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# GUIDELINES ON TOXICOLOGICAL DATA REQUIREMENTS FOR PESTICIDE REGISTRATION

**Pesticides Board**
**Malaysia**
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## PREFACE

The Pesticides Board in its efforts to further upgrade its services to the public, in particular companies applying for the registration of pesticides has prepared these guidelines to supplement the existing ones. These supplementary guidelines provide information in greater detail on the requirements for registration on the toxicological aspect.

In the preparation of these guidelines references were made to some international and national guidelines such as those published by FAO/WHO, OECD, JMAFF, US EPA and EC. The Pesticides Board reserves the right to amend any part of the document which ever it deems fits. It is hoped that with these guidelines the time taken for registration of pesticides will be reduced. Applicants who require further clarification on these guidelines or other matters related to registration may contact the Secretary of the Pesticides Board at the following address:

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Chairman
Pesticides Board

## ACRONYMS

| | |
|---|---|
| AChE | Acetylcholinesterase |
| BMD | Benchmark Dose |
| EC | European Community |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| GLP | Good Laboratory Practice |
| IARC | International Agency for Research on Cancer |
| JMAFF | Japan Ministry of Agriculture, Forestry and Fisheries |
| $LC_{50}$ | Lethal Concentration, 50% |
| $LD_{50}$ | Lethal Dose, 50% |
| LLNA | Local Lymph Node Assay |
| MoGA | Mode of Genotoxic Action |
| NTE | Neuropathy Target Esterase |
| NOAEL | No Observed Adverse Effect Level |
| OECD | Organization for Economic Co-operation and Development |
| OCSPP | Office of Chemical Safety and Pollution Prevention |
| TGAI | Technical Grade Active Ingredient |
| US EPA | United States Environmental Protection Agency |
| WHO | World Health Organization |

# TOXICOLOGICAL DATA REQUIREMENT FOR PESTICIDE REGISTRATION

## 1. INTRODUCTION

In order to assess risks to human health from a pesticide proposed for registration, Pesticides Board consider the toxicity of the compounds and of any relevant impurities, metabolites or degradates, and potential exposure to the technical material, impurities, metabolites or degradates during or after application and resulting from the proposed uses.

The scientific data and other types of information that Pesticides Board require to determine whether the expected exposure is acceptable or is a concern are described below:

**i. Toxicity of technical material**

Data on the acute toxicity of the active ingredient are generated for classification and labelling. Such data indicate any health hazards likely to arise soon after, and as a result of, short-term exposure. Other tests, such as for sub chronic and chronic effects, mutagenicity, carcinogenicity, and reproductive and developmental toxicity, provide information that allows Pesticides Board to determine the risk that the pesticides pose to human health after prolonged or repeated exposure. Such studies are generally conducted with the active ingredient to test whether it induces adverse effects in mammals.

The results of these studies, with data or estimates of exposure and uncertainty factors to account for extrapolation of data, are used to assess the risk to human health resulting from exposure to the active ingredients of pesticides in specific use scenarios. Applicants can also perform screening tests to determine whether full testing is required. These tests (which may be performed *in vitro*) can reduce costs and the number of laboratory animals required for testing. For example, information on chemical properties or screening tests may indicate that testing for skin and eye irritation is not necessary.

**ii. Toxicity of formulated product**

Data on the acute toxicity of formulated pesticide products are generated for classification and labelling and also used to determine the immediate hazard to human health and appropriate first aid and medical treatment. For example, data on acute toxicity are used to identify protective measures to prevent accidental poisoning and can be used to prepare precautionary label statements, such as protective clothing requirements for applicators. These data allow the Pesticides Board to consider the toxicity of the entire formulated product, including co-formulants and other active ingredients, if the formulated product contains more than one active ingredient.

## 2. TYPE OF APPLICATIONS

For the purpose of these guidelines, pesticides are divided into two types i.e. commodity and proprietary pesticides.

For commodity pesticides, registrant should provide acute toxicological data (6 pack studies).

For proprietary pesticides, registrant should provide full toxicological data package, containing comprehensive data as listed below:

Impact on human and animal (Mammalian toxicological data)

i. Acute toxicological data
ii. Sub-acute toxicological data
iii. Chronic toxicological data
iv. Supplemental toxicological data
v. Human toxicology data (such as industrial exposure data, accidental data or, volunteer data)
vi. Toxicological information of every ingredient, synergist and major or important impurity of pesticides

The detail requirements are given in **Appendix I.**

## 3. CONDUCT OF STUDIES

3.1 The studies must be conducted in Good Laboratory Practice (GLP) certified laboratory. The study reports should be accompanied with the GLP certification from the National Compliance Monitoring Authority or relevant International recognised body.

3.2 Studies that are not compliant with GLP or recognised guidelines will be considered case by case on their scientific merit.

3.3 Each study should include at least:

i. Name and full address of test facility, test site(s), sponsor, study number, study director, test guidelines and, if applicable, principal investigator and contributing scientists
ii. Full identification and characterization of the test item and reference item
iii. Experimental starting and completion dates
iv. Signed GLP compliance statement of the study director indicating the extent of GLP compliance
v. Identification of any portions of the study not conducted in compliance with GLP (*if applicable*)
vi. Signed statement of quality assurance listing the types and dates of inspections and dates of reporting to test facility management, study director and if applicable, principal investigator
vii. Description in sufficient detail of the methods and materials including reference to test guidelines (*if applicable*)
viii. Presentation of deviations and unexpected results (*if applicable*)
ix. Evaluation and discussion of the results and, where appropriate, conclusions
x. Storage location of all study related documentation and materials

3.4 A complete and accurate English translation must be included for any information that is not in English.

## 4. DATA WAIVER

The Pesticides Board is receptive to data waiver requests. Such requests should be made on a case by case basis and include a scientifically sound and chemical specific rationale. Registrant should submit formal waiver request including all relevant information to support the waiver as part of their registration application through existing process.

