



Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

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

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(A Statutory Authority established under the Food Safety and Standards Act, 2006)
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List of abbreviations

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
CCPR	Codex Committee on Pesticide Residues
CEP	Critical End Point
CIB&RC	Central Insecticide Board and Registration Committee
cGAP	Critical GAP
CXL	Codex MRL
DAT	Days After Treatment
DM	Dry Matter
DT ₅₀	50% dissipation time (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
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 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 Estimated Short-term 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	Lethal Concentration 50
LD ₅₀	Lethal Dose 50
LOAEL	Lowest-Observed-Adverse-Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification
MRL	Maximum Residue Limit
MRDB	Maximum Reasonably Balanced Diet
ND	Not detected
NEDI	National Estimated Daily Intake
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
SC	Suspension Concentrate
SL	Soluble Liquid
SD	Standard Deviation
STMR	Supervised Trials Median Residue
STMR-P	Supervised trials median residue in a processed commodity
TF	Transfer Factor
TLC	Thin-Layer Chromatography
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

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 regulations 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. The pesticides which are efficacious against the target pests (insects, mites, nematodes, weeds and plant diseases); and are 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 Limits (MRLs) 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 on registered crops approved by CIB&RC. The Food Safety and Standard Authority of India (FSSAI) under Ministry of Health and Family Welfare evaluates the supervised trial residue data based on the approved GAP for fixation of MRL, keeping in view the dietary exposure and risk assessment of the pesticide, only after its approval by the Registration Committee (RC).

At International level, Codex Alimentarius Commission (CAC) since 1970s has been periodically prescribing guideline documents for fixation of MRL, based on the recommendations of FAO/WHO Joint Meeting on Pesticide Residues (JMPR) and these are being updated periodically as per scientific development, fresh experience/lessons 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 pesticides.

Keeping in view the above objectives, the Scientific Panel on Residues of Pesticides and Antibiotics of FSSAI in its 36th 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) Late 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 were 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 would 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 the first meeting.
- 4) Necessary infrastructure including secretarial facility was provided by FSSAI to facilitate timely accomplishment of work.
- 5) Since the work assigned involves multidisciplinary approach, working group could co-opt any expert for the purpose. However, prior approval of FSSAI has to 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 47th meeting held on 25.01. 2017. The Panel further constituted a Group comprising 4 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 MRLs of Pesticides in Food Commodities
- b) Prepare the comparative analysis of the MRL recommended by the Scientific panel vis-à-vis Codex
- c) Examine National Institute of Nutrition (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 had requested for a special expert view for finalisation of the SOP. Hence, the following two experts were specially invited for the 4th and 5th 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 10th April, 2018. The Working Group as well as the Review Group placed on record the special contribution made by Late Dr. S.K Handa in the 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 Guidance Document on “Standard Operating Procedures on fixation of Maximum Residue limits” was drafted.

The Document consists of ten Sections as briefed below: -

Section 1: Introduction to the document has been given with emphasis on developments in the field of Science and Technology, need for framing the guidelines and its importance for the scientists, analysts, regulators, policy makers and stakeholders to understand the basic principles of data generation, evaluation, risk assessment, fixation of MRL and implementation thereof.

Section 2: Deals with 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

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 pesticide level of vegetables and fruits at PHI of 3 and 15 days is to be taken as guidance for fixing MRLs and actual PHI depends on the field practices under GAP, the reference body weight of 60 kg based on the recommendations of the NIN-ICMR; adoption of food consumption factor moderated from the latest National Institute of Nutrition (NIN) data.

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: Explains about risk characterization.

Section 9 Describes in detail the commodities that are processed before consumption and calculation of MRLs for processed commodities from the MRL of RAC using processing factor.

Section 10: Describes fixation of MRL on animal feed, meat, milk, offals and eggs

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, and in addition 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 CIB&RC. As per the requirement, 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 guidelines 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. During the same period the registration process for pesticides and the requirement for data on pesticide residues in crops, water and soil were standardized. Pesticide Tolerance Limits were decided and notified by the Central Committee for Food Standards (CCFS) under the PFA 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 developments 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. However, 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 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 derivation of 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/cGAP
- 5) Dietary Exposure and Risk Assessment.
- 6) Fixation of MRL in RACs.
- 7) Fixation of MRL in processed foods.
- 8) Fixation of MRL in animal products (Milk and Milk products, Meat and Meat Products and Eggs)

It 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 (HR) to workout MRLs.

Keeping this in view, the Scientific Committee of FSSAI on the recommendations 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 RACs is given. The document covers only the general guidelines, and the reader may need-based refer the other documents for further details.

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

These 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 MRLs also requires the following :

- ADI derived from Toxicological studies.
- ARfD derived from Toxicological data.
- Toxicological properties of plant metabolites
- Residues from Supervised Trial as per approved cGAP- STMR, HR, MRL
- Analytical methods-LOQ
- Animal metabolism/Animal feeding study
- Processing studies-Processing factor
- Fixation of MRL using OECD Calculator
- Food consumption data
- 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 including agro-chemicals/pesticides, pesticide applicator manufacturers, agricultural commodities and products, and environmental agencies.
2. Research Institutes that study the behaviour of pesticide residues, by conducting supervised field trials and determining pesticide residues in crops and the environment.
3. Regulators such as: CIB&RC, FSSAI
4. Traders in Agricultural commodities-both domestic and international.

5. Food toxicologists and environmentalists

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

1. Pesticide Industry- what type of data is required for fixation of MRL
2. Research Institutes/Laboratories who shall generate data for fixation of MRL
3. CIB&RC Secretariat experts/members for know how to evaluate the data
4. Secretariat of Scientific Panel of FSSAI-what data need to be submitted/what and how to scrutinise
5. Members of FSSAI-Scientific Panel on pesticide residues

SECTION 2

2. FIELD TRIALS

2.1 Good Agricultural Practices (GAPs):

Good Agricultural Practices (GAPs) in 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 statutory 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 discussions 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 overuse 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.

2.2 Field Trial Parameters:

The user of this document is advised to refer OECD document 509¹ for the purpose of planning field trials for MRL fixation.

2.2.1 Number of trials:

Prior to planning 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; 2016²*).

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 meters for 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 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:

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

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 Emulsifiable 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 control, proposed dose (X), cGAP dose (25% higher than proposed dose i.e 1.25X) and each treatment should be replicated as per the statistical principles and requirements.

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, it 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., gram active ingredient (*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 behaviour 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, labelling, 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.

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 collect 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.

3.1.2.1 Sampling Procedure:

‘Sample Collection’ is the process of taking portion(s) of a food or other substance for laboratory analysis so that the resulting analytical data and conclusions can be applied to the original food or substance sampled. The quality results can be obtained as from a true representative sample.

3.1.3.1 Sample Preparation

Once the sample has arrived at the laboratory, the analyst must prepare the samples for an analysable sample portion that represent the whole sample. The first step is to reduce the total sample to a manageable portion i.e. sub sampling. A reserve portion of the original unprepared sample is necessary and hence should be stored for future use, such as for check analysis or for additional analysis required for confirmation test. The final step is to physically homogenize sub sample aggregate by chopping, grinding, blending etc.

