

DISCLAIMER:

This paper written by Chris Geering, a previous Toxicology Assessor with the ACVM Group, was submitted to the University of Surrey as part of the requirements for completing his Professional Year in New Zealand. The Professional Year represent the third of a four year degree in Toxicology at the University of Surrey, Guildford, UK. The statements and views expressed do not necessarily reflect those of the ACVM Group

The Application of Mammalian Toxicological Data to Establish Maximum Residue Limits (MRL) for Novel Active Ingredients in New Zealand Pesticides

Introduction

To facilitate the full registration of a pesticide or animal remedy that contains a new active ingredient under New Zealand legislation, a full toxicology assessment is required. This includes mammalian toxicology data, environmental fate and environmental toxicological data and is undertaken by the Agricultural Compounds and Veterinary Medicines Group (ACVMG). The ACVMG forms part of the Food Assurance Authority, Ministry of Agriculture and Forestry, New Zealand.

The ACVMG is responsible for the registration and licensing of agricultural compounds and veterinary medicines, and monitoring their importation, manufacture, sale and use in conjunction with the Animal Remedy and Pesticide Boards.

This project focuses specifically on how mammalian toxicological data is applied in pesticides in order to set a Maximum Residue Limit. The definition of pest is a living organism that is not wanted. On this basis pesticides are defined as “agents (usually chemicals) intended to kill unwanted species of animals or plants”.

The purpose of the toxicological assessment in a pesticide is to assess the data that has been submitted by a proprietor in order to determine whether the proposed formulation will present an unacceptable risk profile to humans or to the environment. This is when the proposed pesticide formulation is used according to good agricultural practice (GAP) or alternatively through adverse exposure. The extent and nature of the data that is provided from the proprietor is related to the proposed use pattern of the product, and the nature and composition of the product formulation.

The type of toxicological data that is required for a New Zealand registration is essentially that of the OECD (Organisation for Economic Cooperation and Development). The OECD toxicity testing guidelines serve as a quasi-standard for the type and nature of toxicological data that is required by an OECD member’s regulatory body. Worldwide harmonisation of such data in 1st world countries reduces the unnecessary repetition of laboratory experimentation *in vitro* or in laboratory animals, for a particular chemical but also allows the data packages to be internationally portable.

The establishment of a Maximum Residue Limit (MRL) for a particular chemical in a pesticide formulation that has a proposed use on food producing crops is a required outcome of the toxicological assessment. Prior to determination of the MRL from the toxicological assessment is the determination of a No Observed Effect Level (NOEL) and an Acceptable Daily Intake (ADI) both of which are derived from the mammalian toxicological data.

The aims of this project are as follows:

- a) To introduce the type of mammalian toxicological data that is required by the ACVM Group, MAF and explain the key features and significance of the data.
- b) To provide an overview of the key features of toxicity testing.
- c) To explain the risk based approach of assessing the toxicology of chemicals and how an MRL is proposed from this data.
- d) To outline the specific MRL setting process for a pesticide in New Zealand.
- e) The application of actual toxicological data to demonstrate how the MRL is produced and the margin of safety that the MRL provides in respect to public health.
- f) To outline any potential issues for the future related to the project scope.

Section One
The Required Mammalian Toxicological Data

The data that is provided from a proprietor is intended to address the risk that may be posed from a mammalian toxicology, and environmental toxicology perspective. As stated in the introduction this project focuses on the mammalian toxicological data. The totality of the studies that would be expected from a mammalian toxicology perspective is outlined below:

Acute Oral Studies (Formulation/Active Ingredient)
Acute Dermal Studies (Formulation/ Active Ingredient)
Acute Inhalation Studies (Formulation/ Active Ingredient)
Skin Irritation/Corrosion Studies
Eye Irritation/Corrosion Studies
Skin Sensitisation Studies
Subchronic Toxicity Studies
Reproduction Studies
Developmental Studies
Carcinogenicity Studies
Genotoxicity Studies
Long Term Toxicity Studies
Metabolism/Toxicokinetic Studies
Other Target Organ Studies
Occupational Exposure
Human Toxicological Data

The background information that follows has been extracted and interpreted from Reference 6 (OECD Guidelines for the Testing of Chemicals), as it is these guidelines that assist proprietors in ensuring that a proposed pesticide has covered the key toxicological areas.

Acute Toxicity Studies (i.e. Acute Oral, Dermal and Inhalation Studies)

The purpose of acute toxicity studies is to determine the “degree of chemical toxicity”⁶ of a particular chemical substance. Acute studies investigate the relationship of the dose of a chemical administered to the test animal (measured in units = mg/kg body weight [bw]) and the adverse effects that result in that test animal. It also allows a comparison to be drawn to chemical substances that have a known toxicity, allows specific toxic effects to be identified, and finally to provide information on the method of toxic action. Sufficiently designed acute toxicity studies will allow the determination of an LD₅₀.

The LD₅₀ is defined as the dose of a particular toxicant that is acutely lethal to 50% of test organisms under controlled laboratory conditions. The LD₅₀ is a statistically derived value. The smaller the LD₅₀ (i.e. the fewer the milligrams of chemical per kilogram of body weight to kill 50% of organisms) the *greater* the toxicity. Conversely the larger the LD₅₀ the lower the toxicity.