## 5. EXEMPTIONS

The following are pesticides which can be registered without the submission of toxicological data:

i. Borax
ii. Copper oxychloride
iii. Copper sulphate
iv. Cuprous oxide
v. Cupric sulphate pentahydrate
vi. Cupric hydroxide
vii. Sulphur
viii. Sodium dichromate
ix. Sodium chlorate

## 6. CLASSIFICATION OF PESTICIDE PRODUCTS

The classification is based primarily on the acute oral and dermal toxicity to the rat as this is the standard procedure in toxicology. In practice, the majority of classifications will be made on the acute oral $LD_{50}$ value. From these values, pesticide is classified into one of five class as below:

| **Class** | **$LD_{50}$ for the rat (mg/kg body weight)** | |
|---|---|---|
| | **Oral** | **Dermal** |
| Ia | < 5 | < 50 |
| Ib | 5 - 50 | 50 - 200 |
| II | 50 - 2000 | 200 - 2000 |
| III | > 2000 | > 2000 |
| IV | > 5000 | > 5000 |

Dermal toxicity must always be considered since it has been found that, under most conditions of handling pesticides, a high proportion of the total exposure is dermal. Classification based on dermal data in a class indicating a great risk is necessary when the dermal $LD_{50}$ values indicate greater hazard than oral $LD_{50}$ values.

Provision is made for the classification of a particular compound to be adjusted if, for any reason, the acute hazard to man differs from that indicated by $LD_{50}$ assessments alone.

Classification of a formulation should be based on toxicity data obtained on that formulation by the manufacturer.

However, for certain situation/case, $LD_{50}$ values of the compound or formulation may not be used as basis for classification.

# 7. DATA REQUIREMENT FOR TOXICITY STUDY

## 7.1 ACUTE TOXICOLOGICAL DATA

### 7.1.1 ACUTE ORAL TOXICITY STUDY

**Objective of the study**
The studies data and information to be provided should allow the identification of effects following a single exposure to the pesticide, and in particular to establish, or indicate:

- the toxicity of the active ingredient and formulated product
- the time course and characteristics of the effects with full details of behavioral changes and possible gross pathological findings at post-mortem and
- where possible mode of toxic action

While the emphasis should be on estimating the toxicity ranges involved, the information generated should also permit the active ingredient to be classified regarding its acute hazard. The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

**Test systems**
The study is normally conducted in rats.

**Circumstances under which the study is recommended to be required**
The acute oral toxicity study should always be conducted, except if the pesticide is a gas or a highly volatile liquid.

**Test substance**

- Technical grade active ingredient (TGAI)
- Formulated product

**Typical endpoints of the study**
Mortality: oral $LD_{50}$ (mg a.i/kg bw)

**Test guidelines**
The following test guidelines may be used for determination of the acute oral toxicity:

- OECD Guidelines for the Testing of Chemicals. Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure
- OECD Guidelines for the Testing of Chemicals. Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method
- OECD Guidelines for the Testing of Chemicals. Test No. 425: Acute Oral Toxicity - Up and Down Procedure
- US EPA Health Effects Test Guidelines. OCSPP 870.1100: Acute Oral Toxicity
- EC Testing Method B.1bis. Acute oral Toxicity - Fixed Dose Procedure. Council Regulation (EC) No 440/2008 - Annex Part B

### 7.1.2 ACUTE DERMAL TOXICITY STUDY

**Objective of the study**
The study should provide information on health hazard likely to arise from a short-term exposure to the solid or liquid pesticide by the dermal route. The studies, data and information to be provided and evaluated should allow the identification of effects following a single exposure to the pesticide, and in particular to establish:

- the toxicity of the active ingredient and the formulated product
- the time course and characteristics of the effects with full details of behavioral changes and possible gross pathological findings at post-mortem and
- where possible mode of toxic action

While the emphasis should be on estimating the toxicity ranges involved, the information generated should also permit the active ingredient to be classified regarding its acute hazard. The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

**Test systems**
The study is normally conducted in the rat, rabbit or guinea pig.

**Circumstances under which the study is recommended to be required**
The acute dermal toxicity should always be conducted. Both local and systemic effects should be investigated. The study is normally not required if the pesticide is a gas or a highly volatile liquid, or if it is corrosive to skin or has a pH of < 2 or > 11.5.

**Test substance**

- Technical grade active ingredient (TGAI)
- Formulated product

**Typical endpoints of the study**
Mortality: dermal $LD_{50}$ (mg a.i/kg bw)

**Test guidelines**
The following test guidelines may be used for determination of the acute dermal toxicity:

- OECD Guidelines for the Testing of Chemicals. Test No. 402: Acute Dermal Toxicity
- US EPA Health Effects Test Guidelines. OCSPP 870.1200: Acute Dermal Toxicity
- EC Testing Method B.3. Acute Toxicity (Dermal). Council Regulation (EC) No. 440/2008 – Part B

### 7.1.3 ACUTE INHALATION TOXICITY STUDY

**Objective of the study**
The study provides information on health hazard likely to arise from a short-term exposure through exposure by inhalation.

