |
| Standard deviation (SD) | 0.238 |
| Correction factor for censoring (CF) | 1.000 |
| Proposed MRL estimate | |
| - Highest residue | 0.990 |
| - Mean + 4 SD | 1.226 |
| - CF x 3 Mean | 0.816 |
| Unrounded MRL | 1.226 |
| **Rounded MRL** | **1.5** |

In case of multiple harvest crops like vegetables and fruits, it is desirable to construct a residue dissipation curve based on the data to fix appropriate MRL.

### 5.2 Estimation of extraneous maximum residue levels:

Chemicals for which Extraneous maximum residue limits (EMRLs) are most likely to be needed are those which were widely used as agricultural pesticides, persistent in the environment for relatively long periods after its use has been discontinued and are expected to occur in foods or feeds at levels of sufficient concern to warrant monitoring.

Predictions of persistence in the environment (and the potential for uptake by food or feed crops) can often be based on a combination of data sources normally available for chemicals previously approved as pesticides. These may include information on their physical and chemical properties,

metabolism studies and on supervised field trials, data on environmental fate, rotational crop data, the known persistence of similar chemicals, and especially from monitoring data.

In estimating an EMRL a number of factors are taken into account. These include the amount of data, the relative importance of the commodity in international trade, the potential for trade difficulties or accounts thereof, the frequency of positive results, a knowledge of the propensity of a particular crop to take up residues, e.g., the uptake of DDT by carrots, historical monitoring data, e.g., previous study results and the level and frequency of residues in similar crops, especially those in the same crop group. In some cases, the estimate has turned out to be the highest level reported, especially if a relatively good database is available and the spread of results is reasonably narrow.

In the context of EMRLs, there is no need to consider extreme values to be outliers in a statistical sense, because high residue levels are usually not true statistical outliers but values on one tail of a large distribution. The challenge is to decide when it is reasonable to discard those values in order to reflect the expected gradual decline in the levels of chemicals that are typically subject to EMRL recommendations, while not creating unnecessary barriers to trade.

## SECTION 6

# 6. DIETARY EXPOSURE

## 6.1 Estimating dietary intake of pesticide residues (risk assessment)

Dietary exposure of pesticide residues to human beings is estimated based on the consumption of various food commodities (agricultural produces) including processed ones and their presence in the given food item.

The relevant data for consumption of food required for facilitating the dietary exposure risk assessment have been given at **Annexure - I.** The figure in column 3 is based on the higher median value amongst the rural and urban consumption whereas the figure in column 4 is based on the 95$^{th}$percentile value amongst the rural and urban consumption. The figure in the column 4 shall be used for the RA in long term dietary exposure whereas figure of column 5 shall be used for short term dietary exposure.

The values in Table at Annexure – I have been prepared based on surveys conducted by the National Institute of Nutrition, Hyderabad (2012).

The median value in column 3 is used for long term risk assessment by multiplying with STMR derived from OECD calculator whereas the figure in column 4 is used for short term risk assessment by multiplying with HR.

For example as per Case study No. 1 the median consumption of chillies is 3g/day and this value is used in calculation of long term effects. On the other hand 9g/day is the consumption value for evaluation of short term effects.

## SECTION 7

# 7. HEALTH BASED GUIDANCE VALUE (HBGV)

## 7.1 Hazard Identification

Hazard identification is the first and foremost component of Risk assessment. Hazards associated with the pesticide residues are derived from a set of toxicological studies (*In vivo* and *In vitro*) as well as the human epidemiological studies undertaken with appropriate ethical considerations.

The list of toxicological studies need to be evaluated for identification of hazard is given in ENV/JM/MONO(2007)17.

The basic HBGV are Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD). All the toxicological data are thoroughly evaluated and the Critical End Point as well as Reference Point (RP) in terms of NOAEL and LOAEL (full form) are identified. The derivation of Bench Mark Dose (BMD) is ideal.

## 7.2 Derivation of Acceptable Daily Intake (ADI)

Based on the Critical End Point the appropriate NOAELs are identified for use to derive ADI and ARfD. In normal practice identified NOAEL is divided by factor of 100 as uncertainty/ safety factor for derivation of ADI or ARfD.However, the uncertainty factor can be variable based on the chemical structure of the compound, extent of data and clarity available on the subject as well as the kinetics in animal model.

ADI is expressed in a range (e.g. 0-0.1 mg/kg) whereas ARfD used as a number.

## SECTION 8

## RISK CHARACTERIZATION

To characterize the risk the exposure to pesticide residue through all types of commodities for which MRL are prescribed are compared with the Health based guidance value.

### 8.1 Derivation of Theoretical Maximum Daily Intake (TMDI)

After obtaining information on STMR values, dietary exposure and HBGV, the Theoretical Maximum Daily Intake (TMDI) will be calculated. Theoretical Maximum Daily Intake (TMDI) is calculated to assess the long term dietary intake risk analysis, taking residue level equal to calculated MRL into consideration and per capita consumption of food. The dietary intake of any pesticide is calculated by multiplying the STMR in the crop/food by the amount of food consumed (per capita consumption). Dietary intake is calculated by using STMR for long term risk analysis and HR for short term risk assessment. Sum total of TMDI values for all foods will be compared with the ADI.

Theoretical Maximum Daily Intake (TMDI) is the product of STMR and the intake of the appropriate food commodity. This calculation assumes that the entire commodity consumed has been treated and contains pesticide residue level at the appropriate MRL.

TMDI is calculated using following formula:

$$TMDI = \sum STMR \times Fi$$

Where,

STMR = Supervised Trial Median Residue

Fi = Per capita food consumption (g/day/person)

### 8.2 Derivation of Acceptable Daily Intake (ADI) per person

This is obtained by multiplying the value of ADI by 50 kg which is taken as the body weight for the calculation of risk assessment under Indian context. The body weight has been derived from the study conducted by the NIN, ICMR across the age group 16 to 70 years.

### 8.3 Comparison of ∑TMDI and ADI per person

If the comparisons indicate that the use of pesticides under the conditions of supervised field trials would not give rise to intakes that would exceed ADI, the pesticide would be approved for use under those conditions. It means, if the TMDI is ≤ 80% ADI, there is least risk, and hence the pesticide on the crop is approved as per the prescribed use (GAP). Conventionally, maximum TMDI should not exceed 80 per cent, keeping in view, the rest 20 per cent may come from other sources like air, drinking water,

other unapproved uses of pesticide etc. The calculated MRL based on the supervised field trials is fixed as MRL, provided there are no changes in GAP. If any changes are made in GAP, the supervised field trials need to be conducted for generation of fresh residue data and calculation of MRL for risk analysis and fixing MRL thereon. If, however, the estimated intakes would exceed the ADI, (TMDI = or ≥ 100% ADI), the pesticide would not be approved for use under the conditions of supervised field trials. It means, if the TMDI = or ≥ 100% ADI, , and the GAP need to be suitably modified for or, some of the label claims need to be dropped from the approved list of use of that pesticides so that the TMDI comes below the 80% of ADI.

### 8.3.1 Case Study: 1

Cypermethrin is used / sprayed on cauliflower (multi-locations) as per the GAP and *c*GAP, and the pesticide MRLs are calculated taking into consideration of 3 days PHI (Pre-Harvest Intervals) using OECD MRL calculator.

- The calculated / rounded STMR is 1.5 mg/kg.
- The ADI of Cypermethrin is 0.05 mg/kg body weight/day
- Food factor for cauliflower (per capita food consumption in India) is 0.03 kg/day/person.

$$\text{TMDI} = \frac{\text{STMR x Fi}}{\text{MBW}}$$

Where,

TMDI = Theoretical Maximum Daily Intake (mg/kg body weight/day)
STMR= Supervised trial median residue (mg/kg)
Fi = Per capita food consumption (kg/day/person)
MBW = Mean Body Weight (kg)

$$\text{TMDI} = \frac{1.50 \text{ mg/kg x } 0.03 \text{ kg/person /day}}{50 \text{ kgs}}$$

$$= 0.0009 \text{mg/kg body weight/day}$$

$$\% \text{ ADI} = \frac{\text{TMDI}}{ADI} \times 100 = 1.8\%$$

In this case, the calculated MRL for Cypermethrin on cauliflower is 1.5 mg/kg, and based on the risk assessment, the TMDI is 1.8.% of ADI. Hence, the MRL of 1.5 mg/kg can be recommended for Cypermethrin on cauliflower with 3 days pre-harvest interval. This recommendation can be made for the pesticide approved only on this crop. However, there is a scope (98.2%) for fitting other commodities for fixing Cypermethrin MRLs on all other commodities.

### 8.3.2 Case Study: 2

If Cypermethrin is recommended first time on cauliflower, for which MRLs need to be calculated, and the same pesticide is already registered for use on other crops for which MRLs are available, and in such cases, the TMDI from this trial to be added to ∑TMDI, and % ADI is calculated. The use of Cypermethrin is registered on tomato, brinjal, cabbage, okra, wheat, rice, oil seeds and milk and milk products as per Insecticide Act, 1968, and MRLs are fixed on these crops by FSSAI, India. In this case, TMDI from Cypermethrin residues in cauliflower are added to the ∑TMDI, and the % ADI is 10.68%, and hence MRL of Cypermethrin on cauliflower can be recommended as 1.5 mg/kg with 3 days PHI.

**Case 2: Risk characterization of Cypermethrin residue in/on Cauliflower**

| Food Commodity | STMR calculated (rounded) based on Residue Data from Supervised Field Trials (mg/Kg) or MRLs fixed by FSSAI / CAC | Pre Harvest Interval (days) | Food Consumption (kg/person/day) | Mean Body Weight (kg) | TMDI (mg//day) (Col2xCol4/col5) | ΣTMDI (mg/person/day) | ADI (mg/kg bw/) | ADI (mg/kg/person) | % ADI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| **Cauliflower (New)** | **1.5** | **3** | **0.03** | **50** | **0.045** | **0.267** | **0-0.05** | **2.5** | **10.68** |
| Tomato (IA-CAC) | 0.2 | 3 | 0.026867 | 50 | 0.005 | | | | |
| Brinjal (IA-FSSAI) | 0.2 | 3 | 0.011933 | 50 | 0.002 | | | | |
| Cabbage (IA-FSSAI) | 2 | 7 | 0.009033 | 50 | 0.181 | | | | |
| Okra (IA-FSSAI) | 0.2 | 3 | 0.009367 | 50 | 0.002 | | | | |
| Wheat (IA-FSSAI) | 0.05 | 14 | 0.120167 | 50 | 0.006 | | | | |
| Rice (FSSAI) | 0.01 | - | 0.120167 | 50 | 0.001 | | | | |
| Oil Seeds (FSSAI) | 0.2 | 14 | 0.0284 | 50 | 0.006 | | | | |
| Milk, Milk Products (FSSAI) | 0.1 | - | 0.185 | 50 | 0.019 | | | | |
| | Total | | | | 0.267 | | | | 10.68 |

IA = Registered for use as per Insecticide Act, 1968

CAC = MRLs fixed by Codex Alimentarius Commission

FSSAI = MRLs fixed by Food Safety and Standards Authority of India.