3.1.4.1 Storage of the Sample

Polythene bags, glass beakers or vials may be used as containers for holding the samples. It should be borne in mind that if the samples are not stored properly changes may take place during storage leading to unreliable results. Usually, the samples are stored in deep freezer at -10°C or below. Control sample extracts fortified with pesticides can be stored along with sample to know the degradation, if any, during storage.

3.1.3 Sample size:

Under normal circumstances, one sample per plot (one composite sample from many sub-samples collected from throughout the plot as per para 3.1.1 above is sufficient. Additional samples may be taken in case 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 in field for fixation of MRLs - OECD-509 / Protocol for sampling for residue and persistence analytical test studies

3.1.4 Sampling intervals:

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 last spray. For all dissipation studies with foliar sprays, the samples should be collected from zero day (2 hours after spray) onwards till the residues reach the limit of quantification (LoQ) or crop at the harvest stage, whichever is earlier. The

interval of sampling should be spaced somewhat equally and, where possible, sampling should occur at shorter (0,1,3,7,10,15,20 days) and longer time points (5 day intervals) relative to target PHI. (e.g., if the residue on day 3 is at LoQ, the residue need not be estimated further).

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.

MRL need to be fixed both for RAC and for the edible portion 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 general sampling procedures for residue data generation for MRL fixation are given in OECD-409, Crop field trial.

3.2 Residue Definition

The sample analysis for pesticide residues is an important step and should be performed with highest accuracy and reliability. Prior to analysis, the concept of residue definition should be understood clearly. The residue definition of the target parent pesticide compound, and all the compounds (parent and its metabolites) in the definition are 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 of 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 is available in the published literature. In the case of new pesticide compounds, metabolism studies are conducted to establish metabolic pathways and identify major metabolites of toxicological significance. 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 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., RNH₂). For example, sum of the herbicide 2,4 D, its salts and esters expressed as 2,4 D, and sum of methiocarb, its sulfoxide 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 sulfoxides 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 sulfoxides 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 MRL, 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 (CS₂), 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 CS₂ evolved. Therefore, for

example, residue definition of mancozeb for compliance with MRLs can only be expressed as total dithiocarbamates, determined as CS₂ and expressed as mg CS₂/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 LoQ of 0.01 mg/kg or lower. If it is difficult to achieve 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.

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 is 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 residues in various matrices, but as they do not provide quantitative data, they are not taken into account in estimation of residue levels.

Analytical methods are used to generate the data for estimating dietary exposure, to establish 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 purposes, the surveillance laboratories prefer multi-residue methods, which could include a large number of analytes, as the laboratories do not find practical feasibility 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 so as to provide the flexibility to establish two separate residue definitions where appropriate, one for dietary risk assessment and the other one for MRL compliance monitoring. In such cases, wherever possible, one should either separately analyse 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 / to be submitted, methods for determination of residues in products of animal origin should be validated in the 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 2-10× 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 120 percent with Relative Standard Deviation (RSD) (n=6 or more) less than 20%.

Analytical methods provided should include:

- specialized 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 succeeding 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 fate 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 for MRLs in spices.

4.1.1 Basic properties of the Compound:

4.1.1.1 Identity and physico-chemical properties

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

4.1.1.2 Physical 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 temperature)

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 technical material and 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 crops, 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 also, 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 is used in evaluating both the toxicological and residue profiles of pesticides. The metabolism in experimental animals is examined and compared 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 need 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 changes during and after application to crop plants, that falling on soil 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 radiolabelled 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 (^3H) 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 chemical a.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 of the pesticide applied on edible tissues of food and / or forage crops, milk and eggs, as the case may be. In many cases, it may not be possible to identify significant portions of the total radioactive residues, especially when the total amount of residues is low, when the residues get incorporated into biomolecules, or when the a.i. is extensively metabolized 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 radiolabelled studies, such data would generally not be necessary. The radio validation of the sample preparation process should be a part 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 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 and shown. If changes are observed, e.g., disappearance of a particular HPLC/ LC-MS/MS 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 the following 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 authorized pesticide 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 a pesticide differently. Hence, full and detailed information will be required for the GM crop with metabolism differences from the non-GM crop. For GM crops that do not involve the insertion of alien gene(s) to offer pest (insects / plant diseases) 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 and presented 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 in the non-GM crop, 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. Crops that are sometimes consumed at an immature stage, such as baby corn or leafy salads, should also be sampled and taken 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 various RACs, thus indicating the components to be analysed in residue quantification studies, i.e., the residue definition(s) for both risk assessment and enforcement.

- Elucidation of 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 an 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/ live stock metabolism studies are required for ruminants and poultry in line with Guidance Document for Toxicology for Registration of Chemical Pesticides in India, September 2017 published by CIB&RC. 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 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 residues 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.

In general, the studies include examining the hydrolysis rate, photochemical degradation, and aerobic soil degradation, to name some. These studies are normally required for all pesticides except those with a legally specific use only for seed treatment and post-harvest applications 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 residues obtained from supervised field trials conducted according to the most critical GAP. The field residue data for all agricultural pesticides shall be used in order to calculate and fix MRL. However, monitoring data shall be used for certain specific commodities wherever GAP does not exist and need is felt.

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

- (1) Value 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 = varies 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+/-4SD” methodology. The output value of the OECD calculator formula is based on one of the three approaches viz: Mean+/-4SD, 3x mean x correction factor (F) or HR whichever is higher. 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 (Table 1).

The residue data collected at 3 days after application X dose (recommended) is taken into consideration for calculation of MRLs using OECD MRL calculator.

Pesticide	: Cypermethrin
Crop	: Cauliflower
Waiting Period	: 3 days
Number of locations	: 10
Recommended dose (X)	: 50 grams active ingredient / hectare (GAP)
2X dose	: 100 grams active ingredient / hectare (cGAP)
Number of sprays	: 2
First spray	: Curd formation stage
Second spray	: 10 days after first spray

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

Sampling Interval Days after application	Residue (mg/kg) (Mean Value)* at X Dose,									
	PAU, Ludhiana	CCS, HAU, Hisar	Dr. YSPUHF, Solan	IARI, New Delhi	MPKV, Rahuri	AAU, Anand	IIHR, Bangalore	KAU, Vellayani	CSAUAT, Kanpur	PJTSAU, Hyderabad
0	0.40	0.40	0.53	0.12	0.34	0.34	0.60	0.66	0.63	0.38
1	0.26	0.34	0.36	0.08	0.25	0.19	0.53	0.43	0.42	0.31
3	0.17	0.20	0.13	0.05	0.16	0.16	0.44	0.20	0.31	0.24
5	0.06	0.15	0.08	BDL	0.09	BDL	0.34	0.15	0.20	0.19
7	BDL	0.09	BDL	BDL	0.06	BDL	0.29	0.09	0.09	0.10
10	BDL	0.05	BDL	BDL	BDL	BDL	0.15	0.07	0.03	BDL
15	BDL	BDL	BDL	BDL	BDL	BDL	0.11	BDL	BDL	BDL
20	-	-	-	-	-	-	0.05	-	-	-
25	-	-	-	-	-	-	BDL	-	-	-
30	-	-	-	-	-	-	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 replications) at proposed PHI (say 3 days) after last application from various locations is fed into OECD MRL calculator and the rounded MRL is considered for MRL fixation subject to passing of chronic dietary risk at STMR and Short Term Dietary risk assessment at HR or STMR.