The risk profile is determined via different pathways of exposure after administration to a test animal. Any sex difference in a response can be subsequently established by the use of male and female test animals. From this, chemicals that are either highly toxic, or are of low toxicity can be highlighted. Any adverse human toxicity is inferred from the results of the animal studies, in particular the LD₅₀ values. These values however, can only act as a guideline to the effects of single exposure of a particular chemical and should not be taken as an absolute representation as they can vary within species as well as between species.

Skin and Eye Local Effects

The effects on the skin and eye by a given chemical are important due to the possibility of accidental exposure. These include hazards that may be associated with a particular chemical in its physical form, for example the pH of the chemical.

Allergic Sensitisation

Exposure via the dermal or inhalation routes problems may pose problems typically allergenicity to susceptible humans. This allergenicity can vary, but typically involve one exposure which triggers sensitisation.

Short Term Repeated Dose and Subchronic Toxicity

The purpose of these studies is to provide detailed information on toxic effects, target organs, reversibility or otherwise of effects and a general indication of a “**No Observed Effect Level**” (NOEL). This is also known as the **No Observed Adverse Effect Level (NOAEL)**. Sub-chronic studies are also used to establish the long term effects of pesticides on humans in terms of food safety and are relevant to the development of an MRL.

Unlike the acute toxicity studies which deal with adverse effects of single doses, a common form of human exposure is via the administration of repeated doses of a test substance which do not cause sudden toxicological effects that could occur in an acute study. Sub-chronic studies assess the “toxic effects that are associated with repeated doses of a chemical over part of an average lifespan of an experimental animal.”⁶

Subchronic and chronic dosing regimes differ in that sub-chronic dosing regimes are not longer than 10% of the animals average lifespan. Between acute toxicity study (durations) and sub-chronic study (durations) are the short-term repeated dose studies. These studies are of duration 14, 21 and 28 days.

The principal study durations for sub-chronic studies are 14, 28 and 90 days.

Reproductive Toxicity

The above term covers the areas of “reproduction, fertility and teratogenicity”⁶ based on OECD guidelines. These studies are necessary to assess the potential effects of a chemical with respect to impairment of the male and female reproductive system.

Developmental Toxicity

These studies cover any adverse effect that is caused by exposure to developing organisms during the embryonic development stage.

Carcinogenicity

In order to assess whether a chemical is carcinogenic, the study animals are investigated for a significant period of their lifetime. The development of neoplastic lesions are examined whilst being exposed or after being exposed to various doses of a test substance by a given route.

Genotoxicity

Genotoxicity studies allow the identification of chemicals that may affect “the hereditary effects of living organisms.”¹¹. A agent that can cause alterations in nucleic acids at subtoxic exposure levels which can cause altered hereditary characteristics or even inactivation of DNA is classified as genotoxic.

Chronic Toxicity or Long Term Toxicity

The chronic toxicity studies are designed to “determine the effects of a test substance in a mammalian species following prolonged and repeated exposure”.⁶ Such studies will allow slow toxicological effects to manifest, and subsequent cumulative exposure to be determined.

Toxicokinetics and Metabolism

Toxicokinetics can be defined “as the study of the rates of absorption, distribution, metabolism and excretion of toxic substances or substances that are under toxicological study”⁶. Metabolism itself has a broad definition and includes the fate of a substance in an organism (i.e. absorption, tissue distribution, biotransformations and excretion by all routes.

Toxicokinetics covers the rate of all the processes included under metabolism. The data that is obtained from these studies aids evaluation in other toxicology studies and in the extrapolation of data from animals to man. Studies of this type are conducted on chemicals which are predicted to be of toxicological concern. The toxicological concern itself can be predicted on the level and type of toxicity that is noted or expected and by the severity of potential human exposure.

Other Target Organ Studies

This term covers any studies on a given chemical that may have a significant toxicological effect on a given organ that is not covered by any of the other studies that have been previously stated, which would be useful in the overall toxicology assessment of that chemical.

Occupational Exposure

Occupational exposure specifies suitable or appropriate limits which would indicate a suitable safety threshold to humans (e.g. in the applicator of a given chemical).

Human Toxicological Data

The majority of toxicological studies is conducted on laboratory animals, but any toxicological information of a chemical directly in humans can improve the toxicological characterisation of that chemical.

In the aforementioned testing it is noted that toxicological testing of chemicals is subject to limitations, with respect to the fact that in vitro studies or animal studies extrapolated to humans. Extrapolations of this nature can lead to inaccuracies. Although the findings of a toxicological study are likely to provide a good indication of a particular hazard or risk in humans, it does not mean that a different risk profile will not be observed in humans.

Section Two

Key Features in Toxicity Testing

The following factors are key features extracted and interpreted from Reference 6 (OECD Guidelines for the Testing of Chemicals) that need to be taken into account when a toxicological assessment of a given chemical is performed. These features are highly significant in ensuring that the toxicological data generated is applicable and meaningful.

Chemical Analysis of Test Material

By having a suitable chemical analysis of the proposed test material allows for the selection of the correct route of administration, and overall planning for the proposed toxicological studies. Furthermore, necessary hazard assessments and precautions can be devised with respect to material handling and storage.