### 8.3.3Case Study 3:

In continuation to the existing data given in Case Study 2, and the risk analysis performed, in some cases, on ad-hoc basis, the MRLs for cypermethrin on other commodities can also be based on the National Monitoring Data. For example, the Cypermethrin residues are detected in / on other commodities for various reasons, and in such cases, the monitoring data can be fed in to OECD MRL Calculator, and MRL calculated will be subjected to Dietary Risk Assessment for fixing MRLs provided the ∑TMDI is less than the ADI.

**Monitoring data for MRL:** The working group recommended for the utilization of national monitoring data for the fixation of MRL of spices.

### 8.4 Short term Exposures:

For short term exposure assessment, estimates of high intake of pesticide residue on a single day are based on the HR values from supervised trials. The short term intake is calculated for each food separately (Food quantity x HR x a variability factor in some cases) and compared with ARfD. When an estimate of short term exposure for a pesticide in a food commodity exceeds ARfD, it is necessary to alter the GAP.

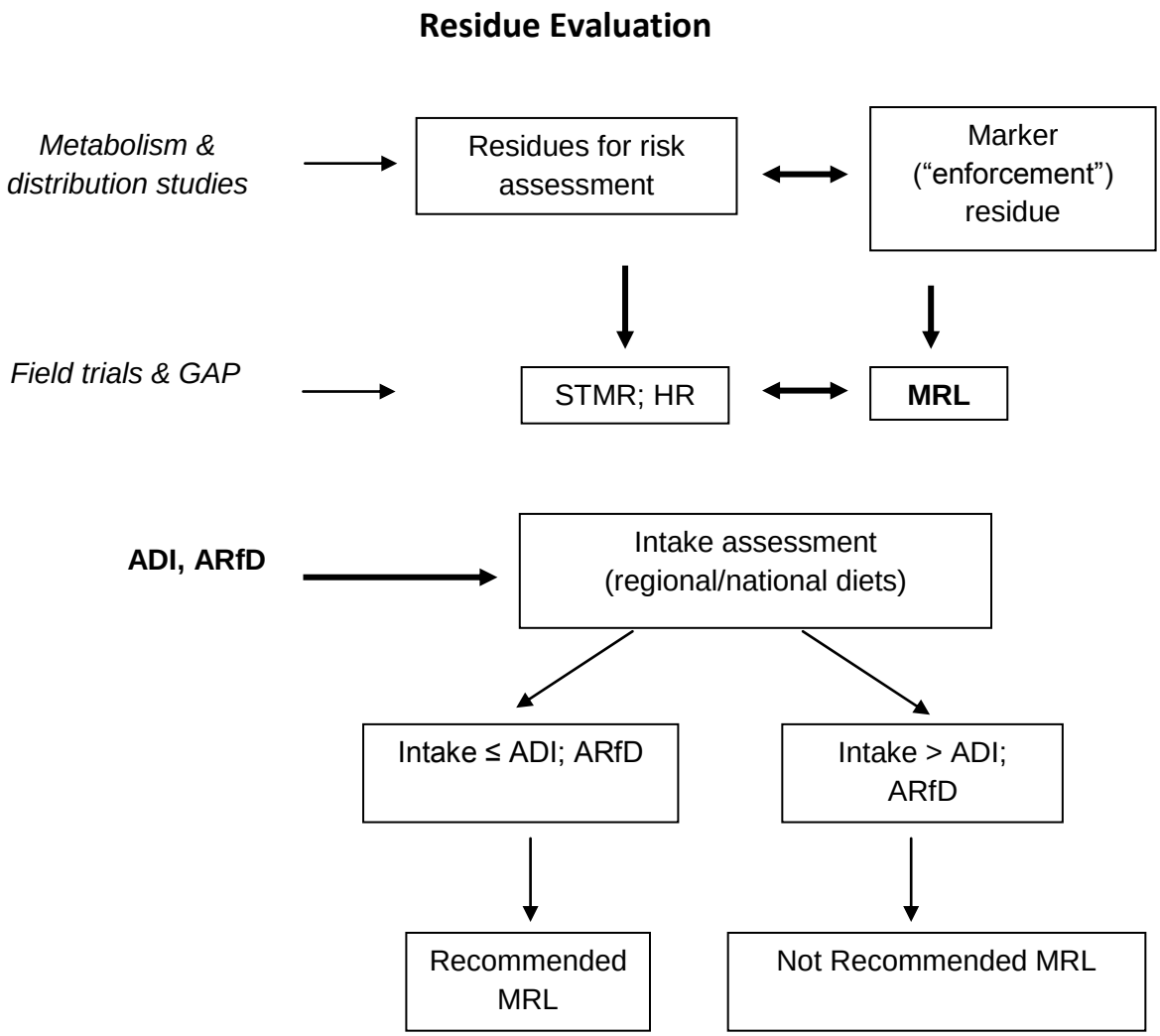



**Fig. Evaluation of residue data for fixation of MRL**

* Subject to refinement by established methods. If after refinement the intake becomes less than ADI it may be recommended

## SECTION 9

## FIXING OF MRL OF PESTICIDES IN PROCESSED FOODS

### Introduction

Foods that are subjected to technological modifications either for preservation or for converting into ready-to-use/eat foods, are designated as "processed foods". Food processing sector is one of the largest sectors in India in terms of production, growth, consumption, and export. Maximum Residue Limits (MRLs) of pesticides are generally fixed on raw agricultural commodities (RAC). Often, agricultural commodities are not eaten raw but undergo processing operations prior to human consumption. These may significantly affect the residue levels of pesticides contained therein and/or thereon. Due to the physico-chemical properties of the residue, its concentration may decrease/remain same or increase in processed fractions compared to the initial concentration in the RAC. The resulting ratio between processed fraction and RAC is denoted as processing factor.

Since considerable processed foods are being traded in the market and consumed, it is mandatory to establish MRLs for processed commodities or evaluate dietary risk assessment of pesticide in processed food. Information obtained from processing studies may serve for 2 different purposes: to ascertain the extent of compliance of residues in processed products with that of legal standards for the RAC, and to refine dietary exposure estimation of consumer with respect to residues in processed products.

### Processing Factor

Raw agricultural commodities are subjected to physical, chemical or biological processes to obtain processed foods e.g., milled grains, fruit juices, and edible oils etc. Various simple culinary processes i.e., washing, trimming, peeling, cooking,baking etc are now considered to refine dietary intake estimates. In most cases processing leads to reduction in the residues, however in few cases there is a built-up of residues, e.g., oil extraction from oilseeds. It is also possible that during processing the pesticide is converted to metabolite (s) having higher toxicity. Thus, every case of processed foods is required to be studied carefully for dietary risk assessment.

Considering variety of processed foods, it is certainly not possible to establish MRLs for all the processed commodities. However, it has been the practice to recommend MRLs for the cases where residue concentrates in the processed food. There are even cases where residues do not concentrate but MRL is desirable. Such cases are considered when toxic metabolite is formed during processing and when the residues in the processed food result due to use of pesticides during processing/storage. JMPR has published a list of processed commodities which can harbour higher residues in the processed portion and such studies should be conducted routinely. Extrapolation of the study is also suggested for the matching commodity having similar processing treatments.

Processing factors are derived from processing studies. Processing can lead to an increase / remain same or a decrease in residues, depending on the specific processing conditions and physicochemical properties of the active substance. They indicate the ratio of the residue in the processed product to that in the corresponding unprocessed product. An enrichment of the residue is

indicated by processing factors greater than 1, whereas a reduction in the residue concentration in the processed product is expressed in a factor of less than 1.

It has been recommended that when there is a significant decrease in the residues from RAC to the processed food the MRL for RAC shall hold good for processed food,

In all cases, the processing factors are required to be worked out to refine the dietary risk assessment. Processing factor is calculated as follows;

$$PF = \frac{\text{Residues in processed commodity (ppm)}}{\text{Residues in raw agricultural commodities (ppm)}}$$

When, the residues are ≤ LOQ in the RAC (as per *c*GAP), no processing study is required. However, there are exceptions e.g., when oil is solvent extracted from the oilseed, residues are frequently higher in oil.

Processing factors are an indispensable tool, which primarily serve two purposes:

1. To provide information to *regulatory authorities* on the *extent /scope of changes in residue levels* during food processing operations, they are crucial for assessing whether the starting material has been in compliance with legal standards.

2. To provide information to *risk assessors* for *refined dietary exposure estimates*, to allow a more realistic assessment in cases when commodities are mainly consumed after processing.

**Processing procedures-Guidelines**

Processing procedures may have a significant impact on pesticide residues, not only related to the magnitude of residue concentration, but also to the chemical transformation in the parent residue during processing (impact on the nature of residue). Several processing operations have been identified in the OECD Guidance ‘‘Document on Overview of Residue Chemistry Studies’’ as being representative of the most widely used industrial and domestic food processing technologies. Further, a larger assortment of processed commodities is published in the OECD Guidance Document on “Magnitude of Pesticide Residues in Processed Commodities”. To each core procedure and processed matrix, the corresponding OECD procedure code has been assigned. In addition to the fractions produced for human consumption, by-products are obtained from some processing operations that are not discarded but may be used for livestock feeding. Residues in those fractions also need to be taken into account when predicting the dietary burden of livestock animals and evaluation of the residue transfer into animal commodities.

**Objectives of processing studies**

**1.** To obtain information about breakdown or reaction products (metabolites/degradation products) which require a separate risk assessment.

**2.** To determine the quantitative distribution of residues in the various processed products, allowing the estimation of processing factors for products which may be consumed.

3. To allow more realistic estimates to be made for the chronic or acute dietary intake of pesticide residues.

**Criteria which need to be addressed by processing studies**

Each processing factor used either in risk assessment of pesticides or enforcement of legal standards should be derived in a study which complies with a minimum of *quality criteria*, as regulatory decisions may largely depend on that piece of information.