The following is the output using the above input data.

	A	B	C	D	E	F	G	H	I	J	K	L
1												
2		Cypermethrin										
3		Cauliflower										
4		India										
5		GAP										
6												
7		Residues (mg/kg)										
8		0.170										
9		0.200										
10		0.130										
11		0.050										
12		0.160										
13		0.160										
14		0.440										
15		0.200										
16		0.310										
17		0.240										
18												
19												
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Cypermethrin
Cauliflower
India
GAP

Residues (mg/kg)	n
0.05	1
0.13	1
0.16	2
0.17	1
0.2	2
0.24	1
0.31	1
0.44	1

Cypermethrin
Cauliflower
India
GAP

Total number of data (n)	10
Percentage of censored data	0%
Number of non-censored data	10
Lowest residue	0.050
Highest residue	0.440
Median residue	0.185
Mean	0.206
Standard deviation (SD)	0.107
Correction factor for censoring (CF)	1.000
<u>Proposed MRL estimate</u>	
- Highest residue	0.440
- Mean + 4 SD	0.633
- CF x 3 Mean	0.618
Unrounded MRL	0.633
Rounded MRL	0.7

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 Dietary Exposure Estimation of Pesticide Residues

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 data have been prepared based on surveys conducted by the National Institute of Nutrition, Hyderabad (2020).

For example, as per Case study No. 1, the median consumption of cauliflower is 78 g/day and this value is used in calculation of long-term effects. On the other hand, 215 g/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 (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level) are identified. The derivation of Bench Mark Dose (BMD) is ideal.

7.2 Derivation of Acceptable Daily Intake (ADI)

Based on the Toxicological 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/kgbw/day) whereas ARfD used as a number.

SECTION 8

8. RISK CHARACTERIZATION

To characterize the risk, the exposures to pesticide residue through all types of commodities for which MRLs are prescribed are compared with the health based guidance value.

8.1 Derivation of National Estimated Daily Intake (NEDI)

After obtaining information on STMR values, dietary consumption and HBGV, the NEDI will be calculated. NEDI is calculated to assess the long-term dietary intake risk analysis, taking residue level equal to calculated STMR 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 STMR/ HR for short term risk assessment. Sum total of NEDI values for all foods will be compared with the ADI.

NEDI 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 STMR and the consumers are consistently exposed during their entire life.

NEDI is calculated using following formula:

$$\text{NEDI} = \sum \text{STMR} \times \text{Fi}/bw$$

Where,

STMR = Supervised Trial Median Residue

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

bw = Mean Body Weight

8.2 Derivation of Acceptable Daily Intake (ADI) per person

This is obtained by multiplying the value of ADI by 60 Kg which is taken as the reference body weight for the calculation of risk assessment under Indian context. The reference body weight has been derived from the study conducted by the NIN, ICMR and Ministry of Health across the age group 16 to 70 years.

8.3 Comparison of NEDI 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 NEDI is $\leq 80\%$ ADI, there is least risk, and hence the pesticide on the crop is approved as per the prescribed use (GAP). Conventionally, maximum NEDI 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, NEDI = or \geq 100% ADI), the pesticide would not be approved for use under the conditions of supervised field trials. It means, if the NEDI = or \geq 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 NEDI comes below the 80% of ADI.

e.g. in Case Study 1

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

Case 1: If pesticide is approved only on this crop.

- The calculated / rounded STMR is 0.7 mg/kg
- The ADI of Cypermethrin is 0.05 mg/kg body weight/day. = 0.05 x 60 = 3 mg/person
- Food factor for cauliflower (per capita food consumption in India) is 0.078 kg /day/person.

$$\text{NEDI} = \Sigma \text{STMR} \times \text{Fi}/\text{bw}$$

Where,

NEDI = National Estimated 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)

$$\text{NEDI} = \frac{0.7 \text{ mg/kg} \times 0.078 \text{ kg/person/day}}{60 \text{ kgs}}$$

$$\text{NEDI} = 0.00091$$

$$\% \text{ ADI} = \frac{\text{NEDI}}{\text{ADI}} \times 100$$

$$\% \text{ ADI} = \frac{0.00091}{3} \times 100 = 0.03 \%$$

In this case, the calculated MRL for cypermethrin on cauliflower is 0.7 mg/kg, and based on the risk assessment, the NEDI is 0.03% of ADI. Hence, the MRL of 0.7 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.

Case 2: If pesticide is already approved on other crops and first time registration on this crop.

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 NEDI from this trial to be added to \sum NEDI 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. In this case, NEDI from cypermethrin residues in cauliflower are added to the \sum NEDI and the % ADI is 0.20 % and hence MRL of cypermethrin on cauliflower can be recommended as 0.7 mg/kg with 3 days PHI (Table 2)

Calculations for Cypermethrin on cauliflower:

NEDI = 0.00091 mg/person/day

Sum of NEDI from all other approved uses = 0.005826 mg/person/day

ADI for Cypermethrin = 0.05 mg/kg/day

Reference body weight = 60 kg

ADI per person = $0.05 \times 60 = 3$ mg/person/day

%ADI (cypermethrin on all approved crops) = $0.005843 \times 100 / 3 = 0.20$ % (approx)

Case 3: Use of National Monitoring Data

In continuation to the existing data given in Case Study 1, 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 \sum NEDI is less than the ADI.

Table 2: Risk characterization of cypermethrin residue on Cauliflower

Food Commodity	STMR calculated (rounded) based on Residue Data from Supervised Field Trials (mg/kg) or MRLs fixed by FSSAI	Pre Harvest Interval (days)	Food Consumption (kg/person/day)	Reference Body Weight (bw) (Kg)	NEDI (mg/person/day) (Col 2xCol 4)/bw	ΣNEDI (mg/person/day)	ADI(mg/kg body Weight)	ADI per person (mg/per person (ADI*60)	% ADI (Col 7*100/Col 8)
1	2	3	4	5	6	7	8	9	10
Cauliflower	0.7	3	0.078	60	0.00091	0.005826	0.05	3	0.1942
Tomato	0.2		0.29	60	0.000966667				
Brinjal	0.2		0.049	60	0.000163333				
Cabbage	2		0.087	60	0.0029				
Okra	0.2		0.068	60	0.000226667				
Wheat	0.05		0.192	60	0.00016				
Rice	0.01		0.257	60	0.0000257				
Oil Seeds	0.2		0.034	60	0.000113333				
Milk, Milk Products	0.1		0.216	60	0.00036				