Determination of Study Animals

When the study animal is not human the interpretation of animal test results in the assessment of a possible human health hazard requires suitable judgement. The choice of test species that are used can depend on factors such as ease of breeding/purchasing, animal husbandry, speed of growth/development and handling under the experimental conditions. As rodents most suitably meet the above requirements they are used extensively for the purpose of toxicological studies. In the acute oral, dermal and inhalation the rat is the OECD preferred species. In the case of the dermal study the rabbit also serves as a viable option.

In eye and skin irritation/corrosivity testing, the methods employed were developed by Draize and the preferred study animal is the rabbit. In the skin sensitisation studies the species preferred in the six recommended methods are the guinea pig, rabbit, mouse and dog. As some neurotoxicity studies are dependent on a cholinesterase inhibition process, the hen is considered to be the most suitable test species for that element. For subchronic and chronic studies, the OECD recommendation is that both rodent and non rodent species can be included.

In carcinogenicity studies, a compound that has an unknown carcinogenic activity “should be tested in both sexes in each of two animal species”.⁶ Rats and mice tend to be more commonly used than the hamster in these studies.

Maintenance of Study Animals

It is essential that adequate environmental conditions for the test species, correct animal care techniques should be employed to ensure that meaningful results are obtained. The diet administered should provide all the nutrients required for the given species, and constituents of the diet that are could have an effect of toxicity in the study should not be present at a level that could affect the results of the study.

Number and Size of Groups

Number of groups and numbers of animals tested is specified in the OECD Standard.(ref 6)

Limit Testing

Limit test values are not absolute but are useful benchmarks in order to establish the basic toxicity of a given chemical. Limit tests can establish “the presence or absence of a toxic hazard”⁶.

Section Three

Risk Based Approach to Assessing the Toxicology of Chemicals and the Development of an MRL

The risk assessment for food borne pesticide residues consists of assessing the toxicological risk of oral exposure to any potential residues of a given compound and identifying the maximum residue levels that the compound(s) should not exceed. Companies that wish to market pesticides for use in New Zealand are required to submit significant scientific toxicological data which must demonstrate that the compound will, under the proposed use pattern, not cause an appreciable adverse effect to human health.

Risk Assessment when assessing the toxicology profile of a compound and subsequent development of an MRL is based upon the four following stages:

1. Hazard identification

A risk assessment identifies the hazards, principally the real or potential adverse effects in humans that could arise from the exposure to chemicals. This should be a scientific, ideally quantitative, assessment of the potential effects at given exposure levels. The results of a risk assessment together with any other factors is used to elaborate all risk management options e.g. hazard reduction or hazard elimination. The risk assessment report should answer the following questions:

- a) Will an agent pose a health hazard to human beings?
- b) Under what circumstances is an identified hazard expressed?

The result of the hazard identification process is a scientific judgement as to whether the chemical that is being evaluated can under the exposure conditions cause an adverse health effect in humans. Usually toxicity is observed in one or more target organs. Frequently multiple end points are observed following exposure to a given chemical. The critical effect which is usually the first significant adverse effect that occurs with increasing dose is determined.

2. Dose Response Assessment

A **dose response assessment** is the process in which the relationship between the dose of a agent administered or received and the incidence of an adverse health effect is determined. For most types of toxic effects (i.e. organ specific, neurological/behavioural, immunological, non genotoxic carcinogenesis, reproductive or developmental) it is generally considered that there is a dose or concentration below which adverse effects will not occur (i.e. a threshold). For other types of toxic effects it is assumed that there is some probability of harm at any level of exposure (i.e. that no threshold exists). At current levels of understanding the latter assumption is generally applied primarily for mutagenesis and genotoxic carcinogenesis. If this is the case a threshold based on the NOAEL and uncertainty factors is estimated.

The Establishment of an ADI from a NOEL (No Observed Effect Level) / NOAEL (No Observed Adverse Effect Level)

The lowest No Observed Effect Level is obtained from the most sensitive animal species used in the appropriate study. For compounds where the long-term (chronic) hazard is of concern relevant studies are of a long term or lifetime duration. For compounds that pose a short term (acute) hazard, then short term studies are appropriate.

The ADI itself is defined by the World Health Organisation (WHO) as:

“The daily intake which, during an entire lifetime, appears to be without appreciable risk on the basis of all known facts at the time” (WHO 1987)

Without appreciable risk can be further defined to mean:

“The practical certainty that injury will not result even after a lifetime of exposure” (WHO 1987)

The acute reference dose (ARD) is a quantitative expression of the acceptable amounts of residue that persons may ingest in short term situations, such as from a single portion or during a single day, without any expectation of harm.

The final ADI or ARD incorporates two “uncertainty” factors or “safety” factors that are applied to the NOEL. The first factor is always taken to be 10 and accommodates the variation between species or **interspecies variability**. This safety factor takes into account that humans may be more sensitive to the toxic effects of the test material than the most sensitive species in the test system. Species differences result from metabolic, functional and structural variations.

The second safety factor also taken to be 10, accommodates variation in human species or **intraspecies variability**. This is based on the rationale that the human species is potentially more diverse and lives for a greater length of time than a laboratory test species. This second factor allows for the possibility that some individuals within the general population may be more sensitive to the toxic effects of a chemical than the majority of the population. Young children or the elderly, for example, are considered to be part of this group of sensitive individuals.