The eligibility criteria employed are outlined below:

**Conditions for processing procedures**

The procedures to be used in processing studies should always correspond as closely as possible to those that normally occur in practice. Thus products of household preparation, e.g., cooked vegetables, should be produced using the equipment and preparation techniques normally used in households, whereas industrial items such as cereal products, preserves, fruit juices or sugar should be produced by procedures representative of commercial food technology.

Processing Studies are not normally required if:

1. The plant or plant product is normally only eaten raw, e.g., head lettuce.
2. Only simple physical operations such as washing and cleaning are involved.
3. No residues above the limit of quantification occur.

Studies are necessary if significant residues occur in plants or plant products which are processed. "Significant residues" normally it means residues above 0.1 mg/kg in RAC. If the pesticide concerned has a low ARfD or ADI, consideration has to be given to conduct processing studies with analyses for residues below 0.1 mg/kg. In the case of hops this level should be 5 mg/kg (residues in beer are then < 0.01 mg/kg because of the dilution factor). For residues of a fat-soluble pesticide in oilseeds, the possibility of concentration in the oil has to be taken into account.

In some cases, more than one commercial process may be routinely used, and reasons should be provided for the chosen process. Importance should be attached to carrying out processing studies for commodities included in Indian diet and for animal feedstuffs derived from crops, e.g., products of cereals, oilseeds, apples, citrus and tomatoes.

The processing studies to determine residues in aqueous tea infusion are often carried out under an artificially "worst case" scenario, which cannot be used for the estimation of realistic processing factors.

The studies should be designed so that processing factors can be derived and MRLs recommended for processed foods and feed important in trade. For consistent processing factors the results of more than one study are necessary. Processing studies should simulate commercial or household practices as closely as possible. The RAC used in the studies should be a field-treated commodity containing quantifiable residues, so that processing factors for the processed products can be determined. This may require field treatment at an exaggerated application rate to obtain sufficiently high residue levels. Processing studies with spiked samples are not acceptable unless it can be demonstrated that the residue in the RAC is entirely on the surface.

**Representativeness of the employed processing procedures**

The applied experimental procedure should simulate industrial or domestic standards as closely as possible. Since the processing conditions are very versatile and are subjected to continuous technological advancement, product properties as defined by food norms should be reflected in the processed product. In view of the importance of industrial products in trade, industrial procedures may be preferred over domestic scale operations in order to obtain more representative results.

**Minimum number of trials**

The number of replicate trials within a processing study is a key parameter for robustness of the derived Pf, particularly when each individual Pf is significantly different. Even within the same study, Pfs derived from two replicate trials may show a considerable degree of variability. When individual Pfs from the 2 trials differ by more than 50 % (with a main focus on the relevant processed fraction), it is recommended to carry out a 3rd trial to enhance the consistency of the data and strengthen confidence in the finally derived factor.

**Validity of the analytical method**

The analytical method used in the processing study should be described in sufficient detail. Parameters like recovery rates, repeatability, reproducibility and sensitivity should be in line with generally agreed requirements for analytical methods for pre-registration purposes. The procedural recovery should be within the range of 70–120 %. In addition, the coefficient of variation should be below 20%. If these parameters are not fulfilled, the study is not accepted.

**Compliance of Good Laboratory Practices (GLP) standards**

Only processing studies conducted in accordance with GLP standards are to be considered so as to ensure the uniformity, consistency, reliability, reproducibility, quality, and integrity of chemical safety tests.

**Sample storage conditions**

Information on the sample storage conditions and the time elapsed between sampling and extraction/analysis is highly essential. The data should also include information about the duration of freezer storage of the samples**.**

**Calculation of Pf**

Pfs are generally reported after rounding to 2-digit accuracy.

If more than one Pf is derived for a processed fraction in a study, the median value is used. If only 2 processing factors are reported, no 3rd replicate is required when they do not deviate by more than 50 % as per the OECD Guideline 508. When the residue concentrations in the RAC and in the processed product are both below the analytical LOQ in all trials, a Pf may not be applicable **(OECD, 2008)**

**Test conditions**

Processing study representative of the potential uses of a given pesticide on crops in both domestic and industrial preparations of food/feed are usually needed. At least two independent trials, with RAC samples from two separate field sites, are necessary for each processing procedure.

Two trials are not sufficient in those situations where two or more significantly different commercial procedures are practiced for a given commodity. For example, the two independent trials are not sufficient in the cases of wine making, the milling of corn, and oil production.

**Test substance**

RAC samples used in PS should contain quantifiable residues – (≥ LOQ), but preferably up to at least 0.1 mg/kg or 10 times the LOQ – so that processing factors for the various processed products can be determined. The residues in the sample immediately prior to processing must be determined and reported. At least two replicate samples of the RAC should be analysed and the actual weights for the RAC samples to be processed should be reported.

**Processing technology**

The technology to be used in the PS should correspond as closely as possible to the actual conditions that are normally used in practice. A distinction should be made between domestic and industrial processing procedures. A flow chart and/or SOP describing the main process are highly recommended for both domestic and industrial processing.

**Products to be covered**

A set of processing studies should be conducted for every crop having residues and being processed, it should be possible to extrapolate the processing factor for the given pesticide to all crops within the given group undergoing the same procedure. The possibility of extrapolating this factor to all crops undergoing the same procedure should be carefully examined and discussed with appropriate regulatory authorities

**Sampling**

RAC samples for analysis must be taken from the bulk sample immediately prior to processing and stored frozen before subsequent analysis. Samples should be taken at the end of the processing procedure and stored under frozen conditions in inert sealed containers, if they need to be stored. Where intermediate samples are required for processing factors, these should be taken at appropriate points within the process and stored frozen. Replicate sampling and analyses are always recommended and the total weight of each of the individual processed fractions should be reported.

**Sample analysis**

The analytical method such as sample extraction and clean-up procedures should be described in detail or referenced and should comply with the OECD Guidance Document on Residue Analytical Methods. Spiked samples should be run concurrently with those from the processing study to validate the method. The validation of the analytical method should target an LOQ that is appropriate considering the toxicity of the components of the residue definition and the need of the data for use in dietary exposure assessment.

**Storage stability data**

For pre-harvest uses, samples should be processed as soon as possible following harvest in order to keep the integrity of the RAC. For post-harvest uses, (e.g. on cereal grains), processing should take place after an interval simulating commercial storage times, e.g. 3-6 months or more after field application of pesticides in the crop, to allow the residues to "age", which may influence the profile of the residues in processed commodities.

If there is no observed decline of residues across the range of the five different crop categories (including animal matrices, if applicable) from the RAC storage stability study, then specific residues freezer stability data for processed foods will not be needed. However, if instability is shown after a certain length of storage, commodities (RAC, animal tissue or processed commodity) are analysed within the demonstrated time period for stable storage.

**Guidelines for the conduct of processing studies: Specific examples**

The objective of studies of the nature of residues is to establish whether or not breakdown or reaction products of residues in the raw commodities are formed during processing which may require a separate risk assessment.

When examining the effects of processing on pesticide residues one will find that the main procedures, e.g., preparation of fruit juices, preserves, wine, will be mainly hydrolytic, because processes involving heating would generally inactivate enzymes present in the commodity. Studies of hydrolysis are therefore chosen as the model for degradation in processing. Since the substrate itself is not likely to have a major effect, the presence of the commodity during such studies is not required. Studies of hydrolysis are not required if the water solubility of the substance is ≤ 0.01 mg/L.

Hydrolysis data (required as part of the physical-chemical properties of an active ingredient) are normally generated at temperatures between 0 - 40℃ C for a time chosen to allow observance of degradation up to at least 70% at pH 4, 7 and 9. The objective of these studies is primarily related to environmental conditions. Therefore, they are not interchangeable with the required data needed to assess residue behaviour during processing, where higher temperatures but normally much shorter periods and, in some cases, at more extreme pH values are typically involved. Reactions are therefore faster and may lead to the formation ofdifferent degradation products.

Typical conditions (temperature, time and pH) which prevail for each of the processing operations are given in the table below (OECD 2008)

**Table**. Typical parameters during processing operations

| **Type of process** | **Critical operation** | **Temperature ($^0$C)** | **Time (min)** | **pH** |
|---|---|---|---|---|
| Cooking vegetables, cereals | Boiling | 100[a] | 15– 50b | 4.5– 7 |
| Fruit preserves | Pasteurisation | 90– 95[c] | 1– 20d | 3– 4.5 |
| Vegetable preserves | Sterilisation | 118– 125[e] | 5– 20f | 4.5– 7 |
| Fruit Juice | Pasteurisation | 82– 90 g | 1– 2h | 3– 4.5 |
| Oil | Raffination | 190– 270i | 20– 360j | 6– 7 |
| Beer | Brewing | 100 | 60– 120 | 4.1– 4.7 |
| Red wine k | Heating of grape mash | 60 | 2l | 2.8– 3.8 |
| Bread | Baking | 100– 120m | 20– 40n | 4– 6 |
| Instant noodle | Steam and dehydration (by frying or hot air) | 100<br>140– 150 (frying)<br>□ 80 (air) | 1– 2<br>1– 2(frying)<br>120(air) | 9o |

a Temperature of the vegetables during cooking
b Time the vegetables or cereals are kept at 100 ℃
c Temperature within the fruit preserves during pasteurization
d Time the fruit preserves are kept at 90–95 ℃
e Temperature within the vegetable preserves during sterilisation

f Time the preserves are kept at 118–125 °Cg Temperature of the fruit juice during pasteurisation

h Time the fruit juice is kept at 82–90 °C

i Temperature of the deodorization during raffination

j Time of the deodorization

k White wine is not heated

l Subsequently either chilled quickly or allowed to cool slowly (overnight)

m Temperature within the loaf and on the surface during 20–40 minutes

n Time the loaf and the surface is kept at 100–120 °C

o Wheat flour is kneaded with 0.1–0.6% Kansui (alkaline water containing 20% K2CO3 and 3.3% Na2CO3)

Based on the details given in Table above, three representative sets of hydrolytic conditions can be considered appropriate to investigate the effects of hydrolysis for the relevant processing operations.