The information to be provided should allow the identification of effects following a single exposure to the pesticide, and in particular to establish:

- the toxicity of the active ingredient and formulated product
- the time course and characteristics of the effects with full details of behavioral changes and possible gross pathological findings at post-mortem and
- where possible mode of toxic action

While the emphasis should be on estimating the toxicity ranges involved, the information generated should also permit the active ingredient to be classified regarding its acute hazard.

The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

**Test systems**
The study is normally conducted in the rat.

**Circumstances under which the study is recommended to be required**
The inhalation toxicity study is recommended if the end-use product consists of, or under the conditions of use will result in, a respirable material (e.g. gas, vapour, aerosol, or particulate)

Inhalation toxicity of the active ingredient shall be reported where any of the following apply:

- the active ingredient has a vapour pressure > $1 \times 10^{-2}$ Pa at 20°C
- the active ingredient is a powders containing a significant proportion of particles of diameter < 50 µm (> 1% on a weight basis)
- the active ingredient is included in products that are powders or are applied by spraying. The head/nose only exposure shall be used, unless whole body exposure can be justified.

**Test substance**

- Technical grade active ingredient (TGAI)
- Formulated product

**Typical endpoints of the study**
Mortality: inhalation $LC_{50}$ (mg a.i/L or mg a.i/$m^3$)

**Test guidelines**

The following test guidelines may be used for determination of acute inhalation toxicity:

- OECD Guidelines for the Testing of Chemicals. Test No. 403: Acute Inhalation Toxicity
- OECD Guidelines for the Testing Of Chemicals. Test No. 436: Acute Inhalation Toxicity – Acute Toxic Class Method
- US EPA Health Effects Test Guidelines. OCSPP 870.1300: Acute Inhalation Toxicity
- EC Testing Method B.2. Acute Toxicity (Inhalation). Council Regulation (EC) No. 440/2008 – Part B

### 7.1.4 SKIN IRRITATION STUDY

**Objective of the study**
The test will provide the potential of skin irritancy of the active ingredient and the formulated product, including the potential reversibility of the effects observed.

**Test systems**
The study *in vivo* is normally conducted with the rabbit. Alternatively, studies with skin disks of the rat or human skin models are used (*in vitro)*.

**Circumstances under which the study is recommended to be required**
The skin irritation study should always be conducted. The study is normally not required if the pesticide is a gas or highly volatile liquid or if it is corrosive to skin or has a pH of < 2 or > 11.5.

**Test substance**
- Technical grade active ingredient (TGAI)
- Formulated product

**Typical endpoints of the study**
Animal irritant responses can be quite variable, but may include values/categories for erythema/eschar formation, oedema formation, inflammation, as well as reversibility of skin lesions.

**Test guidelines**
The following test guidelines may be used for determination dermal irritation:
- OECD Guidelines for the Testing of Chemicals. Test No. 404: Acute Dermal Irritation/Corrosion
- OECD Guidelines for the Testing of Chemicals. Test No. 439: *In Vitro* Skin Irritation - Reconstructed Human Epidermis Test Method
- OECD Guideline for the Testing of Chemicals. Test No. 431: *In Vitro* Skin Corrosion: Reconstructed Human Epidermis (RhE).
- US EPA Health Effects Test Guidelines. OCSPP 870.2500: Acute Dermal Irritation
- EC Testing Method B.4. Acute Toxicity: Dermal Irritation/Corrosion. Council Regulation (EC) No. 440/2008 – Part B
- EC Testing Method B.46. *In Vitro* Skin Irritation: Reconstructed Human Epidermis Model Test. Council Regulation (EC) No. 761/2009

### 7.1.5 EYE IRRITATION STUDY

**Objective of the study**
The test should provide information on the potential of the active ingredient and the formulated product to cause eye irritation, including if relevant, the potential reversibility of the effects observed.

**Test systems**
The study *in vivo* is normally conducted in the rabbit. Alternatively, corneas from cattle eyes, or chicken eyes are also used.

**Circumstances under which the study is required**
The eye irritation study should always be conducted. The study is normally not required if the pesticide is corrosive to the eye, or has a pH of < 2 or > 11.5.

**Test substance**
- Technical grade active ingredient (TGAI)
- Formulated product

**Typical endpoints of the study**
Animal irritant responses may include values/categories for corneal opacity, iritis, conjunctival redness, conjunctival oedema (chemosis), as well as reversibility of eye lesions.

**Test guidelines**
The following test guidelines may be used for determination of eye irritation:
- OECD Guidelines for the Testing of Chemicals. Test No. 405: Acute Eye Irritation/Corrosion
- OECD Guideline for the Testing of Chemicals. Test No. 492: Reconstructed Human Cornea-like Epithelium (RhCE)
- OECD Guidelines for the Testing of Chemicals. Test No. 437: Bovine Corneal/ Opacity and Permeability Test Method for Identifying Ocular Corrosiveness and Severe Irritants
- OECD Guidelines for the Testing of Chemicals. Test No. 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosiveness and Severe Irritants
- US EPA Health Effects Test Guidelines. OCSPP 870.2400: Acute Eye Irritation
- EC Testing Method B.5. Acute Toxicity: Eye Irritation/Corrosion. Council Regulation (EC) No. 440/2008 – Part B
- EC Testing Method B.47 Bovine Corneal Opacity and Permeability Test Method For Identifying Ocular Corrosives and Severe Irritants. Council Regulation (EC) No. 1152/2010
- EC Testing Method B.48 Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants. Council Regulation (EC) No. 1152/2010

### 7.1.6 DERMAL/SKIN SENSITISATION STUDY

**Objective of the study**
The study should provide information to assess the potential of the pesticide to provoke skin sensitisation reactions.