An additional safety factor of 10 is sometimes used where the toxicological data pack is deficient in some way (but further studies are in progress to rectify the deficiency) or if there are other circumstances which indicates that an even greater margin of safety is required.

The ADI or ARD equals the NOAEL divided by the combined factor, which is usually 100 or 1000, depending on the specific details that are presented in the toxicological evaluation.

The ACVM Group evaluates the residue and toxicological data that is supplied by applicants in order to identify any adverse health effect that may occur if consumers were exposed to excessive levels of a pesticide residue.

3. Exposure Assessment

The third step in risk assessment is the **exposure assessment**, which has the aim of determining the nature and extent of contact with chemical substances experienced or anticipated under different conditions.

The maximum exposure assessment in the development of Maximum Residue Limit (MRLs) for a particular substance estimates the theoretical maximum residue consumers could ingest from all the existing and proposed new uses for a pesticide. The ACVM group calculates the theoretical maximum daily intake (TMDI) and compares this with the ADI.

The TMDI calculation itself includes the consideration of the amount of foods eaten from national and overseas food consumption data and estimates the dietary intake of pesticides to assist the prediction of low level, long term exposure (i.e. – chronic exposure). The TMDI calculation deliberately overestimates the potential intake by using data from consumers that are potentially exposed to the highest level of residues (via their diet) to err on the side of public safety.

For the majority of agricultural compounds it is the cumulative effects of long term exposure that are of concern to the general population, where potential exposure exists only through the diet. There are few agricultural compounds that pose an acute (short term) hazard to consumers. The assessment of TMDI is based on the FAO/WHO procedures, Guidelines for Predicting Dietary Intake of Pesticide Residues.

Essentially a residue is deemed acceptable provided that the TMDI is less than the ADI.

Calculation of a TMDI

The TMDI is calculated by multiplying the established or proposed MRLs (for permissible proportions) by the average New Zealand daily per capita estimated consumption for each food commodity. The products are then added up.

$$\text{TMDI} = \sum F_i * \text{MRL}_i$$

where:

F_i = New Zealand per capita food consumption for a given food commodity

and

MRL_i = permissible proportion (MRL) corresponding to that food commodity.

The consumption estimates are derived from food balance sheets based on New Zealand dietary surveys. If food consumption data are not available for a given commodity, then the consumption value for a similar food is used. The calculated TMDI is estimated assuming an average persons weight of 60 kg. This is compared with the pesticides ADI. Three possibilities arise:

$$\text{TMDI}/ 60 \text{ (kg)} = \text{ADI (mg/kg body weight)}$$

$$\text{TMDI}/ 60 \text{ (kg)} < \text{ADI (mg/kg body weight)}$$

$$\text{TMDI}/ 60 \text{ (kg)} > \text{ADI (mg/kg body weight)}$$

4. Risk Characterization

Risk Characterization is the last step in risk assessment. This is designed to support risk managers by providing the essential scientific evidence and rationale about risk required for decision making. In risk characterisation, estimates of the risk to human health under relevant exposure scenarios are provided.

Risk management encompasses all of those activities required to reach decisions on whether an associated risk requires elimination or necessary reduction.

In the case of an MRL development it involves the proportion of the ADI that is represented by the TMDI. The health risks from pesticides and most other chemicals that are not regarded as carcinogens are assumed to have a threshold below which no adverse effects are expected in the general population. This assumption is **highly contentious** and many people in the world of consumers do not accept it. The risk characterisation relies on the ADI as the benchmark for the protection of public health.

The ADI adds a large margin of safety, that results in a level significantly below what is observed to cause the slightest effects in animal studies. To further ensure that a health protective MRL value is reached, the TMDI deliberately overestimates the true agricultural compound residue intake as it does not take into account a number of reduction factors including:

- a) At harvest most of the treated crops contain residues that are significantly below the Maximum Residue Limit (MRL)
- b) Residues are normally reduced through storage, preparation, commercial processing and cooking.
- c) It is unlikely that each and every commodity for which an MRL is proposed will have been treated with the pesticide during the lifetime of the consumer.

As the TMDI must be set less than the ADI then it is highly unlikely that the ADI would be exceeded in practice as long as the major crop uses are covered by permissible proportions (or MRLs). This is particularly true, given that the TMDI greatly overestimates the actual pesticide residue intake.