**Table:** The hydrolysis conditions listed below are selected to cover most processing procedures.

| **Temperature($^0$C)** | **Time-min** | **pH** | **Processed represented** |
|---|---|---|---|
| 90 | 20 | 4 | Pasteurisation |
| 100 | 60 | 5 | Baking, brewing, boiling |
| 120[a] | 20 | 6 | Sterilization |

[a]Closed system under pressure (e.g. Autoclave or similar)

For other processing practices involving more extreme conditions (deodorization during raffination, high pH of instant noodles, the temperature and time for preparation of meat and fish) specific studies should be considered on a case-by-case basis. The effects of processes other than hydrolysis, e.g., oxidation, reduction, enzymic or thermal degradation, may also have to be investigated if the properties of the pesticide or its metabolites indicate that such processes may produce toxicologically significant degradation products.

Depending upon the potential range of pesticide uses, one or more of the representative hydrolysis situations should be investigated. The studies are normally conducted with a radio labeled form of the active substance or the residue in question. The desired goal of such a study is the identification and characterization of at least 90% of the remaining TRR.

It is required to take into account the nature of the major products in the hydrolysis study, dilution or concentration factors during processing, and the initial residue levels in the raw agricultural commodity when evaluating the results of the studies.

Processed products can be classified according to certain types of process. The studies have to take into account the importance of the processed product in human or animal diets. Degradation products of toxicological significance occurring in the hydrolysis studies have to be taken into consideration as well as residues of concern found in plant metabolism studies. For a core set of data on an active ingredient the processing studies should be conducted on representative commodities such as citrus fruits, apples, grapes, tomatoes, potatoes, cereals and oilseeds. By

using core processing procedures and selected crops it should be possible to extrapolate to other crops processed by the same procedure. Only in cases where it is not possible to derive consistent processing factors or where a very low ADI is established it would be necessary to conduct processing studies on every crop (OECD Guidelines for the Testing of Chemicals, Test No. 508.)

In some cases, further trials may be necessary to cover particular circumstances. Examples are the determination of residues in oil produced from oilseeds with no significant residues where the active substance has a log Pow above 4, and extended studies on active substances with a very low ADI.

**Dehydration factors**

Dehydration factors are recommended for the commodities where only loss of water is involved during processing and there is no degradation of the pesticide. Such factors have also been recommended for spices and herbs by European Spice Association (Ref: http://www.esa-spices.org/documents). The dehydration factor is calculated as follows;

$$\text{Dehydration Factor} = \frac{1}{\left[1-\left(\frac{\%\ \text{Water content}}{100}\right)\right]}$$

The best estimate of the processing factor should be applied for the estimation of maximum residue level, HR-P and STMR-P in processed commodities.

To estimate a maximum residue level for a processed product the MRL or maximum residue level of the RAC is multiplied by the processing factor derived from the residue definition for enforcement **(PfENF).**

For the purpose of IEDI and IESTI estimation, the STMR and HR of the RAC is multiplied by the processing factor derived from the residue definition for dietary risk assessment (PfRISK) to give the median and highest residue in the processed commodity. The HR, STMR value estimated in this way for the processed commodity should be referred to as the HR-P and STMR-P of the processed product.

Maximum residue level for the processed commodity will only be recommended if the resulting residue value is higher than the maximum residue level proposed for the corresponding RAC.

HR-Ps and/or STMR-Ps for commodities for human consumption are estimated regardless of the availability of consumption data.

If data are available for the residues in the edible portion of the commodity, e.g., in banana pulp, the HR and STMR should be estimated directly from the residues in the edible portion found in supervised trials at the maximum registered use rate (as opposed to using pesticide residue values for the whole commodity).

If these data are not available for the edible portion, the whole commodity residue values are used in the dietary intake estimations, even though this may result in a gross over-estimate of the actual residues likely to be consumed.

**Special considerations for dried chilli pepper:**

A concentration factor of10 for the estimation of residue levels of pesticides in dried chilli pepper is used from the HR values estimated for residues in or on fresh chilli pepper;

**It is recommended that:**

- where representative processing studies on residues in or on chili peppers are available, the residue levels for dried chili peppers should be estimated based on the actual experimental data.
- relevant concentration factor should be applied to multiply the actual measured residue values in fresh chili peppers and estimate the maximum residue and median residue levels from the converted data set.

## SECTION 10

## Animal forage feed, meat, milk, offals and eggs

### 10.1 MRL fixation for animal feed, meat, milk, offals and eggs

The present guideline document reflects brief account of livestock residue studies for pesticides. Such studies help estimating pesticide residues in meat, milk, eggs and meat by-products following exposure through feed/fodder or direct treatment to livestock and /or dwelling area. The MRLs of pesticides in animal feeds are calculated based on HR, STMR, or STMR-P, following use of pesticides as per the GAP or GFP (Good Feeding Practice). The levels of pesticide residues thus transferred to animal products are used for risk assessment for consumers to establish MRLs. Feeding trials are predominantly conducted on cattle,sheep,goat,pigs and chicken. The animals used in the study are usually in the egg or milk producing stage or are close to slaughter (e.g., last 100 days for beef cattle). However, when rat metabolism study reveals different metabolites of toxicological significance in ruminants, swine feeding trial is considered.

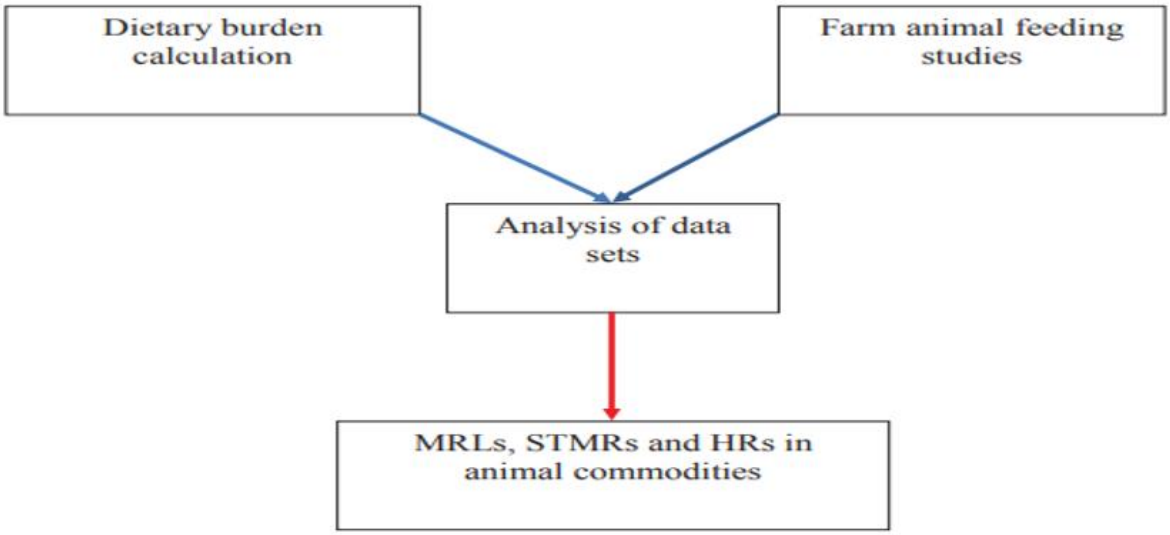



**Fig. Estimation of residues in animal commodities**

Livestock feed comprised of variety of agricultural commodities and by-products. EU has published a detailed information on crop commodities representing forages, roots and tubers, cereal grains/crop seeds and by-products. In order to estimate the maximum residues that will arise in animal commodities, the highest residues in individual feed items are used by considering STMR or STMR-P to each of the component of mixed commodities. In order to avoid variation in calculation due to differing moisture levels, the residues in feed/fodder are always expressed on dry matter basis. When total diet contributions exceed 100 %, the contribution is reduced to 100% in such a way that the highest possible dietary burden is retained. Following tables explain the steps involved in calculation of maximum dietary burden.

**Maximum dietary burden of beef cattle**

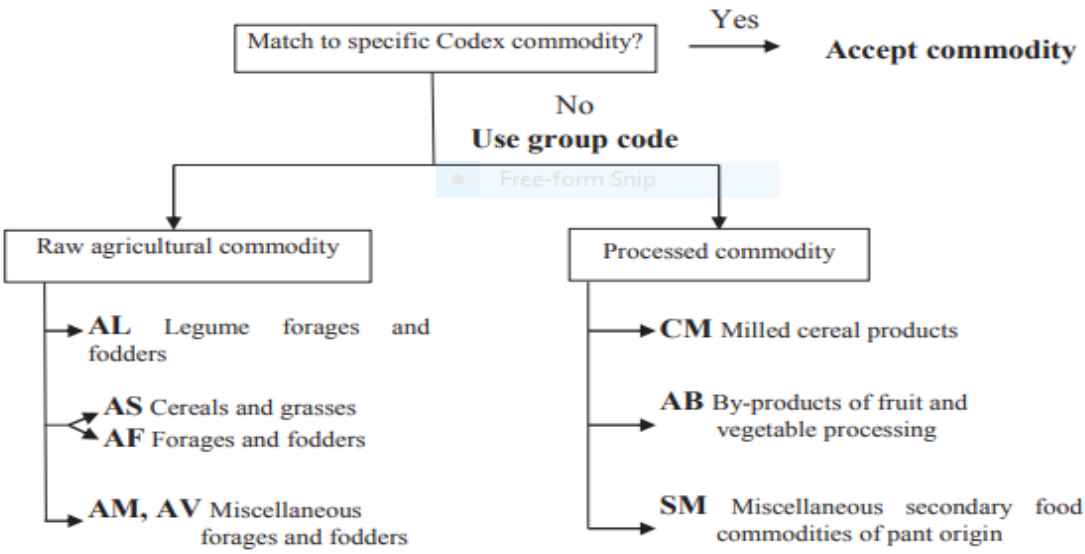
| Commodity/ Crop | Commodity group | Residue mg/kg | Basis | % Dry matter | Residue dw mg/kg | Diet content (%) | | | Residue contribution (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | US-CAN | EU | AUS | US-CAN | EU | AUS |
| Grape pomace, dry | AB | 0.038 | STMR-P | 100 | 0.038 | | | 20 | | | 0.01 |
| Bean forage(green) | AL | 2.1 | high residue | 35 | 6.000 | 30 | | 60 | 1.80 | | 3.60 |
| Alfalfa fodder | AL | 4 | high residue | 89 | 4.494 | 60 | | 80 | 2.70 | | 3.60 |
| Pea vines(green) | AL | 0.86 | high residue | 25 | 3.440 | 20 | 20 | 60 | 0.69 | 0.69 | 2.06 |
| Maize fodder | AS AF | 4.3 | high residue | 83 | 5.181 | 25 | 25 | 40 | 1.30 | 1.30 | 2.07 |
| Wheat straw & fodder, dry | AS AF | 4.3 | high residue | 88 | 4.886 | 10 | 20 | 80 | 0.49 | 0.98 | 3.91 |
| Barley forage | AS AF | 1.4 | high residue | 30 | 4.667 | 30 | 30 | 50 | 1.40 | 1.40 | 2.33 |
| Wheat milled(bran) | CM | 0.084 | STMR-P | 88 | 0.095 | 40 | 30 | 40 | 0.04 | 0.03 | 0.04 |
| Rice | GC | 0.57 | STMR | 88 | 0.648 | 20 | | 40 | 0.13 | | 0.26 |
| Wheat | GC | 0.035 | STMR | 89 | 0.039 | 20 | 40 | 80 | 0.01 | 0.02 | 0.03 |
| **Total** | | | | | | ***255*** | ***165*** | ***550*** | ***8.54*** | ***4.40*** | ***17.91*** |