Residue Evaluation

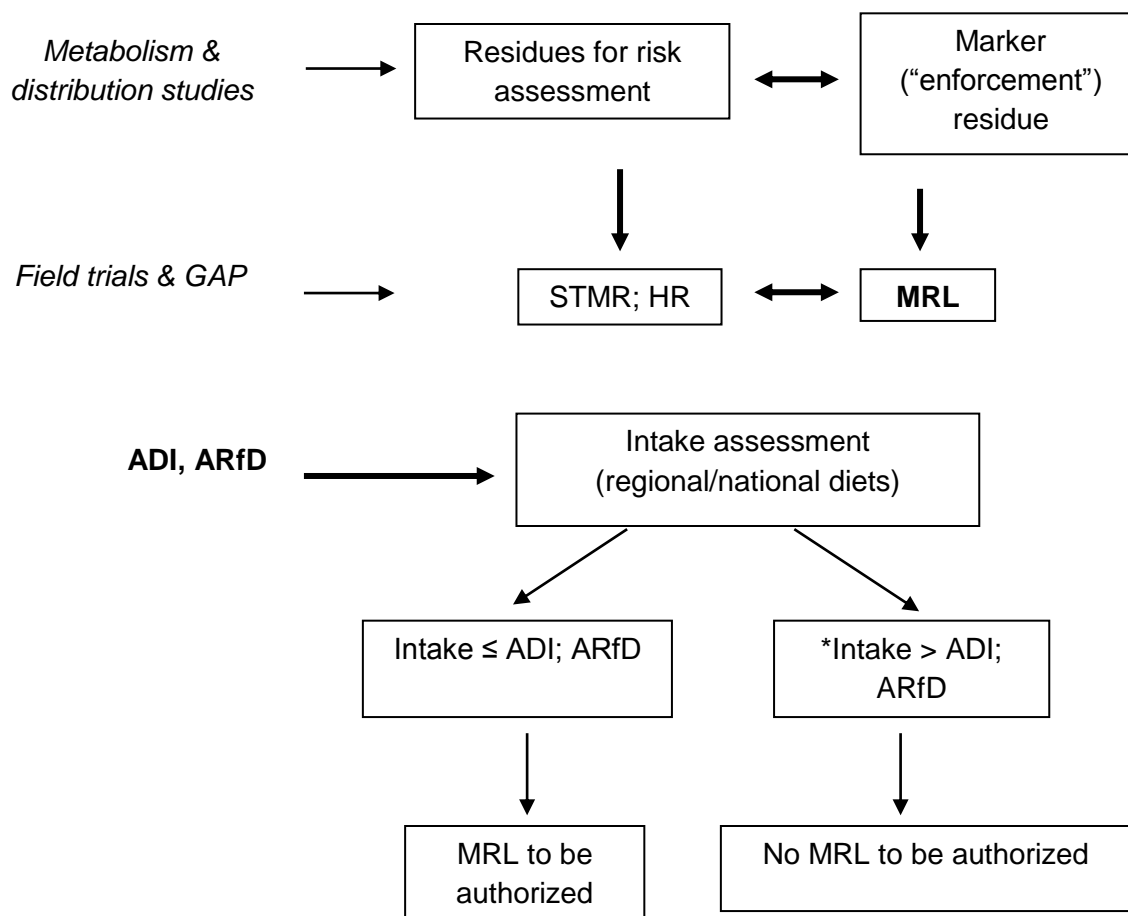


Fig. Evaluation of residue data for fixation of MRL

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

SECTION 9

9. FIXING OF MRL OF PESTICIDES IN PROCESSED FOODS

9.1. Introduction

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). RAC are subjected to various processing methods and can be designated as Processed Agriculture commodity (PAC) which are further processed for preparation of various processed foods for human consumption. In general foods that are subjected to technological modifications either for preservation or for converting into ready-to-use foods are designated as “processed foods”. Processing of an RAC may have significant impact on the pesticide residue levels contained therein and/or there-on. The levels may decrease/remain the same or increase in the processed fractions depending on the physico-chemical properties of the pesticide and its concentration in RAC. 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 commodity/ fraction and RAC is denoted as processing factor(pf).

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 pesticides in processed food. Information obtained from processing studies may serve 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.

9.2 Processing Factor (pf)

Raw agricultural commodities are subjected to physical, chemical or biological processes to obtain processed commodities/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. Currently no harmonized list of processing factors is available within Europe and worldwide. Considering variety of processed foods, it is certainly not possible to establish MRLs for all the processed commodities which are employed in their preparation. 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 reduction in pesticide residue levels 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 as 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 commodity/ food. However, 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 \leq LOQ in the RAC (as per cGAP), 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 considered as an indispensable tool and primarily serve two major purposes:

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

9.3 Processing Studies - General Guidelines

Processing studies are almost exclusively conducted on a very limited number of representative commodities. 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.

9.3.1 Objectives of processing studies

1. To obtain information about breakdown or reaction products (metabolites/degradation products) that requires a separate risk assessment.
2. To determine the quantitative distribution of residues in various processed commodities /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.

9.3.2 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:

9.3.3 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.

Processing Studies are necessary if significant residues occur in plants or plant products which are processed. “Significant residues” normally 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 that *are* important in trade. In order to obtain consistent processing factors the results of more than one study are necessary. 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.

9.3.4 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.

9.3.5 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 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.

9.3.6 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.

9.3.7 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.

9.3.8 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.

9.3.9 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)

9.3.10 Test conditions

Processing study(PS) 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.

9.3.11 Test substance

RAC samples used in PS should contain quantifiable residues – (\geq 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.

9.3.12 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.

9.3.13 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

9.4 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.

9.4.1 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.

9.4.2 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.

9.5 Guidelines for the conduct of processing studies: Specific examples

When examining the effects of processing on pesticide residues, one will find that the vital parameter which is most likely to affect the nature of the pesticide residue during many processing operations is hydrolysis, e.g., preparation of fruit juices, preserves, wine etc will be mainly hydrolytic. Processes such as heating would generally inactivate enzymes present in the substrate, leaving primarily simple hydrolysis as the degradation mechanism. Therefore, within a core

procedure, it is *hydrolysis--characterised by temperature*, time and pH--that influences the nature of residues. For example, pasteurisation of juice from pressed fruit is the core procedure in preparation of fruit juice. Studies of hydrolysis are therefore chosen as the model for degradation in processing.

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 behavior 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 of different 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.3 Typical parameters during processing operations

Type of process	Critical operation	Temperature (°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)	100 140– 150 (frying) □ 80 (air)	1– 2 1– 2(frying) 120(air)	9o

a Temperature of the vegetables during cooking

b Time the vegetables or cereals are kept at 100 °C

c Temperature within the fruit preserves during pasteurization

d Time the fruit preserves are kept at 90–95 °C

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 refining

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

Temperature(°C)	Time-min	pH	Processed represented
90	20	4	Pasteurisation
100	60	5	Baking, brewing, boiling
120 ^a	20	6	Sterilization

^aClosed system under pressure (e.g. Autoclave or similar)

9.5.1 Processing practices involving more extreme conditions

For other processing practices involving more extreme conditions (e.g. deodorization during refining, 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, enzymatic 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 Total Radioactive Residues (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 RAC when evaluating the results of the studies. These 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.

9.6 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;

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

9.7 Specific processing operations

Special considerations for dried chilli pepper: As a special case, for dried chilli peppers, a generic factor can be used for conversion of residues from fresh peppers to dried chilli peppers. Based on the available data, JMPR recommended a concentration factor of 10 for the estimation of pesticide residue levels in dried chilli pepper (JMPR 2007).

Other recommendations are:

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

Oil extraction process: In the oil extraction process, the concentration/dilution factors depend on the type of processing and the extent to which meal and crude oil picks up a specific pesticide during crushing. The solubility of a pesticide in water or fat, and in the solvents used in oil extraction, influences the concentration of a pesticide in the processed products.