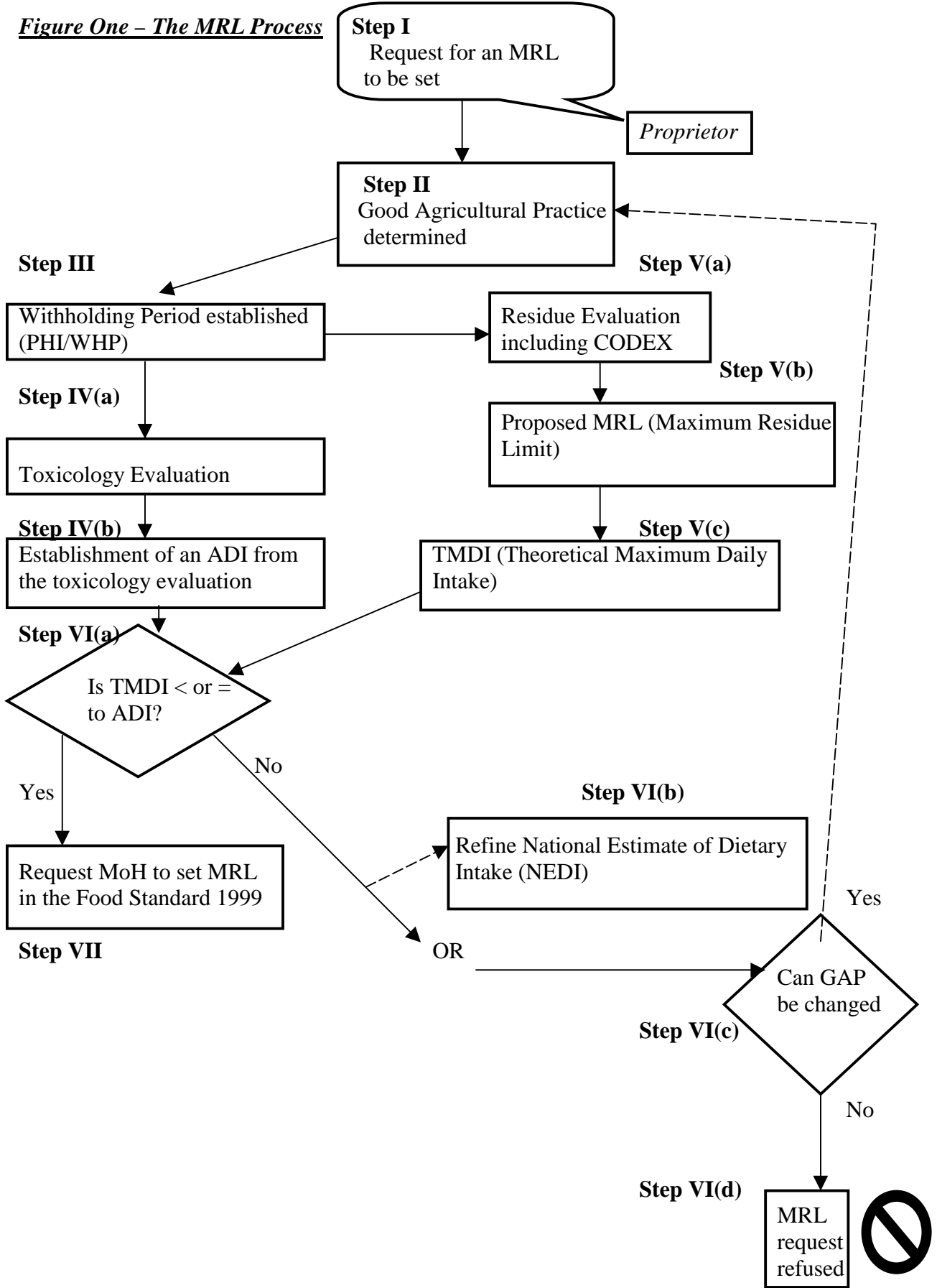
If the TMDI is greater than the ADI this does not necessarily mean that adverse health effects would be expected in consumers. However the dietary intake must be refined using the national estimated daily intake (NEDI) calculation. The NEDI calculation takes into account a number of factors to refine the estimates of long term dietary intake of agricultural compound residues, for example the median residue data from supervised trials, the proportion or part of crop or food commodity treated and the residue definition.

When all the refinements to the calculation have been made, using the best available data, and the estimated intake still indicates the potential for consumers to be exposed to residues above the ADI for significant periods of time, then the proposed and existing uses are reviewed. If at this point the intake concerns still cannot be resolved, then the proposed new use may not be accepted.

Management of exposure (oral ingestion of food borne residues) is largely through the label directions for use and especially the establishment of a pre-harvest interval (PHI) which meets Good Agricultural Practice (GAP).

Section Four
The Specific MRL Setting Process for a Pesticide in New Zealand

Figure One – The MRL Process



The risk based approach to assessing the toxicology of chemicals and subsequent development of an MRL has been discussed in section three. The purpose of section four is to demonstrate how this process is applied in New Zealand.

Explanation of Figure One - The MRL Process in New Zealand Step by Step

Step I - The request for an MRL usually arises from the applicant for a product containing a new active ingredient which they wish to register as a pesticide in New Zealand which contains a compound that will exceed the default MRL of 0.1 mg/kg. The significance of the default MRL is explained on page 14.

Step II - Good Agricultural Practice (GAP) of the use of that particular active ingredient is established. For example, if the active ingredient was to be used in apples as a fungicide, the purpose of GAP would be to ensure that the residues of that active ingredient in apples are minimised, but are present at a high enough concentration to be efficacious against the fungi. Pesticides that are used at too low a concentration in a particular food commodity can cause the development of resistance.