**Commodities selected to contribute to the maximum burden of beef cattle**

| **Commodity/Crop** | **Commodity group** | **Residue mg/kg** | **Basis** | **% Dry matter** | **Residue dw mg/kg** | **Diet content (%)** | | | **Residue contribution (ppm)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | US-CAN | EU | AU | US-CAN | EU | AU |
| Grape pomace,dry | AB | 0.038 | STMR-P | 100 | 0.038 | | | 20 | | | 0.01 |
| Bean forage(green) | AL | 2.1 | high residue | 35 | 6.000 | 30 | | 60 | 1.80 | | 3.60 |
| Alfalfa fodder | AL | 4 | high residue | 89 | 4.494 | **30** | | **20** | **1.35** | | **0.90** |
| Pea vines(green) | AL | 0.86 | high residue | 25 | 3.440 | - | 20 | - | - | 0.69 | - |
| Maize fodder | AS AF | 4.3 | high residue | 83 | 5.181 | 25 | 25 | 40 | 1.30 | 1.30 | 2.07 |
| Wheat straw and fodder, dry | AS AF | 4.3 | high residue | 88 | 4.886 | - | - | **40** | - | - | **1.95** |
| Barley forage | AS AF | 1.4 | high residue | 30 | 4.667 | **5** | **5** | - | 0.23 | **0.23** | - |
| Wheat milled(bran) | CM | 0.084 | STMR-P | 88 | 0.095 | 40 | 30 | 40 | 0.04 | 0.03 | 0.04 |
| Rice straw | GC | 0.57 | STMR | 88 | 0.648 | 20 | | 40 | 0.13 | | 0.26 |
| Wheat straw | GC | 0.035 | STMR | 89 | 0.039 | - | 40 | **40** | - | 0.02 | **0.02** |
| **Total** | | | | | | **150** | **120** | **300** | **4.84** | **2.26** | **8.85** |

**Final table with 100% diet calculation for maximum residue burden for beef cattles**

| **Commodity/Crop** | **Commodity group** | **Residue mg/kg** | **Basis** | **% Dry matter** | **Residue dw mg/kg** | **Diet content (%)** | | | **Residue contribution (ppm)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | US-CAN | EU | AU | US-CAN | EU | AU |
| Bean forage(green) | AL | 2.1 | high residue | 35 | 6.000 | 30 | | 60 | 1.80 | | 3.60 |
| Alfalfa fodder | AL | 4 | high residue | 89 | 4.494 | **30** | | - | **1.35** | | - |
| Pea vines(green) | AL | 0.86 | high residue | 25 | 3.440 | - | 20 | - | - | 0.69 | - |
| Maize fodder | AS AF | 4.3 | high residue | 83 | 5.181 | 25 | 25 | 40 | 1.30 | 1.30 | 2.07 |
| Barley forage | AS AF | 1.4 | high residue | 30 | 4.667 | **5** | **5** | - | 0.23 | **0.23** | - |
| Wheat milled(bran) | CM | 0.084 | STMR-P | 88 | 0.095 | - | 30 | - | - | 0.028 | - |
| Rice straw | GC | 0.57 | STMR | 88 | 0.648 | 10 | | - | **0.06** | | - |
| Wheat straw | GC | 0.035 | STMR | 89 | 0.039 | - | **20** | - | - | 0.008 | - |
| **Total** | | | | | | **100** | **100** | **100** | **4.74** | **2.25** | **5.67** |




Grouping feed items for calculation of dietary burden of livestock

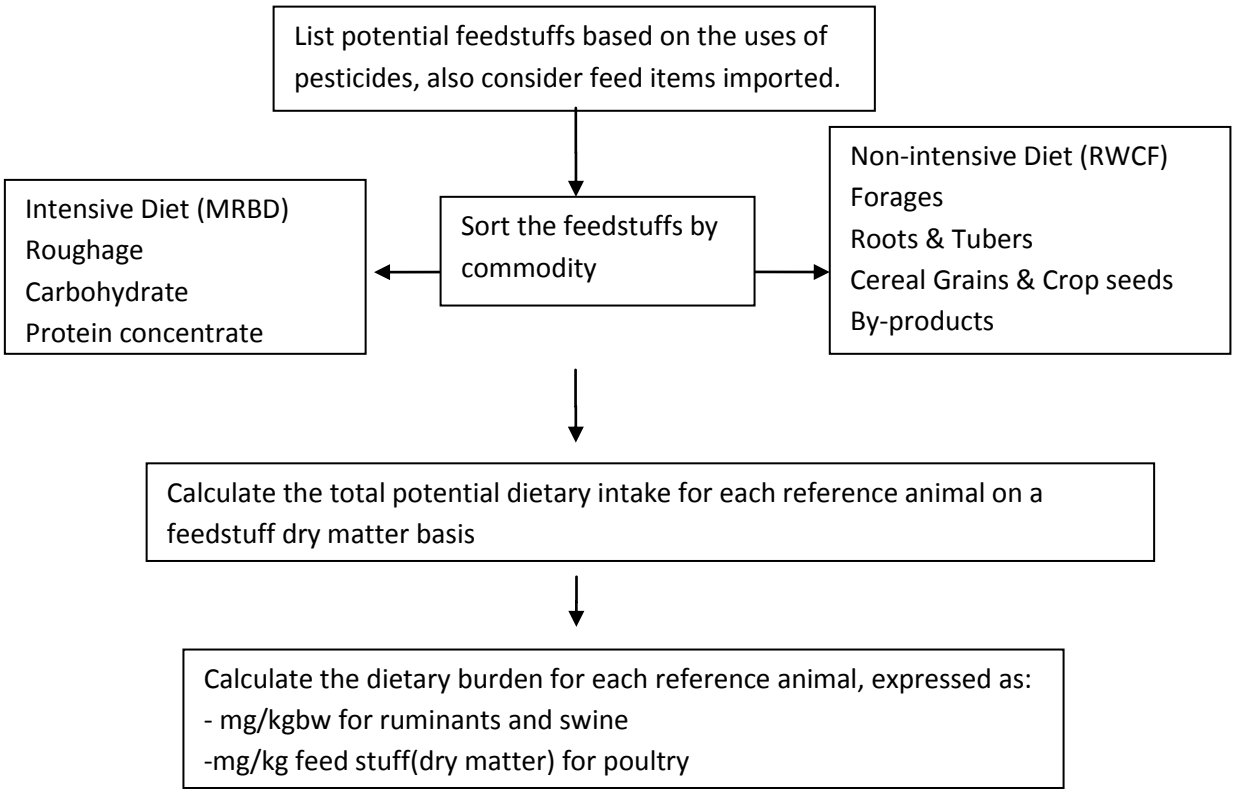
Non-intensive livestock production system as pasture and crop-based grazing and intensive livestock production system include lot feeding arrangements. In fact globally dietary burdens may vary significantly and to account for this variability at the international level, feed items can be grouped according to their nutritional relevance. Following table explains dietary burden calculation for beef cattle using USA/CAN input values.

| Category | Commodity | % Diet | Highest Dietary Contribution (mg/kg bw) |
|---|---|---|---|
| 15% Roughage | Alfalfa hay | 15 | 0.031 |
| 80% Carbohydrate | Potato cull | 30 | 0.025 |
| | Aspirated grain fraction (corn) | 5 | 0.002 |
| | Corn milled by-products | 45 | 0.010 |
| 5% Protein | Alfalfa meal | 0 | 0 |
| **Total** | | **95** | **0.068** |

In regions where non-intensive (e.g. grazing) livestock production systems predominate, anticipated dietary burdens are calculated based on a "reasonable worstcase diet/feed" approach (RWCF). In regions where intensive feeding practices predominate, the anticipated dietary burden is calculated based on a "maximum reasonably balanced diet (MRBD)" approach. The approach uses fixed percentages of roughage, carbohydrate concentrate, and protein concentrate of various livestock types. In India, MRBD approach is rarely practised for meat, (except for poultry), but a substantial

population of livestock is reared with less intensive to non-intensive diets. Therefore, it is advisable to calculate dietary burden according to the RWCF approach.

**Dietary burden is calculated by the steps mentioned in the following figure.**




Livestock feeding studies are conducted with laying hen or dairy cattle and results of these studies can be extrapolated to other domestic animals e.g., laying hen feeding study data to poultry and cattle feeding study data to all ruminants (e.g., goats and sheep) and other animals such as horses, pigs and rabbits.

### 10.2 Direct application to farm animals

For the protection of the livestock against ecto-parasites e.g., lice, flies, mites and ticks, pesticides are directly applied as sprays, dips, dust, dust-bags, back-rubber, ear-tags, pour-ons or jetting. These treatments might result in the detection of pesticide residues in animal products. Therefore, Good Veterinary Practice (GV)P trials are conducted on farm animals considering the maximum conditions as per the label. The highest residues in the products will support the MRL recommendations. Unlike animal feeding trials, extrapolation of residue levels is generally not justified and separate studies are required for each species of livestock. When premise treatment trials are conducted, animals are not removed from their housing, however, they can be taken to milking sheds.

### 10.3 Interpretation of the results

Several general points to consider when interpreting data from livestock feeding studies are described below. Note that in the case of fat-soluble compounds data interpretation might be handled differently by different jurisdictions.