**Test systems**
The conventional study is conducted in guinea pig. The local lymph node assay (LLNA) study is conducted in mice. Alternative none animal assay are also acceptable for submission to address skin sensitisation. Not that multiple alternative assays maybe necessary to satisfy the data requirement.

**Circumstances under which the study is recommended to be required**
The dermal sensitisation study is recommended if repeated dermal exposure is likely under conditions of use. The study is normally not required if the pesticide is corrosive to skin or has a pH of < 2 or > 11.5. If an active ingredient is identified as a skin sensitizer, it can potentially induce a hypersensitivity reaction. Therefore, potential respiratory sensitisation should be taken into account when appropriate tests are available or when there are indications of respiratory sensitisation effects.

**Test substance**
- Technical grade active ingredient (TGAI)
- Formulated product

**Typical endpoints of the study**
Conventional test: Skin reaction (e.g. erythema) is assessed.
LLNA: Proliferation of lymphocytes in the lymph nodes is assessed.
*In chemico*: Protein reactivity is assessed.
*In vitro* method: luciferase gene induction is assessed.

**Test guidelines**
The following test guidelines may be used for determination dermal irritation:
- OECD Guidelines for the Testing of Chemicals. Test No. 406: Skin Sensitisation
- OECD Guidelines for the Testing of Chemicals. Test No. 429: Skin Sensitisation : Local Lymph Node Assay
- OECD Guidelines for the Testing of Chemicals. Test No. 442: Skin Sensitisation : Local Lymph Node Assay: 442A: DA; 442B: BrdU-ELISA
- OECD Guidelines for the Testing of Chemicals. Test No. 442C: *In Chemico* Skin Sensitisation. Direct Peptide Reactivity Assay (DPRA)
- OECD Guidelines for the Testing of Chemicals. Test No. 442D: *In Vitro* Skin Sensitisation. ARE-Nrf2 Luciferase Test Method
- US EPA Health Effects Test Guidelines. OCSPP 870.2600 Skin Sensitisation

- EC Testing Method B.42. Skin Sensitisation: Local Lymph Node Assay. Council Regulation (EC) No. 440/2008
- EC Testing Method B.6. Skin Sensitisation. Council Regulation (EC) No. 440/2008
- OECD Guidelines for the Testing of Chemicals. Test No. 442E: *In Vitro* Skin Sensitisation - The Human Cell Line Activation Test or h-CLAT Method

### 7.1.7 ACUTE DELAYED NEUROTOXICITY IN HENS

**Objective of the study**
The objective of this study is to obtain information on possible delayed neurotoxicity likely to arise from acute exposure. This study will provide information on certain classes of substances to cause specific types of neurotoxicity that might not be detected in other toxicity studies.

**Circumstances under which the study is recommended to be required**
All new organophosphorus and carbamate compounds.

**Test systems**
Young adult domestic laying hen (*Gallus gallus domesticus*) free from any viral infection and without abnormality of gait.

**Test substance**
- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**

| | |
|---|---|
| Behavioral | : Sign/symptoms of neurobehavioral toxicity |
| Biochemical | : Decreased AChE and decreased NTE activity |
| Histopathological | : Lesion in nervous system |

**Test guidelines**
The following test guidelines may be used for determination:
- OECD Test Guideline 418: Delayed Neurotoxicity of Organophosphorus Substances following Acute Exposure
- OCSPP 870.6100 Delayed Neurotoxicity of Organophosphorus Substances Following Acute and 28-day Exposure Delayed Neurotoxicity of Organophosphorus Substances after Acute Exposure (Annex to Regulation (EC) No. 440/2008)

## 7.2 SUB-ACUTE TOXICOLOGICAL DATA

### 7.2.1 REPEATED DOSE 21 OR 28 DAYS DERMAL TOXICITY STUDY

**Objective of the study**
A 21 or 28 day repeated dose dermal study will provide information on possible health hazards likely to arise from repeated dermal exposure to a test substance for a period of 21/28 days.

Such sub-chronic dermal toxicity studies are primarily used to derive regulatory concentrations for assessing operator and worker risk in occupational settings.

**Circumstances under which the study is recommended to be required**
The 21/28-day dermal toxicity study is recommended if the pesticide is intended for agricultural uses or if repeated human dermal exposure may occur.

The study is not required if an acceptable 90 days dermal toxicity study is performed and submitted, or if the pesticide is a severe irritant.

**Test systems**
The study is normally conducted with the adult rat, rabbit or guinea pig. If another species is used, the applicant should provide a justification for its selection.

**Test substance**
- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**
The principle of the test is that a substance is applied daily to the skin in graduated doses to several groups of experimental animals, one dose per group, for a period of 21/28 days. During the period of application the animals are observed daily to detect signs of toxicity. Animals which die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied.

Typical endpoints include mortality, toxic response, haematology, clinical pathology, and histopathology. Where possible, the NOAEL will be reported.

**Test guidelines**
The following test guidelines may be used for the short-term 21/28 day dermal toxicity studies:
- OECD Guidelines for the Testing of Chemicals. Test No. 410: Repeated Dose Dermal Toxicity: 21/28-day Study
- US EPA Health Effects Test Guidelines. OCSPP 870.3200. 21/28-day Dermal Toxicity

- EC Testing Method B.9 Repeated Dose (28 days) Toxicity (dermal). Council Regulation (EC) No. 440/2008

### 7.2.2 REPEATED DOSE 28 DAYS ORAL DELAYED NEUROTOXICITY IN HENS

**Objective of the study**
The objective of this study is to obtain information on possible delayed neurotoxicity likely to arise from repeated exposures over a limited period of time. This study will provide information on dose response and can provide an estimate of a NAOEL which can be of use for establishing safety criteria for exposure. Usually the delayed neuropathic potential of repeated exposure is determined after acute tests.