Step III

Residues of a particular compound within a given food commodity typically exhibit the following graph as displayed in Figure Two:

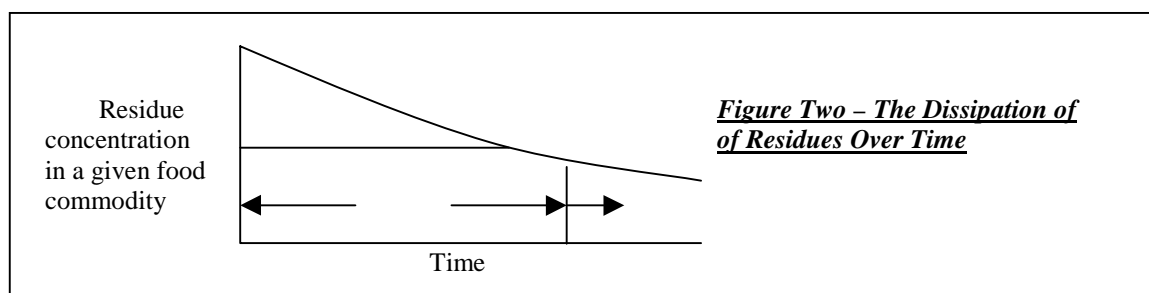


Figure Two demonstrates that over time, residues dissipate within a particular commodity. After a crop has been sprayed with a pesticide, the crop will contain residues of that particular pesticide for a certain length of time and will not be sprayed again after that point for a certain length of time to allow the concentration of residues to fall. It is this aforementioned length of time that is termed as the **Withholding period** for a particular chemical.

Steps IV(a) and IV(b)

As explained on page 9 the ADI is established from the NOAEL from the toxicology assessment.

Step V(a)

A residue evaluation is conducted in order to determine potential residues of an active ingredient of a pesticide within a given food commodity that the pesticide will be applied on.

Step V(b)

A suitable MRL is proposed from the residue evaluation for an active ingredient in a given food commodity.

Step V(c)

The TMDI of that particular food commodity containing residues of the active ingredient is determined through dietary surveys.

Step VI(a)

The TMDI is compared with the ADI. If the value of the TMDI is less than or equal to the ADI then a suitable MRL can be established on this basis.

If the NEDI is found to exceed the ADI then the two options available are as follows:

- a) Determine whether the NEDI can be refined. (**Step VI(b)**)
- b) Can GAP be altered. (**Step VI(c)**)

With pesticides in food commodities, the Withholding Period usually cannot be extended to lower the residue to a value less than the ADI especially for products applied very close to harvest are only ripe for a limited period of time.

It may be possible to reduce the proposed application rate as long as efficacy concerns are resolved.

Step VI(d)

If steps VI(b) or VI(c) are not determined to be viable options then the MRL request is subsequently refused.

Step VII

An application for the proposed MRL for a given active ingredient in a pesticide is sent to the Ministry of Health for inclusion into the New Zealand (Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999.

MRLs that are proposed to the Ministry of Health act as a “worst case scenario” and take into account the highest residue concentration that could theoretically be found in particular food commodity. Residues that are found in food crops at a given WHP or PHI exhibit a distribution curve as follows:

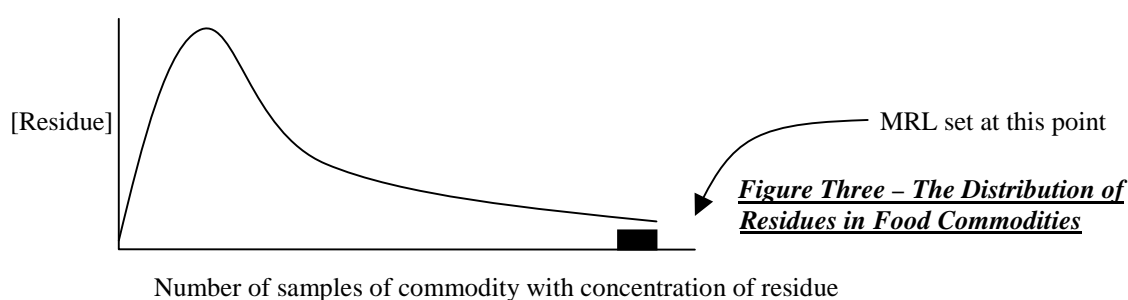


Figure three essentially demonstrates that the MRL is set based upon the highest concentration of residues that may be found in the minority of food crops.

The Default Maximum Residue Limit in New Zealand

Where a chemical has not been registered for use in particular crops in New Zealand it is not illegal to use that chemical “off label” provided that the residue does not exceed the default MRL of 0.1 parts per million (e.g. 0.1 mg/kg). The term “off label” can be defined as using a registered product on a particular commodity that has not been approved by the ACVM Group, MAF. Off label use is common on crops that are not abundant (e.g. asparagus), and thus few products if any are registered for use on such minor crops. Off label use does not carry any advice about GAP or WHP (PHI)

An MRL itself is not an threshold of food safety. The MRL itself usually has at least has a 100 fold safety factor built into it. When the 0.1 mg/kg default was defined in the New Zealand Food Regulations 1984, it was approximately the limit of detection for most chemicals. It had not been checked to ensure that this was an acceptable level based on the toxicology data submitted in support of the pesticides concerned.

The methods of detection have improved significantly which now means that the default MRL is just a number – in the majority of cases there will not be a problem (market access for example) in adopting the default value without assessment but there are a few rare cases in which an MRL must be set below the level of the default MRL. The toxicological acceptability of this is checked when applications for registration of new pesticides are being processed by the ACVM Group.

Reports from other regulatory bodies e.g. EPA, CCPR and are also used in the assessment.