In the case of crude oils, maximum residue levels can be approximated based on the physico/chemical properties of the pesticides and on the oil content of the raw materials. *Pesticides with high solubility in fat (or in the extraction solvents) will concentrate in crude oil. In this case, the MRL for crude oil will be the seed MRL multiplied by a processing factor.* This factor is inversely related to the oil content of the seed or lower.

Table 5 :Some theoretical processing factors from seed/fruit to crude oil during crushing to be applied for fat/hexane soluble pesticides.

Table.5 Processing factors to be applied for fat/hexane soluble pesticides

Oil seed	Average oil (%)	Processing factor
Rapeseed	40-45	2.5
Sunflower seed	40-45	2.5
Soybean	18-21	5
Coconut (as it is for fruit incl. coconut water)	20	5
Palm fruit	50-55	2
Palm kernel	45	2
Groundnut/peanut	40-50	2.5

Linseed	40-50	2.5
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Baking process: Bakery, a crucial segment of food processing industry, is one of the oldest and traditional activities in India. The bakery products are widely accepted for enhanced nutrient value and also for range of affordability. Baking operation involves prolonged cooking of food by dry heat normally in an oven. It is primarily used for the preparation of bread, cakes, pastries and pies, tarts, and quiches. It is also used for the preparation of baked potatoes, baked apples, and baked beans.

While conducting processing studies and deriving processing factors (Pfs) for baking, the important parameters that must be considered are the duration of the heating process and the applied temperature. In addition to their influence on the structure and texture of the biscuits, these parameters are also critical with regard to their potential influence on the concentration of pesticide in the end product. The baking parameters might influence the pesticide residues level in processed food, depending on the pesticide properties. Further, it is well known that the volatility, polarity, and thermal stability of a particular active ingredient could be used to understand the underlying basis of processing factors. This understanding will allow the extrapolation of processing factors to a large number of pesticides within different cereal-based processed food, thus minimizing the work and time required to develop processing factors and to reach an international harmonized guidance of processing factors.

Hot beverages: Beverages such as tea and coffee are consumed in the form of water infusions of the prepared leaves or seed as the case may be, and the most relevant part consumed is the percolate or infusion. The RAC in case of tea refers to the “made tea” i.e., processed, fermented and graded dry leaves and the MRLs for processed tea are calculated on the basis of pesticide residue in the infusion obtained by following a standard procedure for preparation of the percolate. In case of coffee the RAC refers to the dried coffee bean and the final consumed product is the infusion obtained from dried and pulverised seed. Residues of more polar pesticides or their metabolites are generally higher in the infusion than nonpolar or lipophilic compounds.

Milk and milk products: Dairy products present a host of processes involving heating, fermentation, coagulation, fat separation etc. and this, combined with the presence of various components of milk makes it difficult to study the behaviour of residues. Therefore, each of the dairy products should be studied individually.

Alcoholic beverages-malting and brewing: These processes involve special treatments, fermentation and distillation in production. Water-soluble components are more likely to get transferred to the beverages. In contrast, wine making involves no dilution and hence the residue pattern will be different. Hence, based on the initial residue levels in the RAC, the levels in the final processed beverage have to be carefully estimated.

Irradiated foods: The pesticide compound residues may behave in a different way in foods subjected to irradiation for preparation/preservation. While fixing the MRLs, special considerations are needed on such irradiated foods.

Processing factors in Complex foods prepared from multiple processed commodities:

Determining the processing factor in a Processed food (ready-to eat food) is a complex process and is challenging since it contains multiple ingredients which are by and large prepared from RACs which have been already Processed. Nevertheless such an exercise may become essential in the long run and would allow risk assessment of processed food /s as consumed by the general population.

9.8 Specific requirements prior to the exercise of fixing of MRLs in Processed foods

Currently, comprehensive datasets have been compiled by various international authorities on the processing factors of various pesticide residues in various crops and crop groups. Equipped with this global data, one can thoroughly analyze and examine the possibilities of directly adopting the processing factors in selected processed foods on a need-only basis. Taking into account the various ramifications and complexities, we may adopt a two-pronged approach which would be highly useful in addressing the issue of processing factors in processed foods in the country.

a) Short term approach

Step 1: Adoption of processing factors: To adopt the existing processing factors from comprehensive databases from reliable scientific bodies such as EFSA or JMPR etc.

Step 2: Ascertain their origin: To ensure that processing factors which are likely to be adopted are generated from standardized processing technologies / operations. More importantly, the processing factors should have been obtained on standardized designated processed fractions.

b) Long term approach

1. A need to develop a database of validated processing factors based on residue definitions for enforcement.
2. To make a compendium of representative processing techniques in the country which can be considered as a standard description of all relevant processes and basis for validation of processing studies.
3. The proposed compendium processing techniques is to be built upon a selection of representative and up-to-date processing studies submitted in the context of regulatory procedures for RACs.
4. It should cover the most important processes in food processing, both with respect to importance in consumption and production.
5. For each process, a typical set of processing conditions are to be provided based on published literature and/or inquiry in the food processing industry.
6. Detailed descriptions of processing conditions and yield factors for the processes are to be provided.

7. Processing studies are conducted on a very limited number of representative commodities. Priority to be given such processed foods which have no similarity with those foods from other countries (such foods which are exclusive to our national/local consumer markets).
8. Extrapolation proposals are to be made based on the comparability of processing conditions, the plant anatomy and the plant part to be processed.

9.9 Future perspective in the area of Pesticide residue levels in processed foods in India

Currently, in India, there is a tremendous emphasis and sustained growth in the food processing sector, and production as well as consumption of processed foods is having a rising trend. Exposure or treatments of an RAC can considerably impact and alter the residue levels of pesticides contained therein/thereon the processed matrix. Depending on the processing method/s and due to the differences in physico-chemical properties of pesticides, the residue concentrations may decrease or increase in the processed fractions when compared to the original raw agricultural product. Hence, obtaining pesticide residue data from processing studies assumes importance for compliance of residues with global legal standards and to refine dietary exposure assessment and risk to humans. It is pragmatic that we should work towards the development of a comprehensive database of processing operations, processing studies, processing factors for various pesticides, and most importantly the extent of processed food consumption in various groups of the general population in order to refine any possible risk associated with consumption of processed foods. To achieve this objective, it may be necessary to adopt a pragmatic approach involving both short-term and long-term approaches as briefly mentioned above.

SECTION 10

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 typically conducted on cattle (ruminants) and laying hens (poultry). The results of laying hen feeding study data may be extrapolated to other types of poultry and cattle feeding study data to all ruminants (e.g., goats and sheep) and other animals such as horses, pigs and rabbits.

The selection of appropriate dose for livestock feeding studies require the detailed information regarding the type of feedstuffs available for livestock, quantities being fed, and which feedstuff components might be used as alternatives and are interchangeable. 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.