- Confirm that the actual dose administered is equivalent to the nominal dose estimated for each feed level. To avoid incorrect interpretation of the results, the actual dose levels reported in the study should be used.
- Plot residues for each matrix versus dose level in the study. Plot all residue values (including multiple results for different animals at a given dose level) versus dose level to determine the spread of residue values (at each dose level) and to ascertain if a linear dose response is observed.
- If a linear relationship exists (through 3 or more data points), i.e., all transfer factors (TF) are approximately the same, interpolation, linear regression, or use of the average transfer factor is acceptable.
- If the calculated dietary burdens are either below the lowest dose or above the highest dose of the feeding study, the transfer factor nearest the calculated burden may be used to calculate the residue value, or linear regression may be used if a linear relationship exists. The experimental dose values should be within about 30% of the dietary burden.
- If a linear relationship does not exist, it may be possible to interpolate between two data points on either side of the exposure, or it may be possible to use the transfer factor from a single data point not more than 30 - 50% removed from the exposure. Generally the approach giving the highest residue value should be used.
- For fat-soluble pesticides (see definition in OECD TG 505 (1)), note whether different fat depots have been analyzed as required. It is important to determine which depot for each fat soluble pesticide has the highest residue so that the MRL is not underestimated. Variability in results should also be considered.
- If milking animals are used in the feeding study, if the pesticide is fat-soluble, and if quantifiable residues in the milk and/or cream exceed residue levels in fat, then milk is indicated as a significant elimination pathway. Non-fat soluble pesticide residues are more likely to be found in the kidney, rather than in fat and in milk and cream, as they are usually eliminated quickly via the kidney. Fat-soluble pesticides will tend to accumulate in milk/cream. Accumulation of the residue in fat from a male animal will be greater than in a milking ruminant because milk/cream provides an alternative elimination pathway for the residue. If the residue is higher in milk and/or cream than in fat, another study using non-lactating animals may be considered to determine accurately residues in fat. Alternatively, it may be possible for an adjustment factor to be applied to the residues observed in the milk producing animal study to account for likely higher residues in meat-only animals. For example, this could be done if adequate data were provided to determine a half-life for elimination from depuration data, collected as part of the milk producing animal study.
- If laying hens are used in the feeding study, if the pesticide is fat-soluble, and if quantifiable residues in the egg (yolk) exceed residue levels in fat, then egg (yolk) is indicated as a significant pathway. In such cases, residues in fat of broilers (meat-bird production) may be higher than residues in the fat of layers. Currently there is no mechanism to account for this possibility in MRL setting.

Thus estimated dietary burdens are compared with the residues obtained from animal transfer studies for estimating maximum residue levels and STMR for animal commodities. MRLs/EMRLs for fat-

soluble pesticide residues in milk and milk products are expressed on a whole product basis. For a "milk product" with a fat content less than 2%, the MRL applied should be half of those specified for milk. The MRL for "milk products" with a fat content of 2% or more should be 25 times the maximum residue limit specified for milk, expressed on a fat basis.

**References:**


- Codex Alimentarius Commission, Codex classification of foods and animal feeds, draft revision-1 (2006) Joint FAO/WHO Food Standards Programme.
- FAO Manual 2002. Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed. Food and Agriculture Organization of the United Nations. Rome, 2002. First edition.
- FAO, 2009c. Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed (FAO Manual, 2nd Edition). FAO Plant Production and Protection Paper, 197, 2009.
- FAO, 2020. Understanding international harmonization of pesticide maximum residue limits with Codex standards A case study on rice. 91 p.http://www.fao.org/documents/card/en/c/cb0463en
- "Handbook of Agriculture" 6th revised edition, Indian Council of Agricultural Research KrishiAnusandhanBhavan-I, Pusa, New Delhi-110012.
- INDIA Good Agriculture Practices (INDGAP) Certification Scheme: Quality Council of India Governing Structure, QCI/ INDGAP/ Ver.1 Sept. 14.
- OECD "Guidance Document on Crop Field Trials Series on Pesticides" No 66 / Series on Testing and Assessment No. 164 ENV/JM/MONO(2011)50.
- OECD "Guidance Document on Pesticide Residue Analytical Methods" Series on Testing and Assessment Number 72;Series on Pesticides Number 39;ENV/JM/MONO(2007)17.
- OECD, 2008. OECD Guidelines for the Testing of Chemicals: Magnitude of the pesticide residues in processed commodities. No. 508, OECD, Paris, 2008.
- OECD, 2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial. No. 509, OECD, Paris, 2009.
- OECD, 2010. OECD Guidance document on pesticide residue analytical methods, SANCO/825/00 REV. 8.12, 2010.
- OECD, 2009. OECD Guidance document on the definition of residues, ENV/JM/MONO (2009) 30.
- OECD Guidelines for the Testing of Chemicals, TG 506: Stability of Pesticide Residues in Store Commodities. Organization for Economic Co-operation and Development, 16 October 2007.
- SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.
- Submission and Evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed, FAO Plant Production and Protection Paper 170 (2002).
- Andrew G Renwick, Pesticide residue analysis and its relationship to hazard characterization (ADI/ARfD) and intake estimations (NEDI/NESTI), Pest Management Science 58: 1073-1082 (2002).
- OECD MRL Calculator user guide, Series on Pesticides No: 56, ENV/JM/MONO(2011)2 of Environment Directorate, Joint Meeting Of The Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

- Submission and Evaluation of Pesticide Residue data for the estimation of Maximum Residue Levels in food and feed. FAO Plant Production and Protection paper 225 (2016)
- OECD MRL Calculator-Statistical White Paper, Series on Pesticides No: 57, ENV/JM/MONO(2011)3 of Environment Directorate, Joint Meeting Of The Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology
- Household consumption of various goods and services in India 2011-12, June 2014, National Sample survey office, Ministry of Statistics and Program implementation, Government of India.
- (https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282011%292&doclanguage=en). (Series on Pesticides No: 56, ENV/JM/MONO (2011)2 of Environment Directorate, Joint Meeting Of The Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology;
- Series on Pesticides No: 57, ENV/JM/MONO (2011)3 of Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology).
- R. Scholz, M. Herrmann, B. Michalski (2016) Compilation of Processing Factors and Evaluation of Quality Controlled Data of Food Processing Studies. J. Verbr. Lebensm.,doi:10.1007/s00003-016-1043-3
  BfR (2016) http://www.bfr.bund.de. Accessed 1 July 2016
- Holland PT, Hamilton D, Ohlin B, Skidmore MW (1994) Effects of storage and processing on pesticide residues in plantproducts. Pure ApplChem 66:335–356 Kaushik G, Satya S, Naik SN (2009) Food processing a tool to pesticide residue dissipation—A review. Food Res Int42:26–40. doi:10.1016/j.foodres.2008.09.009
- OECD (2008a) OECD Guidance Document on the Magnitude ofPesticide Residues in Processed Commodities ENV/JMMONO(2008)23.
  http://www.oecd.org/env/ehs/pesticidesbiocides/publicationsonpesticideresidues.htm.
  Accessed 28February 2016
- OECD (2008b) Test Guideline No. 508: Magnitude of thePesticide Residues in Processed Commodities, OECDGuidelines for the Testing of Chemicals, Section 5, OECD Publishing. doi:10.1787/9789264067622-

**Annexure I:**

## The relevant data for consumption of food (g/day or ml/day) required for facilitating the dietary exposure risk assessment

| Food Commodity | Rural | Urban | Recommended dietary consumption for long-term effects ( for MRL calculation ). | Consumption based on 95th Percentile | | |
|---|---|---|---|---|---|---|
| | | | | Rural | Urban | Recommended consumption value for short-term assessment based on ARfD |
| **Cereals and Millets** | | | | | | |
| 1. Bajra | 113 | 85 | 113 | 371 | 200 | 371 |
| 2. Barley | 15 | 59 | 59 | 15 | 88 | 88 |
| 3. Maize | 100 | 94 | 100 | 300 | 300 | 300 |
| 4. Ragi | 47 | 56 | 56 | 220 | 95 | 220 |
| 5. Rice (Milled and parboiled) | 257 | 154 | 257 | 818 | 540 | 818 |
| 6. Sorghum (Jowar) | 163 | 94 | 163 | 455 | 162 | 455 |
| 7. Wheat (whole flour) | 192 | 149 | 192 | 478 | 385 | 478 |
| 8. Other cereals / millets | 37 | 45 | 45 | 160 | 174 | 174 |
| **Oils** | | | | | | |
| 9. Groundnut oil | 22 | 24 | 24 | 64 | 64 | 64 |
| 10. Mustard Oil | 14 | 13 | 14 | 37 | 48 | 48 |
| 11. Sesamum Oil (Til) | 5 | 39 | 39 | 20 | 40 | 40 |
| 12. Soybean Oil | 16 | 15 | 16 | 53 | 63 | 63 |
| 13. Coconut oil | 13 | 15 | 15 | 43 | 53 | 53 |
| 14. Sunflower oil | 16 | 21 | 21 | 52 | 61 | 61 |
| 15. Safflower oil | 16 | 20 | 15 | | | |
| 16. Cotton seed oil | 19 | 28 | 28 | 64 | 85 | 85 |
| 17. Rice bran oil | 16 | 20 | 20 | | | |
| 18. Other oils | 16 | 11 | 16 | 33 | 47 | 47 |
| **Oil Seeds** | | | | | | |
| 19. Groundnut | 5 | 9 | 9 | 85 | 49 | 85 |
| 20. Mustard | 2 | 2 | 2 | 6 | 6 | 6 |
| 21. Sesamum | 12 | 3 | 12 | 50 | 24 | 50 |
| 22. Coconut | 4 | 5 | 5 | 20 | 37 | 37 |
| 23. Sunflower | 2 | 2 | 2 | 5 | 5 | 5 |
| 24. Safflower | 4 | 4 | 4 | 5 | 5 | 5 |
| **Sugar** | | | | | | |
| 25. Sugar/Jaggery | 20 | 12 | 20 | 68 | 39 | 68 |
| 26. Sugarcane juice | 220 | 220 | 220 | | 220 | 220 |
| **Fruits** | | | | | | |
| 27. Apple | 75 | 100 | 100 | 120 | 200 | 200 |