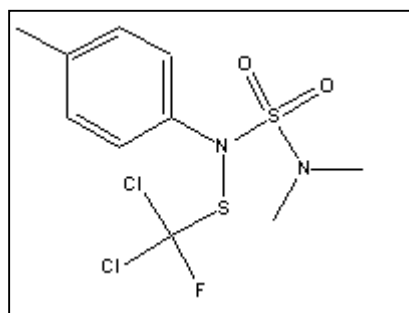
Section Five
**The Application of Actual Toxicological Data and how this Corresponds to the
Registration of a Particular Product**

This section uses actual publicly available toxicological data for two specific active ingredients, **tolyfluanid** and **indoxacarb**, and the MRLs that have been recently proposed for inclusion into the New Zealand (Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999.

Case Study One – Tolyfluanid

Chemical Abstract Services (CAS) Number – 731-27-1

Figure Four – Chemical Structure of Tolyfluanid



Tolyfluanid is a broad spectrum fungicide and is mainly used in pome fruit to control mould, powdery mildew and various leaf spot for vegetables and ornamentals. The tolyfluanid residue is made up of two components, the parent compound “tolylfluanid” and the DMST metabolite (N,N-dimethyl-N-p-tolysulphamide). This active ingredient is currently registered in one pesticide formulation in New Zealand, Euparen Multi at a active ingredient concentration of 500 g/kg.

Molecular Weight – 347.3

Molecular Formula – C₁₀H₁₃Cl₂FN₂O₂S₂

Form – Colourless, odourless, crystalline powder

Melting Point - 93°C

Boiling Point – Decomposes on distillation

Biochemistry – A non specific thiol reactant that inhibits respiration

Mode of Action – A foliar basic fungicide with a protective action.

Mammalian Toxicology

Acute oral toxicity (LD₅₀)

Rats >5000 mg/kg

Mice >1000 mg/kg

Guinea pigs 250-500 mg/kg.

Based on the above acute oral data tolyfluanid is of low acute oral toxicity and on this basis the sudden onset of toxic effects in humans via the oral route of food commodities containing this active ingredient is unlikely.

Skin and Eye – Acute percutaneous LD₅₀ for rats >5000 mg/kg – Severely irritating to the skin and moderately irritating to the eyes (rabbits).

Skin Sensitisation

Tolyfluanid has been determined to be a skin sensitiser.

Acute Inhalation Toxicity

Acute Inhalation (LC₅₀) (4 hours) for rats 0.265 mg/l air (aerosol)

NOEL – 5 mg/kg bw (Determined from subchronic toxicological study in rats)

Acceptable Daily Intake (ADI) – 0.05 mg/kg bw day

This acceptable daily intake value demonstrates the application of the 100 fold safety factor, 10 for interspecies variability, and 10 for intraspecies variability as explained in Section Three.

TMDI – 0.00506 mg/kg bw day

This Theoretical Maximum Daily Intake (TMDI) calculates the likely maximum consumption of all food commodities that are treated with products containing the active ingredient tolyfluanid.

TMDI as % of ADI – 10.12 %

The TMDI as a percentage of the ADI demonstrates a large margin of safety, as it does not exceed the ADI.

MRL Proposed for Tolyfluanid:

The MRL that has been proposed for the New Zealand (Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999 is 1.0 mg/kg in apples, with a 14 day withholding period, adopting a worst case scenario. On this basis the MRL proposed is unlikely to pose any risk to human health.

Case Study Two – Indoxacarb

Chemical Abstract Services (CAS) Number – 144171-61-9

Figure Five – Chemical Structure of Indoxacarb



Indoxacarb is an oxadiazine insecticide that is effective against lepidopteran insects such as caterpillars. The compound has a low volatility and it is made up of a 75:25 ratio of active (S) isomer to inactive (R) isomer. Within residue analysis both the active and inactive isomer are considered. Indoxacarb is currently registered within one pesticide, Steward 150 SC Insecticide at an active ingredient concentration of 150 g/litre.

Molecular Weight – 527.8

Molecular Formula – $C_{22}H_{17}ClF_3N_3O_7$

Form – White powder

Melting Point – 88.1°C

Biochemistry – The active component works by blocking sodium channels in nerve cells.

Mode of Action – The insecticide is made active by contact and ingestion. Insects that are affected cease feeding with poor co-ordination, paralysis and ultimately death.

Mammalian Toxicology

Acute oral toxicity (LD₅₀)

Male rats – 1732 mg/kg

Female rats – 268 mg/kg

On the basis of the above acute oral data indoxacarb is of fairly high toxicity via the oral route.

Skin and Eye – The acute percutaneous LD₅₀ for rabbits >5000 mg/kg. Indoxacarb is not found to be an eye or skin irritant in rabbits.

Skin Sensitisation – Indoxacarb is found to be a dermal sensitiser in guinea pigs.

Acute Inhalation Toxicity – The acute inhalation (LC₅₀) (4 hours) for rats >2 mg/l.

NOEL – 2 mg/kg bw (Determined from subchronic toxicological study in rats)

Acceptable Daily Intake (ADI) – 0.02 mg/kg bw day

This acceptable daily intake value again demonstrates the application of the 100 fold safety factor, 10 for interspecies variability, and 10 for interspecies variability as explained in Section Three.