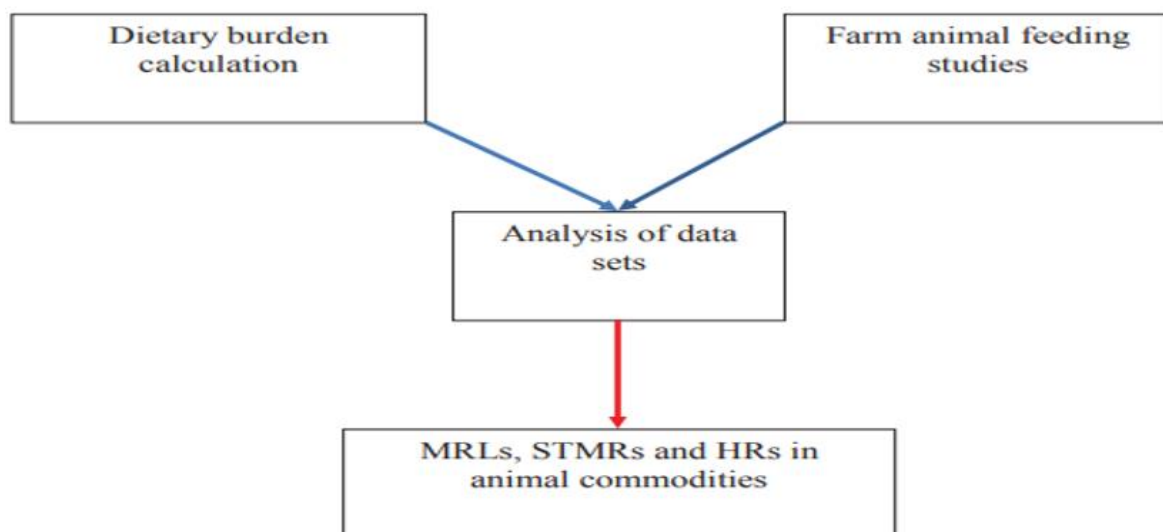


Fig. Estimation of residues in animal commodities

Livestock feed comprises of variety of agricultural commodities and by-products. EU has published 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.

The total potential dietary contribution (e.g., for cattle) for each commodity can be calculated according to the following equation:

$$\text{Dietary Contribution (mg/kg bw)} = \frac{\text{Residue level (mg/kg)} \times \% \text{ Diet} \times \text{Daily Feed Intake (kg)}}{\% \text{ DM} \times \text{Animal Body wt (kg)}}$$

The individual dietary contributions from feedstuffs are then summed up to calculate the dietary burden for each reference animal based on the predominant feeding practices globally and/or in the region of interest. Note that livestock bodyweights and daily feed intake differ among regions and should be taken into consideration as per the region of interest while calculating the dietary burden. Following tables explain the steps involved in calculation of maximum dietary burden.

Note: These tables are for illustration purpose only.

Table 6 Maximum dietary burden of beef cattle (Body weight: 500kg, Daily intake: 12kg)

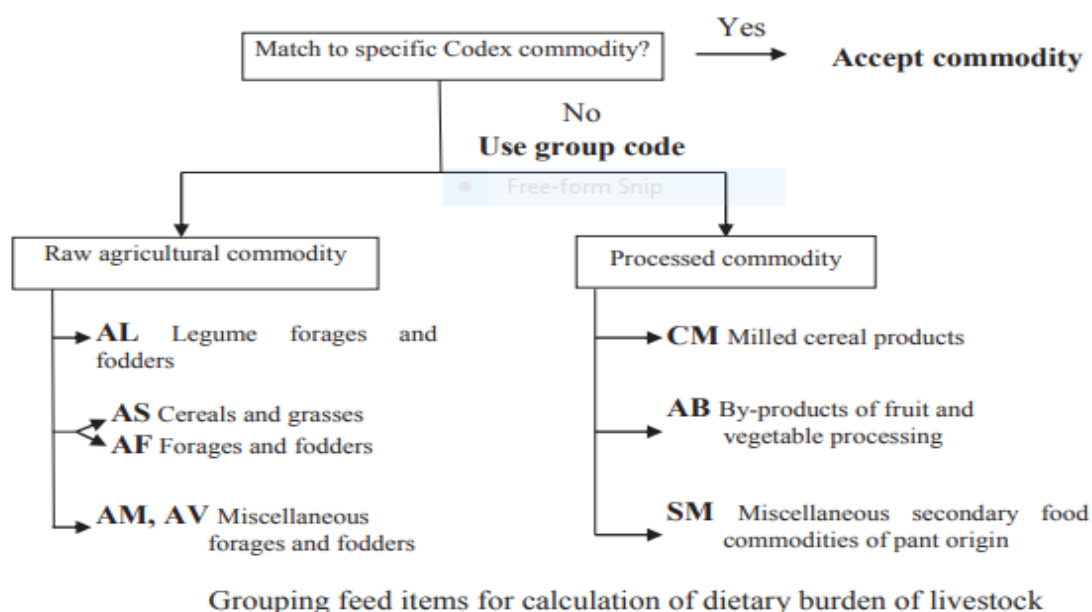
Commodity/ Crop	Commodity group	Residue (mg/kg)	Basis	% Dry Matter(DM)	Residue (mg/kg, DM)	Diet content (%)			Dietary contribution (mg/kg bw)		
						US-CAN	EU	AUS	US-CAN	EU	AUS
Grape pomace,dry	AB	0.038	STMR-P	100	0.038			20			0.0002
Bean forage(green)	AL	2.1	high residue	35	6.000	30		60	0.0432		0.0864
Alfalfa fodder	AL	4	high residue	89	4.494	60		80	0.0647		0.0863
Pea vines(green)	AL	0.86	high residue	25	3.440	20	20	60	0.0165	0.0165	0.0495
Maize fodder	AS AF	4.3	high residue	83	5.181	25	25	40	0.0311	0.0311	0.0497
Wheat straw & fodder, dry	AS AF	4.3	high residue	88	4.886	10	20	80	0.0117	0.0235	0.0938
Barley forage	AS AF	1.4	high residue	30	4.667	30	30	50	0.0336	0.0336	0.0560
Wheat milled(bran)	CM	0.084	STMR-P	88	0.095	40	30	40	0.0009	0.0007	0.0009
Rice	GC	0.57	STMR	88	0.648	20		40	0.0031		0.0062
Wheat	GC	0.035	STMR	89	0.039	20	40	80	0.0002	0.02	0.0008
Total						255	165	550	0.205	0.106	0.430

Table 7 Commodities selected to contribute to the maximum dietary burden of beef cattle (Body weight:500kg, Daily intake: 12kg)

Commodity/Crop	Commodity group	Residue (mg/kg)	Basis	% Dry Matter (DM)	Residue (mg/kg, DM)	Diet content (%)			Dietary contribution (mg/kg bw)		
						US-CAN	EU	AU	US-CAN	EU	AU
Grape pomace,dry	AB	0.038	STMR-P	100	0.038			20			0.0002
Bean forage(green)	AL	2.1	high residue	35	6.000	30		60	0.0432		0.0864
Alfalfa fodder	AL	4	high residue	89	4.494	30		20	0.0324		0.0216
Pea vines(green)	AL	0.86	high residue	25	3.440	-	20	-	-	0.0165	-
Maize fodder	AS AF	4.3	high residue	83	5.181	25	25	40	0.0311	0.0311	0.0497
Wheat straw and fodder, dry	AS AF	4.3	high residue	88	4.886	-	-	40	-	-	0.0469
Barley forage	AS AF	1.4	high residue	30	4.667	5	5	-	0.0056	0.0056	-
Wheat milled(bran)	CM	0.084	STMR-P	88	0.095	40	30	40	0.0009	0.0007	0.0009
Rice straw	GC	0.57	STMR	88	0.648	20		40	0.0031		0.0062
Wheat straw	GC	0.035	STMR	89	0.039	-	40	40	-	0.0004	0.0004
Total						150	120	300	0.1163	0.0543	0.2123