**Test systems**
Adult domestic laying hen (*Gallus gallus domesticus*). The animals should be healthy, free from any viral disease and any abnormalities of gait.

**Circumstances under which the study is recommended to be required**
All new organophosphorus and carbamate compounds. The 28-days oral delayed neurotoxicity in hens study is required if the pesticide is intended for food or non-food uses.

**Test substance**
- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**
Determination of NOAEL

**Test guidelines**
The following test guidelines may be used for determination:
- OECD Test Guideline 419: Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study

### 7.2.3 SUB-ACUTE 90 DAYS DIETARY FEEDING STUDY

**Objective of the study**
The 90-day study provides information on the possible health hazards likely to arise from repeated exposure over a prolonged period of time, covering post-weaning maturation and growth well into adulthood. The study will provide information on the major toxic effects, indicate target organs and the possibility of accumulation.

The study can provide an estimate of a NOAEL, which can be used for establishing safety criteria for human exposure. Short-term toxicity studies will thus provide useful data on the risks for operators who are handling and using the pesticide, as well as for other persons who may be exposed sub-chronically. In addition, the outcome of the 90-day study can be used in selecting dose levels for chronic toxicity studies.

**Test systems**
The study is normally conducted with rats. If other rodent species are used, a justification should be provided by the applicant.

The test in mice is not recommended, but the responsible authority may wish to strongly encourage the applicant to conduct a 90-day range-finding study for the purposes of dose selection for the mouse carcinogenicity study.

**Circumstances under which the study is recommended to be required**
The 90-day oral toxicity study (rodent) is always required, both if the pesticide is to be applied for food or for non-food uses.

**Test substance**
- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**
The principle of the test is that the test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 90 days. During the period of administration the animals are observed closely for signs of toxicity. Animals which die or are killed during the test are necropsied and, at the conclusion of the test, surviving animals are also killed and necropsied.

Typical endpoints are mortality, signs of toxicity, including time of onset, duration, and severity of any toxic effects, lesions and pathological changes, target organs. Where possible, the NOAEL will be reported.

**Test guidelines**

The following test guidelines may be used for the 90-day oral short-term toxicity study with rodents:

- OECD Guidelines for the Testing of Chemicals**.** Test No. 408: Repeated Dose 90-day Oral Toxicity Study in Rodents
- US EPA Health Effects Test Guidelines. OCSPP 870.3100. 90-Day Oral Toxicity in Rodents
- EC Testing Method B.26. Sub-chronic Oral Toxicity Test. Repeated Dose 90-day Oral Toxicity Study in Rodents. Council Regulation (EC) No. 440/2008

## 7.3 CHRONIC TOXICOLOGICAL DATA

### 7.3.1 CHRONIC DIETARY FEEDING STUDY
**(24 months in rat, 18 months in mouse and 1 year in dogs)**

**Objective of the study**
The objective of a long-term, or chronic, oral toxicity study is to determine the effects of a substance in a mammalian species following prolonged and repeated exposure. More specifically, the long-term oral study aims to:

- identify the chronic toxicity of a chemical
- identify target organs
- characterise the dose-response relationship
- identify a NOAEL or point of departure for establishment of a BMD

If possible, the long-term oral toxicity study should be combined with the carcinogenicity study

**Test systems**
The long-term oral toxicity study is normally conducted with rats. Studies designed to simultaneously fulfill the requirements of both the chronic oral and carcinogenicity studies (i.e. a combined study) may be conducted. Minimum acceptable study durations are:

1. Chronic rodent study (food use): 24 months
2. Chronic rodent study (non-food use): 12 months
3. Chronic dog study: 12 months
4. Mouse carcinogenicity study: 18 months
5. Rat carcinogenicity study: 24 months

**Circumstances under which the study is recommended to be required**
The long-term oral toxicity study is recommended if either:

1. The use of the pesticide is likely to result in repeated human exposure over a considerable portion of the human lifespan
2. The use requires a maximum residue limit or an exemption from the requirement of a maximum residue limit.

If it is claimed that such testing is unnecessary, that claim shall be fully justified.

**Test substance**

- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**
The NOAEL is reported whenever possible. Other typical endpoints of the study include, but are not limited to survival, body weight, toxic response, necropsy findings, histopathological findings.

**Test guidelines**

The following test guidelines may be used for the long-term oral toxicity study:

- OECD Guidelines for the Testing of Chemicals. Test No. 452: Chronic Toxicity Studies
- OECD Guidelines for the Testing of Chemicals. Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies
- US EPA Health Effects Test Guidelines. OCSPP 870.4100. Chronic Toxicity
- US EPA Health Effects Test Guidelines. OCSPP 870.4300. Combined Chronic Toxicity/Carcinogenicity.
- EC Testing Method B.30. Chronic Toxicity Test. Council Regulation (EC) No. 440/2008
- EC Testing Method B.33. Combined Chronic Toxicity/Carcinogenicity Test. Council Regulation (EC) No. 440/2008

### 7.3.2 CARCINOGENICITY STUDY

**(Not less than 24 months for rats and 18 months for mouse. This study can be combined with chronic feeding study, if appropriate)**

**Objective of the study**

The objective of carcinogenicity study is to determine the carcinogenic effects of a substance in a mammalian species following prolonged and repeated exposure. More specifically, the carcinogenicity study aims to:

- identify the carcinogenic properties of a chemical, resulting in an increased incidence of neoplasms, increased proportion of malignant neoplasms or a reduction in the time to appearance of neoplasms, compared with concurrent control groups
- identify target organ(s) of carcinogenicity
- identify the time to appearance of neoplasms
- characterise the tumors dose-response relationship
- identify of a NOAEL or point of departure for establishment of a BMD

If possible, the carcinogenicity study should be combined with the long-term oral toxicity study.