TMDI – 0.0031 mg/kg bw day

This Theoretical Maximum Daily Intake (TMDI) calculates the likely maximum consumption of all food commodities that are treated with products containing the active ingredient indoxacarb.

TMDI as % of ADI – 15.5 %

The TMDI as a percentage of the ADI demonstrates a large margin of safety, as it does not exceed the ADI for indoxacarb.

MRL Proposed for Indoxacarb

The MRL that has been proposed for the New Zealand (Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999 is 0.5 mg/kg in pomefruit and 0.5 mg/kg in vegetable brassicas. If growers follow the suggested method of treatment as stated on the product, then the expected residues for vegetable brassicas will range from 0.2 mg/kg for broccoli to 0.5 mg/kg for cabbage. The MRLs proposed are unlikely to pose a risk to human health.

Section Six - Issues for the Future

This final section outlines some of the issues that may affect the subject scope that has been discussed in this project.

Review of Acceptable Daily Intakes (ADIs)?

During the 1990s legislators in the USA forced some changes to the legislation regulating pesticides requiring the re-assessment of pesticides to ensure that residues in food meet an increased safety factor when exposure to children was likely to be significant. The particular chemicals concerned have been or are in the process of being “re-assessed”. The proprietors of the products are required to produce defence data to the regulators in order to facilitate the product’s continued registration. Some of this data (e.g. – toxicology data) is very expensive to generate. Furthermore the products that are concerned are typically “mature” and are not the high revenue earners that the newer products are. With many of these mature products the product registrations are being allowed to lapse.

Subsequently the Acceptable Daily Intakes (ADIs) for the majority of the pesticides reviewed have been reduced. This has caused many MRLs for these chemicals to be reduced where increased withholding periods are possible within GAP. Where no management options are available to reduce the TMDI then the product registration in New Zealand would be reviewed and possibly cancelled.

Limitations of the Acceptable Daily Intake (ADI)

The ADI is a consensual value with a high subjective component and should be considered as an indicative number than an absolute one as the crux of the definition by the WHO is "without appreciable risk". The question then, is to what degree and to whom? Better models are required to set the true ADI given the extrapolation of laboratory animals to humans. A perception of the true ADI for a given chemical is zero for only then can no adverse effects to a given chemical be expected.

The ADI calculation is based on subchronic toxicity studies on healthy animals to model healthy humans - most humans are not healthy in the sense a good proportion now suffer chronic illness for which permanent medication is taken. There is no allowance for inclusion of compound interaction in the setting of the ADI.

Contaminants Influencing the Acceptable Daily Intake (ADI)

The cumulative effect of persistent environmental contaminants and the residue of a compound (e.g. - interactions with DDE, Phthalates, fungal toxins, certain heavy metals such as Cadmium and Lead) may heighten the risk of potential adverse exposure of humans consuming a given food commodity.

References

The following resources were used in the completion of this project:

1. **The Pesticides Manual** – CDS Tomlin (12th edn) – British Crop Protection Council (2000)
2. **Environmental Health Criteria 210** - World Health Organization – Principles for the Assessment of Risks to Human Health from Exposure to Chemicals – WHO (1999)
3. **Handbook of Human Toxicology** – Edward J Massaro – CRC Press (1997)
4. **Understanding Toxicology** – Chemicals, Their Benefits and Risks – H. Bruno Schiefer *et al* - CRC Press (1997)
5. **Environmental Health Criteria 70** – World Health Organization Principles for the Safety Assessment of Food Additives and Contaminants in Food – WHO (1987)
6. **OECD Guidelines for the Testing of Chemicals** – Paris – Organization for Economic Cooperation and Development – (1981)
7. **Guidelines for Predicting Dietary Intake of Pesticide Residues** – GEMS/Food – Geneva: World Health Organisation – WHO (1989)
8. **Recommendations for the Revision of the Guidelines for Predicting Dietary Intake of Pesticide Residues** – Report of a FAO/WHO consultation – WHO/FNU/FOS/95.11. – Geneva: World Health Organisation – WHO (1995)
9. **Codex Alimentarius Commission Procedural Manual** – 8th edn. – Rome: Food and Agriculture Organization – FAO (1993)
10. **Annual Review of Pharmacology and Toxicology** – Arthur K. Cho *et al* - Volume 38, 1998 – Annual Reviews (1998)
11. **Principles and Methods of Toxicology** – 3rd edn. – A. Wallace Hayes – Raen Press (1994)

Appendix

The following is an expansion of the acronyms that have been used in the completion of this project:

ACVM(G) = Agricultural Compounds and Veterinary Medicines (Group)

ADI = Acceptable Daily Intake

ARD = Acute Reference Dose

CAS = Chemical Abstract Services

GAP = Good Agricultural Practice

MAF = Ministry of Agriculture and Forestry

MoH = Ministry of Health

MRL = Maximum Residue Limits

NEDI = National Estimate of Dietary Intake

NOAEL = No Observed Adverse Effect Level

NOEL = No Observed Effect Level

OECD = Organisation for Economic Co-operation and Development

PHI = Preharvest Interval.

TMDI = Theoretical Maximum Daily Intake

WHO = World Health Organization

WHP = Withholding Period