Table 8 Final table with 100% diet calculation for maximum dietary contribution for beef cattles (Body weight: 500kg, Daily intake: 12kg)

Commodity/Crop	Commodity group	Residue (mg/kg)	Basis	% Dry Matter (DM)	Residue (mg/kg, DM)	Diet content (%)			Dietary contribution (mg/kg bw)		
						US-CAN	EU	AU	US-CAN	EU	AU
Bean forage(green)	AL	2.1	high residue	35	6.000	30		60	0.0432		0.0864
Alfalfa fodder	AL	4	high residue	89	4.494	30		-	0.0324		-
Pea vines(green)	AL	0.86	high residue	25	3.440	-	20	-	-	0.0165	-
Maize fodder	AS AF	4.3	high residue	83	5.181	25	25	40	0.0311	0.0311	0.0497
Barley forage	AS AF	1.4	high residue	30	4.667	5	5	-	0.0056	0.0056	-
Wheat milled(bran)	CM	0.084	STMR-P	88	0.095	-	30	-	-	0.0007	-
Rice straw	GC	0.57	STMR	88	0.648	10		-	0.0016		-
Wheat straw	GC	0.035	STMR	89	0.039	-	20	-	-	0.0002	-
Total						100	100	100	0.1139	0.0541	0.1361



Non-intensive livestock production systems are defined as pasture and crop-based grazing whereas intensive livestock production system includes 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 based on “maximum reasonably balanced diet (MRBD) approach using USA/CAN input values.

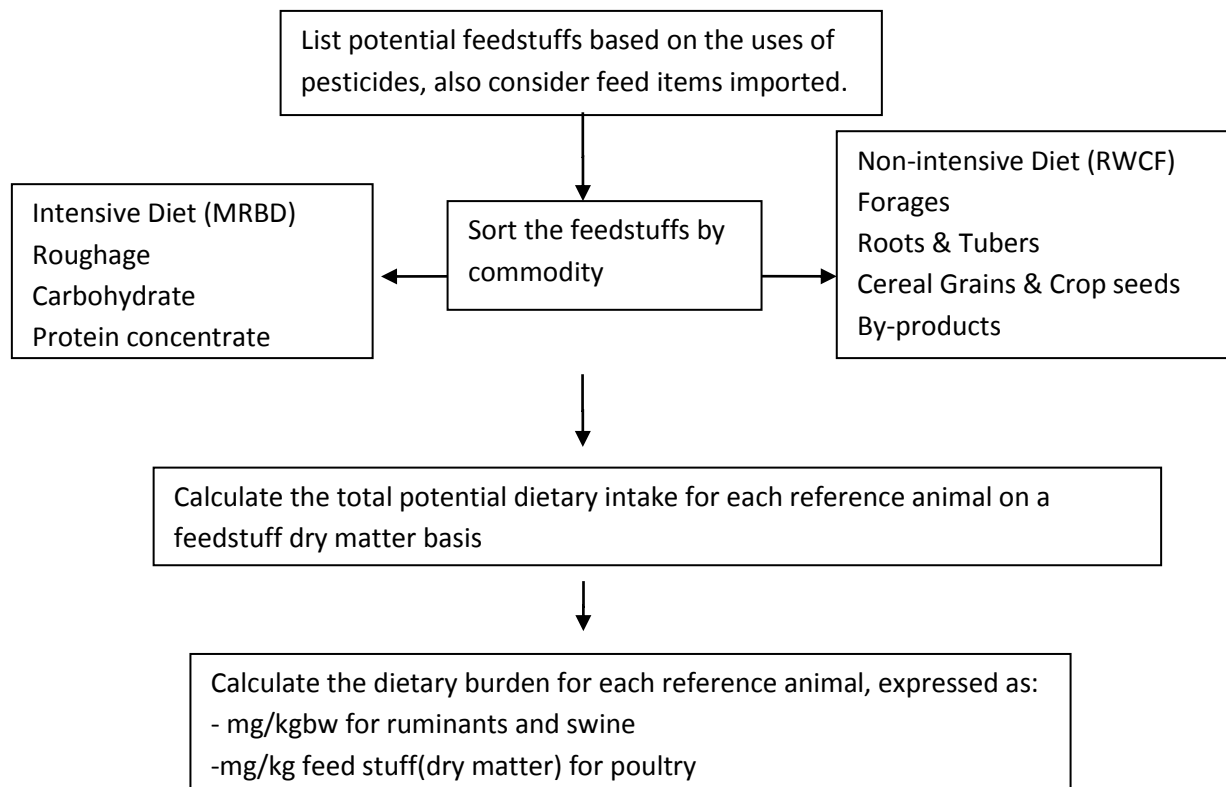
Table 9:

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 (e.g., EU, Australia, Japan), anticipated dietary burdens are calculated based on a “reasonable worst-case diet/feed” approach (RWCF). In regions where intensive feeding practices predominate (e.g., USA/CAN), the anticipated dietary burden is calculated based on a MRBD approach. The MRBD 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. The RWCF method groups commodities based on forages, roots and tubers, cereal grains& crop seeds and by-products.

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



Livestock feeding studies are typically conducted with laying hen (poultry) or dairy cattle (ruminants) and results of these studies can be extrapolated to other domestic animals e.g., laying hen feeding study data to other types of poultry and cattle feeding study data to all ruminants (e.g., goats and sheep) and other animals such as horses, pigs and rabbits. Dose levels used in the feeding study should cover the lowest and highest dietary burdens identified across all relevant regions.

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 (GVP) 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 to 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 assuming all milks to contain 4% fat. Therefore, the information of fat content of milk and milk products is of high importance. For milk and milk products, an adjustment of the MRL is necessary, taking into account the fat content of the product. For a "milk product" with a fat content less than 2%, the MRL applied should be half of those specified for milk on a whole product basis. The MRL for "milk products" with a fat content of 4% or more should be 25 times the maximum residue limit specified for milk, expressed on a fat basis.