**Test systems**

The carcinogenicity study is normally conducted in two rodent species; rat and mouse are preferred. Studies designed to simultaneously fulfill the requirements of both the carcinogenicity and the chronic oral studies (i.e. a combined study) may be conducted. Minimum acceptable study durations are:

1. Chronic rodent study (food use): 24 months
2. Chronic rodent study (non-food use): 12 months
3. Mouse carcinogenicity study: 18 months
4. Rat carcinogenicity study: 24 months

If comparative metabolism data indicate that either rat or mouse is an inappropriate model for human cancer risk assessment, an alternative species should be considered.

**Circumstances under which the study is recommended to be required**

The carcinogenicity study is recommended if:

1. The use of the pesticide is likely to result in significant human exposure over a considerable portion of the human lifespan which is significant in terms of either frequency, duration or magnitude of exposure; or
2. The use requires a maximum residue limit or an exemption from the requirement of a maximum residue limit; or
3. The active ingredient, metabolite, degrade, or impurity (a) is structurally related to a recognized carcinogen, or (b) causes mutagenic effects as

demonstrated by *in vitro* or *in vivo* testing, or (c) produces a morphologic effect in any organ (e.g. hyperplasia, metaplasia) in sub-chronic studies that may lead to a neoplastic change.

If it is claimed that such testing is unnecessary, that claim shall be fully justified.

**Test substance**

- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**

The NOAEL is reported whenever possible. Other typical endpoints of the study include, but are not limited to: survival, body weight, toxic response, necropsy findings, histopathological findings.

Conventional histopathological terminology such as that published by the IARC should be used in the nomenclature and reporting of tumors.

**Test guidelines**

The following test guidelines may be used for the long-term oral toxicity study:

- OECD Guidelines for the Testing of Chemicals. Test No. 451: Carcinogenicity Studies
- OECD Guidelines for the Testing of Chemicals. Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies
- US EPA Health Effects Test Guidelines. OCSPP 870.4200. Carcinogenicity
- US EPA Health Effects Test Guidelines. OCSPP 870.4300. Combined Chronic Toxicity/Carcinogenicity
- EC Testing Method B.32. Carcinogenicity Test. Council Regulation (EC) No. 440/2008
- EC Testing Method B.33. Combined Chronic Toxicity/Carcinogenicity Test. Council Regulation (EC) No. 440/2008

## 7.4 SUPPLEMENTAL TOXICOLOGICAL DATA

### 7.4.1 DEVELOPMENTAL STUDY
**(2 species, one rodent and one non-rodent)**

**Objective of the study**
The objective of the prenatal developmental toxicity study is provide general information concerning the effects of prenatal exposure on the pregnant test animal and on the developing organism. More specifically, the developmental toxicity study aims to:

- identify direct and indirect effects on embryonic and foetal development resulting from exposure to the active ingredient
- identify any maternal toxicity
- establish the relationship between observed responses and dose in both dam and offspring
- establish NOAELs for maternal toxicity and pup development

**Test systems**
The prenatal developmental toxicity study is normally conducted with rat and rabbit by the oral route. The rat study may not be required if developmental toxicity has been adequately assessed as part of an extended one-generation reproductive toxicity study. Additional testing by other routes may be recommended if the pesticide is determined to be a prenatal developmental toxicant after oral dosing.

**Circumstances under which the study is recommended to be required**
The prenatal developmental toxicity study is recommended if the pesticide is intended:

- for food uses
- for non-food uses if use of the product is likely to result in significant human exposure over a portion of the human lifespan in terms of frequency, magnitude or duration of exposure.

If it is claimed that such testing is unnecessary, that claim shall be fully justified.

**Test substance**

- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**
The NOAEL for maternal toxicity and pup development are reported whenever possible. Other typical endpoints of the study include, but are not limited to: Maternal toxic response data developmental endpoints for litters with implants; developmental endpoints for litters with live foetuses.

Diagnostic criteria for malformations and variations should be given in the report.

**Test guidelines**

The following test guidelines may be used for the developmental toxicity study:

- OECD Guidelines for the Testing of Chemicals. Test No. 414: Prenatal Development Toxicity Study
- OECD Guidelines for the Testing of Chemicals. Test No. 426: Developmental Neurotoxicity Study
- US EPA Health Effects Test Guidelines. OCSPP 870.3700. Prenatal Developmental Toxicity Study
- EC Testing Method B.31. Prenatal Developmental Toxicity Study. Council Regulation (EC) No. 440/2008

### 7.4.2 REPRODUCTIVE STUDY
### (2 generations of rodents and one litter)

**Objective of the study**
The objective of the two-generation reproductive toxicity study is to determine the effects of a pesticide on the integrity and performance of the male and female reproductive systems and on the growth and development of the offspring. The two-generation study aims to establish the NOAEL for parental toxicity, reproductive outcome and pup development.

**Test systems**
The two-generation reproductive toxicity study is normally conducted with rats. If another mammalian species is used, a justification should be provided by the applicant.