| Food Commodity | Rural | Urban | Recommended dietary consumption for long-term effects ( for MRL calculation ). | Consumption based on 95th Percentile | | |
|---|---|---|---|---|---|---|
| | | | | Rural | Urban | Recommended consumption value for short-term assessment based on ARfD |
| 28. Grapes | 50 | 55 | 55 | 250 | 350 | 350 |
| 29. Mango | 167 | 125 | 167 | 500 | 386 | 500 |
| 30. Banana | 112 | 80 | 112 | 258 | 160 | 258 |
| 31. Pineapple | 15 | 87 | 87 | | 380 | 380 |
| 32. Pomegranate | 12 | 50 | 50 | 125 | 200 | 200 |
| 33. Guava | 100 | 63 | 100 | 200 | 100 | 200 |
| 34. Litchi | 62 | 30 | 62 | 62 | 100 | 100 |
| 35. Peach | 8 | 8 | 8 | 8 | 50 | 50 |
| 36. Citrus-Orange | 75 | 91 | 91 | 120 | 142 | 142 |
| 37. Lime | 4 | 5 | 5 | 37 | 20 | 37 |
| 38. Other Citrus fruits | 21 | 35 | 35 | 60 | 261 | 261 |
| 39. Other fruits (papaya, water melon, etc) | 115 | 100 | 115 | 315 | 250 | 315 |
| **Spices** | | | | | | |
| 40. Cardamom | 2 | 2 | 2 | 4 | 4 | 4 |
| 41. Black pepper | 2 | 2 | 2 | 3 | 4 | 4 |
| 42. Coriander | 2 | 3 | 3 | 5 | 5 | 5 |
| 43. Cumin | 2 | 2 | 2 | 4 | 5 | 5 |
| 44. Fenugreek | 2 | 2 | 2 | 79 | 5 | 79 |
| 45. Ginger | 3 | 3 | 3 | 10 | 10 | 10 |
| 46. Chillies (dried) | 3 | 2 | 3 | 9 | 8 | 9 |
| 47. Garlic | 2 | 2 | 2 | 8 | 9 | 9 |
| 48. Other spices | 2 | 2 | 2 | 8 | 8 | 8 |
| **Vegetables** | | | | | | |
| 49. Tomato | 28 | 29 | 29 | 90 | 100 | 100 |
| 50. Okra | 52 | 68 | 68 | 251 | 205 | 251 |
| 51. Brinjal | 49 | 42 | 49 | 165 | 143 | 165 |
| 52. Cabbage | 87 | 74 | 87 | 206 | 230 | 230 |
| 53. Cauliflower | 78 | 58 | 78 | 215 | 190 | 215 |
| 54. Knol-khol | 13 | | 13 | 356 | | 356 |
| 55. Potato | 86 | 69 | 86 | 253 | 203 | 253 |
| 56. Radish | 61 | 50 | 61 | 201 | 135 | 201 |
| 57. Beetroot | 59 | 51 | 59 | 211 | 158 | 211 |
| 58. Tapioca | 223 | 100 | 223 | 444 | 231 | 444 |
| 59. Carrot | 44 | 30 | 44 | 157 | 122 | 157 |
| 60. Cowpea | 24 | 56 | 56 | 89 | 120 | 120 |
| 61. Beans, French beans, Field beans, | 57, 89, 60 | 58 / 48 | 89 | 148 | 80 | 148 |

| Food Commodity | Rural | Urban | Recommended dietary consumption for long-term effects ( for MRL calculation ). | Consumption based on 95th Percentile | | |
|---|---|---|---|---|---|---|
| | | | | Rural | Urban | Recommended consumption value for short-term assessment based on ARfD |
| Cluster beans | | | | | | |
| 62. Pea | 46 | 33 | 46 | 115 | 124 | 124 |
| 63. Bell pepper | 21 | 25 | 25 | 179 | 162 | 179 |
| 64. Chillies (green) | 3 | 3 | 3 | 11 | 11 | 11 |
| 65. Cucurbits: Cucumber, Melons, Pumpkin, Ash gourd, Snake gourd, Bitter gourd, Bottle gourd, Ridge gourd, Coccinia, Gherkin | 57 | 42 | 57 | 250 | 175 | 250 |
| 66. Onion | 21 | 23 | 23 | 68 | 85 | 85 |
| **Pulses** | | | | | | |
| 67. Red gram | 32 | 34 | 34 | 92 | 100 | 100 |
| 68. Bengal gram | 40 | 51 | 51 | 99 | 150 | 150 |
| 69. Green gram | 42 | 52 | 52 | 123 | 111 | 123 |
| 70. Black gram | 30 | 27 | 30 | 65 | 75 | 75 |
| 71. Horse gram | 28 | 25 | 28 | 128 | 90 | 128 |
| 72. Cowpea | 37 | 41 | 41 | 140 | 144 | 144 |
| 73. Soybean | 18 | 20 | 20 | 95 | 100 | 100 |
| 74. Other pulses | 28 | 25 | 28 | 65 | 160 | 160 |
| **Plantation Crops / Beverages** | | | | | | |
| 75. Coffee beans (dry basis) | 2 | 3 | 3 | | | 3 |
| 76. Tea | 10 | 10 | | | | 10 |
| **Foods of Animal Origin** | | | | | | |
| 77. Egg | 42 | 40 | 40 | 93 | 100 | 100 |
| 78. Milk and Milk products | 25, 216, 91 | 90 | 216 | 300 | 316 | 316 |
| 79. Meat | 156 | 109 | 156 | 291 | 220 | 291 |
| 80. Fish and aquatics | 92 | 88 | 92 | 164 | 214 | 214 |
| 81. Other marine products | 20 | 35 | 35 | 98 | 115 | 115 |

## Format for Submitting Data on Pesticide Residue and Toxicity in Crop Commodities by the applicants seeking Registration/MRL Fixation

### I) GENERAL INFORMATION:

**CHEMICAL ( Molecule name)**

**1. Identity**

- ISO common name:
- Chemical name
  IUPAC:
- CAS:
- CAS registry No.:
- Structural formula:
- Molecular weight:

**2) Active Ingredient**

Physical Properties

- PHYSICAL STATE :
- COLOUR :
- DENSITY :
- MELTING POINT :
- STABILITY (Time & Temperature to be mentioned) :

Chemical Properties

- OCTANOL WATER PARTITION COEFFICIENT :
- SOLUBILITY (At $25^0$C) :
- HYDROLYSIS :
- PHOTOLYSIS :
- REFERENCE TO FAO SPECIFICATIONS FOR TC OR TK :

**3). Formulations**

Provide a list of commercially available formulations in India:
Type of proposed formulation:

Properties:

- Physical State :
- Colour :
- Strength Of Formulation :
- Density :
- Solubility/ Miscibility :

## SUMMARY OF GOOD AGRICULTURAL PRACTICES FOR PESTICIDE USE IN CROPS

| | |
|---|---|
| Responsible body for reporting:<br>(Company Name, Address, email and telephone number)<br>Date | |
| Pesticide (Common name): | |
| Trade name: | |
| Type of Pesticide<br>(Insecticide/ Fungicide/ Herbicide / Others) | |

### (I). Proposed Pesticide Use Pattern and Pre-Harvest Interval

| Crop and/or stage when pesticide is applied | Targeted Pest or group of pests | Formulation | | Spray application on the target crop | | | | | Pre-harvest interval (PHI) (days) |
|---|---|---|---|---|---|---|---|---|---|
| | | Type | Conc. of ai | Method Type of pesticide sprayer | Crop growth stage | number (range) | kg ai/ha | Water l/ha | |
| | | | | | | | | | |

**II) METABOLISM AND ENVIRONMENTAL FATE:**

**(A) Crop Metabolism studies**

**(B) Animal metabolism studies**

**III) FATE AND BEHAVIOUR IN SOIL:**

**IV) FATE AND BEHAVIOR IN WATER/ /WATER-SEDIMENT SYSTEMS:**

**V) DATA ON RESIDUES-**

**A) INFORMATION ON SUPERVISEDFIELD TRIALSFOR RESIDUE STUDIES**

| **Details** | **Location-1** | **Location -2** | **Location-3** | **Location-4** |
|---|---|---|---|---|
| Name of the institute where residue field trial has been carried out | | | | |
| Name of the institute where residue analysis has been carried out | | | | |
| **Application Data** | | | | |
| Name of the crop including variety | | | | |
| Crop sowing date | | | | |
| Crop transplanting date, as applicable | | | | |
| Trial Layout/ Experimental Design | | | | |
| Plot size (sq m) | | | | |

| | | | | |
|---|---|---|---|---|
| Number of plants per plot / unit area (for orchards) | | | | |
| Number of plots per treatment | | | | |
| Method of application and equipment | | | | |
| Number of applications and application dates | | | | |
| **Application details** | | | | |
| Dose rate | | | | |
| Spray volume | | | | |
| **CLIMATICCONDITIONS** | | | | |
| | **Location-1** | **Location-2** | **Location-3** | **Location-4** |
| Av. Min. temp ($^{o}$C) | | | | |
| Av. Max. temp ($^{o}$C) | | | | |
| Max. Relative Humidity | | | | |
| Min. Relative Humidity | | | | |
| Average Relative Humidity(%) | | | | |
| Rainfall(mm) | | | | |
| **SAMPLINGDATA** | | | | |
| No. of samples taken per test/treatment | | | | |
| Sample treatment, viz., chopping, wrapping, packing, transport from crop field to analytical lab etc. | | | | |
| Sample weight and preparation | | | | |

| | | | | |
|---|---|---|---|---|
| Data of sampling with time | | | | |
| Interval between application and sampling | | | | |
| Sample storage conditions before analysis | | | | |

## B) METHOD OFANALYSIS

| **Details of method** | |
|---|---|
| Name of equipment | |
| Limit of determination | |

**REPLICATION WISE RECOVERY DETAILS**

**(completely randomized design is the statistical tool for 3 treatments and 6 replicates)**

| **Fortification Level (µg/g)** | **Mean Recovery (µg/g)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Name of pesticide chemical molecule** | | | | | | | |
| | **Crop** | | | | **Soil** | | | |
| | R1 R2 | R3 R 4 | R5 R6 | Mean | R1 R2 | R3 R 4 | R5 R6 | Mean |
| | | | | | | | | |
| | | | | | | | | |
| ANALYSIS OF VARIANCE (ANOVA) | | | | | | | | |
| | | | | | | | | |
| **Fortification Level (µg/g)** | **Mean Recovery (µg/g)** | | | | | | | |
| | | | | | | | | |
| | **Crop** | | | | **Soil** | | | |

| | R1 R2 | R3 R 4 | R5 R6 | Mean | R1 R2 | R3 R4 | R5 R6 | Mean |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| ANALYSIS OF VARIANCE (ANOVA) | | | | | | | | |
| **Fortification Level (µg/g)** | **Mean Recovery (µg/g)** | | | | | | | |
| | | | | | | | | |
| | **Crop** | | | | **Soil** | | | |
| | R1 R2 | R3 R 4 | R5 R6 | Mean | R1 R2 | R3 R 4 | R5 R6 | Mean |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| ANALYSIS OF VARIANCE (ANOVA) | | | | | | | | |