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Annexure I:

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

Food Commodity	Recommended dietary consumption for long-term effects (for MRL calculation).	Consumption based on 95th Percentile (Recommended consumption value for short-term assessment based on ARfD)
Cereals and Millets		
1. Bajra	113	371
2. Barley	59	88
3. Maize	100	300
4. Ragi	56	220
5. Rice (Milled and parboiled)	257	818
6. Sorghum (Jowar)	163	455
7. Wheat (whole flour)	192	478
8. Other cereals / millets	45	174
Oils		
9. Groundnut oil	24	64
10. Mustard Oil	14	48
11. Sesamum Oil (Til)	39	40
12. Soybean Oil	16	63
13. Coconut oil	15	53
14. Sunflower oil	21	61
15. Safflower oil	15	
16. Cotton seed oil	28	85
17. Rice bran oil	20	
18. Other oils	16	47
Oil Seeds		
19. Groundnut	9	85
20. Mustard	2	6
21. Sesamum	12	50
22. Coconut	5	37
23. Sunflower	2	5
24. Safflower	4	5
25. Sugar/Jaggery	20	68
26. Sugarcane juice	220	220
Fruits		
27. Apple	100	200
28. Grapes	55	350
29. Mango	167	500
30. Banana	112	258
31. Pineapple	87	380
32. Pomegranate	50	200

Food Commodity	Recommended dietary consumption for long-term effects (for MRL calculation).	Consumption based on 95th Percentile (Recommended consumption value for short-term assessment based on ARfD)
33. Guava	100	200
34. Litchi	62	100
35. Peach	8	50
36. Citrus-Orange	91	142
37. Lime	5	37
38. Other Citrus fruits	35	261
39. Other fruits (papaya, water melon, etc)	115	315
Spices		
40. Cardamom	2	4
41. Black pepper	2	4
42. Coriander	3	5
43. Cumin	2	5
44. Fenugreek	2	79
45. Ginger	3	10
46. Chillies (dried)	3	9
47. Garlic	2	9
48. Other spices	2	8
Vegetables		
49. Tomato	29	100
50. Okra	68	251
51. Brinjal	49	165
52. Cabbage	87	230
53. Cauliflower	78	215
54. Knol-khol	13	356
55. Potato	86	253
56. Radish	61	201
57. Beetroot	59	211
58. Tapioca	223	444
59. Carrot	44	157
60. Cowpea	56	120
61. Beans, French beans, Field beans, Cluster beans	89	148
62. Pea	46	124
63. Bell pepper	25	179
64. Chillies (green)	3	11
65. Cucurbits: Cucumber, Melons, Pumpkin, Ash gourd, Snake gourd, Bitter gourd, Bottle gourd, Ridge gourd, Coccinia, Gherkin	57	250

Food Commodity	Recommended dietary consumption for long-term effects (for MRL calculation).	Consumption based on 95th Percentile (Recommended consumption value for short-term assessment based on ARfD)
66. Onion	23	85
Pulses		
67. Red gram	34	100
68. Bengal gram	51	150
69. Green gram	52	123
70. Black gram	30	75
71. Horse gram	28	128
72. Cowpea	41	144
73. Soybean	20	100
74. Other pulses	28	160
Plantation Crops / Beverages		
75. Coffee beans (dry basis)	3	3
76. Tea	10	10
Foods of Animal Origin		
77. Egg	40	100
78. Milk and Milk products	216	316
79. Meat	156	291
80. Fish and aquatics	92	214
81. Other marine products	35	115

Format for Submitting Data 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) <u>Active Ingredient</u>	
<u>Physical Properties</u>	
Physical State	
Colour	
Density	
Melting Point	
Stability (Time & Temperature to be Mentioned)	
<u>Chemical Properties</u>	
Octanol Water Partition Coefficient	
Solubility (At 25°C)	
Hydrolysis	
Photolysis	
Reference to FAO Specifications for TC Or TK	
3). <u>Formulations</u>	
Provide a list of commercially available formulations in India: Type of proposed formulation: Properties:	

Physical State	
Colour	
Strength Of Formulation	
Density	
Solubility/ Miscibility	

(II). SUMMARY OF GOOD AGRICULTURAL PRACTICES FOR PESTICIDE USE IN CROPS

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

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	

III) Metabolism and Environmental fate:

- (A) Crop Metabolism studies
- (B) Animal metabolism studies

IV) Fate and behaviour in soil:

V) Fate and behavior in water/ /water-sediment systems:

VI) Data on residues

A) Information on Supervised field Trials for 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				
Climatic conditions				

	Location-1	Location-2	Location-3	Location-4
Av. Min. temp (°C)				
Av. Max. temp (°C)				
Max. Relative Humidity				
Min. Relative Humidity				
Average Relative Humidity (%)				
Rainfall(mm)				
Sampling data				
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 of analysis

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 (%)													
	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 (%)													
	Name of pesticide chemical molecule													
	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 (%)													
	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)														

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

Residues of pesticide on Crop commodity (Name of Crop) 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																	
1																	
3																	
5																	
7																	
10																	
Soil																	

T1: Optimum dose																			
Crop (0)																			
1																			
3																			
5																			
7																			
10																			
Crop																			
Soil																			
T-2 dose																			
Crop (0)																			

1																	
3																	
5																	
7																	
10																	
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 MRL (mg/kg)	
Prescribed MRL on registered crop in other countries ((mg/kg))	
National MRLs of the pesticide on other crops ((mg/kg))	

VII. Toxicology

Summary of Acute Toxicity Studies With....:

Species	Strain	Sex	Route	Batch No.; Purity(%)	LD50 (mg/kg bw)	LC50 (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)

SUBACUTE

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/Pen drive

Basis of calculation of ADI:

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

Residue definition:

Labels and Leaflets (attach):

Note: Compact Disk/Pen drive 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

1. Name of pesticides and the crop on which the MRLs is to be fixed in
 - New MRL to be established Yes / No
 - MRL is already established, and application is for revision of MRL Yes / No
 - Justification for revision of MRL, if applicable
 - Already established MRL valuemg/kg (if any)
 - New proposed MRL value.....mg/kg
2. Date on which application was received by the Registration Secretariat.
3. Date on which application with details sent to the Food Safety and Standards Authority of India
4. General information-

A)	Identity	Yes	No
B)	Physical and chemical properties	Yes	No
C)	Technical material	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)	Trial conducted	YES	NO
B)	Commodity	YES	NO
C)	Name of the institutewhere supervised trials were carried out	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)	Number 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 pesticides applied to trial plots with relevant details	YES	NO
O)	Crop growth stage at the last spray treatment	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 Waiting Period/Pre-Harvest Interval	Yes	No

7. Method of analysis:

A)	Complete Method Of 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 (°C)	Yes	No
B)	Average Max. Temperature (°C)	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 LD50	Yes	No
B)	Acute dermal LD50	Yes	No
C)	Acute inhalation IC50	Yes	No
D)	Mutagenicity name of tests doses used Results	Yes	No
E)	Teratogenicity Rat Rabbit	Yes Yes	No No
F)	Effect on reproduction (Rat)	Yes	No
G)	Carcinogenicity (Rat/Mice)Noel	Yes	No
H)	Toxicity to Livestock (Animal name)	Yes	No
I)	ADI	Yes	No
J)	Basis of calculation of ADI	Yes	No
K)	Has the pesticide been reviewed by any other international organisation?	Yes	No
L)	If so, whether details have been provided?	Yes	No
M)	Has the proposed MRL of the pesticide in crop been given?	Yes	No
N)	Has MRL fixed by other countries on the proposed food commodity been submitted	Yes	No

O)	Has the results of the residue analysis submitted as per guidelines (for fungicide & insecticides (4 location 1 season); herbicides (3 Locations and 2 seasons); soil applied insecticides (4 Locations and 1 Season); provided harvest time residue in soil is < LOQ; 2 nd season data is not required).	Yes	No
P)	Have you given information on use patterns	Yes	No
Q)	Have you given information on GAP information	Yes	No
R)	Have you given information on residues resulting from Supervised Trials on crops?	Yes	No
S)	Have you given information on National Maximum Residue Limit?	Yes	No
T)	Have you given information on residue definition?	Yes	No