**Circumstances under which the study is recommended to be required**
The two-generation reproductive study is recommended if the pesticide is intended:

- for food uses
- for non-food uses if use of the product is likely to result in significant human exposure over a portion of the human lifespan in terms of frequency, magnitude or duration of exposure.

If it is claimed that such testing is unnecessary, that claim shall be fully justified.

**Test substance**

- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**
The NOAEL for parental toxicity, reproductive outcome and pup development are reported whenever possible.

Other typical endpoints of the study include, but are not limited to; indices of mating, fertility, gestation, birth, viability, and lactation; offspring sex ratio; precoital interval, including the number of days until mating; duration of gestation calculated from day 0 of pregnancy; sperm number and quality; survival, body weight, necropsy findings and histopathological findings.

**Test guidelines**
The following test guidelines may be used for the two-generation reproductive toxicity study:

- OECD Guidelines for the Testing of Chemicals. Test No. 416: Two-Generation Reproduction Study
- OECD Guidelines for the Testing of Chemicals. Test No. 443: Extended One-Generation Reproductive Toxicity Study

- US EPA Health Effects Test Guidelines. OCSPP 870.3800. Reproduction and Fertility Effects
- EC Testing Method B.35. Two-Generation Reproduction Toxicity Study. Council Regulation (EC) No. 440/2008

### 7.4.3 GENOTOXICITY STUDY

The objective of genotoxicity testing is to exclude or identify potential mutagenic hazards to humans, and, for those substances that are positive, to aid in the elucidation of the mode of genotoxic action (MoGA). This guidance therefore presents a strategy for genotoxicity testing since this term encompasses all the assays included in the strategy. Consequently, it is important to generate information on three types of genetic damage, namely gene mutation, changes to chromosome structure (i.e. clastogenicity) and number (i.e. aneuploidy), to provide comprehensive coverage of the mutagenic potential of a chemical.

**Objective of the study**
The aim of genotoxicity testing is to:

- predict genotoxic potential
- identify genotoxic carcinogens at an early stage
- elucidate the mechanism of action of some carcinogens.

#### 7.4.3.1 Bacterial Reverse Mutation Assay (AMES Test)

The bacterial reverse mutation test uses strains of *Salmonella typhimurium* and *Escherichia coli* that require amino-acids to grow. The principle of this test is that it detects mutations which revert mutations present in the test strains, and restore the functional capability of the bacteria to synthesize an essential amino acid.

Point mutations are the cause of many human genetic diseases and there is substantial evidence that point mutations in oncogenes and tumour suppressor genes of somatic cells are involved in tumour formation in humans and experimental animals.

The bacterial reverse mutation test is commonly employed as an initial screen for genotoxic activity and, in particular, for point mutation inducing activity. An extensive data base has demonstrated that many chemicals that are positive in this test also exhibit mutagenic activity in other tests.

Although many compounds that are positive in this test are mammalian carcinogens, the correlation is not absolute. It is dependent on chemical class and there are carcinogens that are not detected by this test because they act through other, non-genotoxic mechanisms or mechanisms absent in bacterial cells. Mammalian mutation tests may thus be more appropriate for some pesticide groups.

**Circumstances under which the study is recommended to be required**
The bacterial reverse mutation assay is always required.

**Test systems**
At least five strains of bacteria should be used. The recommended combination of strains is:

1. *S. typhimurium* TA1535
2. *S. typhimurium* TA1537 or TA97 or TA97a
3. *S. typhimurium* TA98
4. *S. typhimurium* TA100
5. *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102

**Test substance**

- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**
Data should be presented as the number of revertant colonies per plate. There are several criteria for determining a positive result, such as a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain with or without metabolic activation system. Biological relevance of the results should be considered first; statistical methods may be used as an aid in evaluating the test results.

Positive results from the bacterial reverse mutation test indicate that a substance induces point mutations by base substitutions or frameshifts in the genome of either *Salmonella typhimurium* and/or *Escherichia coli*. Negative results indicate that under the test conditions, the test substance is not mutagenic in the tested species.

**Test guidelines**
The following test guidelines may be used for the bacterial reverse mutation study:

- OECD Guidelines for the Testing of Chemicals. Test No. 471: Bacterial Reverse Mutation Test
- US EPA Health Effects Test Guidelines. OCSPP 870.5100. Bacterial Reverse Mutation Test
- EC Testing Method B.13/14. Mutagenicity - Reverse Mutation Test Using Bacteria. Council Regulation (EC) No. 440/2008

#### 7.4.3.2 Mammalian Cell Assay

Mammalian cell assays include a combined test for structural and numerical chromosome aberrations in mammalian cells and a test for gene mutation in mammalian cells.

The purpose of the *in vitro* chromosome aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian somatic cells. The *in vitro* mammalian cell gene mutation test can be used to detect gene mutations induced by chemical substances.

These tests are used to screen for possible mammalian mutagens and carcinogens. Many compounds that are positive in this test are mammalian carcinogens; however, there is not a perfect correlation between this test and carcinogenicity. Correlation is dependent on chemical class and there may be carcinogens that are not detected by these tests because they appear to act through mechanisms other than direct DNA damage or genotoxic mechanisms.

**Test systems**
The following assays are generally recommended:

- *In vitro* chromosome aberration test: Chinese hamster ovary or Chinese hamster lung fibroblast (V79) cells, hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene locus, accompanied by an appropriate *in vitro* test for clastogenicity
- *In vitro* mammalian cell gene mutation test: Mouse lymphoma L5178Y cells, thymidine kinase (TK) gene locus, with assay conditions maximized for small colony expression or detection
- *In vitro* mammalian cell gene mutation test: Chinese hamster ovary cells strain AS52, xanthine-guanine phosphoribosyl transferase (XPRT) gene locus

If other mammalian cell assays are conducted, the applicant should provide a justification.