## C) RESIDUE DATA- THROUGH FIELD EXPERIMENT (RANDOMISED BLOCK DESIGN)

### Residues of pesticide on Crop commodity and soil

| Sampling Day | Name of crop commodity / | Location 1 (,….) | | | | Location 2 (…) | | | | Location 3 (…) | | | | Location 4 (….) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **T0: Untreated control** | | | | | | | | | | | | | | | | | |
| | | R1 | R2 | R3 | Mean | R1 | R2 | R3 | Mean | R1 | R2 | R3 | Mean | R1 | R2 | R3 | Mean |
| **Crop 0** | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
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| **1** | | | | | | | | | | | | | | | | | |
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| **3** | | | | | | | | | | | | | | | | | |
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| **5** | | | | | | | | | | | | | | | | | |
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| **7** | | | | | | | | | | | | | | | | | |
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| **10** | | | | | | | | | | | | | | | | | |
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| **p** | | | | | | | | | | | | | | | | | |
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| **Soil** | | | | | | | | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | |
| **T1: Optimum dose** | | | | | | | | | | | | | | | | | |
| **Crop (0)** | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| **1** | | | | | | | | | | | | | | | | | |
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| **3** | | | | | | | | | | | | | | | | | |
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| **5** | | | | | | | | | | | | | | | | | |
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| **7** | | | | | | | | | | | | | | | | | |
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| **10** | | | | | | | | | | | | | | | | | |
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| **Crop** | | | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | | | |
| **Soil** | | | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | | | |
| **T-2 dose** | | | | | | | | | | | | | | | | | |
| **Crop (0)** | | | | | | | | | | | | | | | | | |
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| **1** | | | | | | | | | | | | | | | | | |
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| **3** | | | | | | | | | | | | | | | | | |
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| **5** | | | | | | | | | | | | | | | | | |
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| **7** | | | | | | | | | | | | | | | | | |
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| **10** | | | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | | | |
| **Crop commodity** | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| **Soil** | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| **ANALYSIS OF VARIANCE (ANOVA)** | Crop | | | | | | | | | | | | | | | | |
| | soil | | | | | | | | | | | | | | | | |

**BDL – Below Detectable Level**

**D). Proposed Waiting Period/MRL**

| | |
|---|---|
| Proposed waiting period (Pre-harvest interval) | |
| Proposed tolerance limit | |
| Prescribed MRL on registered crop in other countries (ppm) | |
| National MRLs of the pesticide on other crops (ppm) | |

**TOXICOLOGY**

**Summary of Acute Toxicity Studies With....:**

| Species | Strain | Sex | Route | Batch No.; Purity(%) | $LD_{50}$(mg/Kg Bw) | $LC_{50}$ (mg/L) | Results | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

**Test Results of Genotoxicity Studies with …….:**

| Test system | Test compound Strain/species/cell line used | Concentrations | Purity (%) | Result | Reference |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

**SUMMARY OF PIVOTAL TOXICOLOGICAL STUDIES [GIVE DETAILS OF SUB ACUTE, CHRONIC AND SUPPLEMENTAL STUDIES SEPARATELY]**

**SUB ACUTE**

| Studies | Species (Strain) No.of animals | Duration | Purity | Dose Levels/ Regimen and Route Administration | NOAEL (mg/Kg bw/Day) | LOAEL (mg/Kg bw/Day) | Critical Effects | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |

**CHRONIC**

| Studies | Species (Strain) no. of Animals | Duration | Purity | Dose Levels/Regimen and Route of Administration | NOAEL (mg/Kg bw/Day) | LOAEL (mg/Kg bw/Day) | Critical Effects | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |

**SUPPLEMENTAL STUDIES**

| Studies | Species (Strain) No.of Animals | Duration | Purity | Dose Levels/Regimen and Route Of Administration | NOAEL (mg/Kg bw/Day) | LOAEL (mg/Kg bw/Day) | Critical Effects | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |

**Note: Reports to be enclosed as part of CD**

Acceptable Daily Intake (ADI): ..... mg/kg bw/day

Basis of calculations of ADI:.....

National maximum residue limits on other registered crops: .....

Residue definition:.....

Labels and Leaflets (attach):

**Note: Compact Disk containing the above information and data are to be accompanied with the hard copy.**

**General Checklist for submission of information for fixation of MRLs for new pesticides**

(The specific Checklist of documents must be made by the CIB-RC after review of the data of the applicant by the Experts)

For fixation of MRLs complete information/details on various parameters of residues and toxicology of pesticides are essential in the enclosed proforma by Ministry of Health and Family Welfare. The Registration Committee Secretariat may ensure that the information received from the registrants is complete from all aspects so that MRLs are fixed correctly and without any delay.

1. Name of pesticides and the crop on which the MRLs is to be fixed- ….. in ….
2. Date on which application was received by the Registration Secretariat.
3. Date on which application with details sent to the Ministry of Health and Family Welfare.
4. General information-

| | | | |
|---|---|---|---|
| A) | IDENTITY | YES | NO |
| B) | PHYSICALANDCHEMICALPROPERTIES | YES | NO |
| C) | TECHNICALMATERIAL | YES | NO |
| D) | FORMULATION | YES | NO |
| E) | METABOLISM AND ENVIRONMENTAL FATE | YES | NO |

5. Application data on supervised trials (Information in respect of following is provided or not)

| | | | |
|---|---|---|---|
| A) | TRIALCONDUCTED | YES | NO |
| B) | COMMODITY | YES | NO |
| C) | NAME OF THE INSTITUTE WHERE SUPERVISED TRIALS WERE CARRIED | YES | NO |
| D) | NAME OF THE INSTITUTE WHERE RESIDUE ANALYSIS WERE CARRIED OUT | YES | NO |
| E) | CROP PLANTING /SOWING DATA | YES | NO |
| F) | PLOT SIZE IS MENTIONED | YES | NO |
| G) | NUMBER OF PLANTS PER PLOT | YES | NO |
| H) | NUMBERS OF TREATMENTS PROVIDED | YES | NO |
| I) | METHOD OF APPLICATION AND EQUIPMENT | YES | NO |
| J) | NO.OF APPLICATION AND DATES | YES | NO |
| K) | DOSE RATIO | YES | NO |
| L) | SPRAY VOLUME | YES | NO |

| | | | |
|---|---|---|---|
| M) | GROWTH STAGE AT LAST TREATMENT | YES | NO |
| N) | OTHER PESTICIDESAPPLIED TO TRIAL PLOTS WITH RELEVANT DETAILS | YES | NO |
| O) | CROP GROWTHSTAGEAT THE LASTSPRAYTREATMENT | YES | NO |

6. Sampling data

| | | | |
|---|---|---|---|
| A. | DETAILS OF NO. OF SAMPLES TAKEN PER TEST | YES | NO |
| B. | DETAILS OF SAMPLE WEIGHT AND PREPARATION | YES | NO |
| C. | DETAILS OF SAMPLING WITH TIME | YES | NO |
| D. | INTERVAL BETWEEN LAST APPLICATION AND SAMPLING | YES | NO |
| E. | ARE THE DATA ON THE FOLLOWING GIVEN: WAITINGPERIOD PRE-HARVESTINTERVAL | YES | NO |

7. Method of analysis:

| | | | |
|---|---|---|---|
| A) | COMPLETE METHODOF ANALYSIS AS PER **BIS** FORMAT | YES | NO |
| B) | RESULTS OF RECOVERY EXPERIMENTS INDICATING LEVEL FORTIFICATION | YES | NO |
| C) | DETAILS OF EQUIPMENT PROVIDED | YES | NO |
| D) | LIMIT OF DETERMINATION IS INDICATED | YES | NO |

8. Climatic Conditions: whether details of the following are provided:

| | | | |
|---|---|---|---|
| A) | AVERAGE MIN. TEMPERATURE (DEGREE CELSIUS) | YES | NO |
| B) | AVERAGE MAX. TEMPERATURE (DEGREE CELSIUS) | YES | NO |
| C) | MINIMUM RELATIVE HUMIDITY | YES | NO |
| D) | MAXIMUM RELATIVE HUMIDITY | YES | NO |
| E) | AVERAGE RELATIVE HUMIDITY | YES | NO |
| F) | RAINFALL(MM) | YES | NO |

9. Data on toxicity-whether information on the following is provided:

| | | | |
|---|---|---|---|
| A) | ACUTE ORAL RAT $LD_{50}$ | YES | NO |
| B) | ACUTE ORAL MICE $LD_{50}$ | YES | NO |
| C) | ACUTE DERMAL $LD_{50}$ | YES | NO |
| D) | ACUTE INHALATION $LC_{50}$ | YES | NO |

| | | | |
|---|---|---|---|
| E) | MUTAGENICITY NAME OF TESTS DOSES USED RESULTS | YES | NO |
| F) | TERATOGENECITY RAT<br>RABBIT | YES<br>YES | NO<br>NO |
| G) | EFFECTONREPRODUCTION(RAT) | YES | NO |
| H) | CARCINOGENECITY(RAT/MICE)NOEL | YES | NO |
| I) | TOXICITYTO LIVESTOCK(ANIMALNAME) | YES | NO |
| J) | ADI | YES | NO |
| K) | BASIS OF CALCULATION OF ADI | YES | NO |
| L) | HAS THE PESTICIDE BEEN REVIEWED BY JMPR OR ANY OTHER INTERNATIONAL ORGANISATION? | YES | NO |
| M) | IF SO,WHETHER DETAILS HAVE BEENPROVIDED? | YES | NO |
| N | HAS THE PROPOSED MRL OF THE PESTICIDE IN CROP BEEN GIVEN? | YES | NO |
| O) | HAS MRLFIXED BY OTHER COUNTRIES ON THE PROPOSED FOOD COMMODITY BEEN SUBMITTED | YES | NO |
| P) | HASTHE RESULTS OF THE RESIDUE ANALYSISFOR THREE SEASONS/ MULTI LOCATION TRAILS BEEN SUBMITTED? | YES | NO |
| Q) | HAVE YOU GIVEN IN FORMATION ON USE PATTERNS | YES | NO |
| R) | HAVE YOU GIVEN INFORMATION ON GAP INFORMATION | YES | NO |
| S) | HAVE YOU GIVEN INFORMATION ON RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS | YES | NO |
| T) | HAVE YOU GIVEN INFORMATION ON NATIONAL MAXIMUMRESIDUELIMIT | YES | NO |
| U) | HAVE YOU GIVEN INFORMATION ON RESIDUE DEFINITION | YES | NO |