**Circumstances under which the study is recommended to be required**
The mammalian cell assays are always required.

**Test substance**

- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**
Specific endpoints of the various assays can be found in the testing guidelines cited below.

**Test guidelines**

The following test guidelines may be used for the mammalian cell assays:

- OECD Guidelines for the Testing of Chemicals. Test No. 473: *In Vitro* Mammalian Chromosomal Aberration Test
- OECD Guidelines for the Testing of Chemicals. Test No. 476: *In Vitro* Mammalian Cell Gene Mutation Test
- OECD Guidelines for the Testing of Chemicals. Test No. 487: *In Vitro* Mammalian Cell Micronucleus Test
- OECD Guidelines for the Testing of Chemicals. Test No. 490: *In Vitro* Mammalian Cell Gene Mutation Test
- US EPA Health Effects Test Guidelines. OCSPP 870.5300. *In Vitro* Mammalian Cell Gene Mutation Test
- US EPA Health Effects Test Guidelines. OCSPP 870.5375. *In Vitro* Mammalian Chromosome Aberration Test
- EC Testing Method *B.10*. Mutagenicity. *In Vitro* Mammalian Chromosome Aberration Test. Council Regulation (EC) No 440/2008
- EC Testing Method *B.17*. Mutagenicity. *In Vitro* Mammalian Cell Gene Mutation Test. Council Regulation (EC) No 440/2008

#### 7.4.3.3 *In Vivo* Genotoxicity Tests

The mammalian *in vivo* micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts, by analysis of erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals, usually rodents (mice or rats).

The mammalian *in vivo* chromosome aberration test is used for the detection of structural chromosome aberrations induced by test compounds in bone marrow cells of animals, usually rodents (rats, mice and Chinese hamsters).

**Test systems**
The following *in vivo* studies in mammalian somatic cells are generally recommended:

- The mammalian erythrocyte micronucleus test is preferred. Mice or rats are recommended if bone marrow is used, although any appropriate mammalian species may be used.
- The rodent bone marrow chromosome aberration tests are acceptable as an alternative. Rats, mice, and Chinese hamsters are commonly used for this test, although any appropriate mammalian species may be used.

If other *in vivo* studies in mammalian somatic cells are conducted, the applicant should provide a justification.

For most of the active ingredients recognised as *in vivo* somatic cell mutagens no further genotoxicity testing will normally be necessary since they will be considered to be potential genotoxic carcinogens and potential germ cell mutagens. However, in some specific cases germ cells studies may be undertaken to demonstrate whether a somatic cell mutagen is or is not a germ cell mutagen.

**Circumstances under which the study is recommended to be required**
If all the results of the *in vitro* studies are negative, at least one *in vivo* study should be conducted.

A negative result in the first *in vivo* test in somatic cells shall provide sufficient reassurance for active ingredients that are negative in the three *in vitro* tests.

For pesticides for which an equivocal or a positive test result is obtained in any *in vitro* test, the nature of additional testing needed should be evaluated on a case-by-case basis taking into account all relevant information.

**Test substance**

- Technical grade active ingredient (TGAI)

**Typical endpoints of the study**

Specific endpoints of the various assays can be found in the testing guidelines cited below.

**Test guidelines**

The following test guidelines may be used for the mammalian cell assays:

- OECD Guidelines for the Testing of Chemicals. Test No. 474: Mammalian Erythrocyte Micronucleus Test
- OECD Guidelines for the Testing of Chemicals. Test No. 475: Mammalian Bone Marrow Chromosome Aberration Test
- US EPA Health Effects Test Guidelines. OCSPP 870.5385 Mammalian Bone Marrow Chromosome Aberration Test
- US EPA Health Effects Test Guidelines. OCSPP 870.5395. Mammalian Erythrocyte Micronucleus Test
- EC Testing Method *B.12*. Mutagenicity - *In Vivo* Mammalian Erythrocyte Micronucleus Test. Council Regulation (EC) No 440/2008
- EC Testing Method *B.11*. Mutagenicity – *In Vivo* Mammalian Bone-Marrow Chromosome Aberration Test. Council Regulation (EC) No. 440/2008

### 7.4.4 METABOLIC STUDY

**Objective of the study**

The tests should provide sufficient data to permit:

- an evaluation of the rate and extent of absorption
- the tissue distribution and the rate and extent of excretion of the test substance and the relevant metabolites
- the identification of metabolites and the metabolic pathway

The effect of dose level on these parameters and whether results are different after single versus repeated doses, should also be investigated.

**Test systems**

The study is normally conducted in rats. It may be necessary in some cases to perform additional studies on another species (such as goat or chicken).

**Circumstances in which the study is recommended to be required**

The study should be conducted when chronic or carcinogenicity studies are recommended (generally if the pesticide is used on food and feed). The study may be recommended if significant adverse effects are seen in available toxicology studies and these effects can be further elucidated by metabolism studies.

**Test substance**

Pure active ingredient or radio-labelled pure active ingredient.

**Typical endpoints of the study**

Various endpoints should be considered, such as (but not limited to): rate and extent of absorption, tissue distribution, potential for accumulation, rate and extent of excretion, metabolic pathways(s) in animals, toxicologically significant metabolites.

**Test guidelines**

The following test guidelines may be used for the determination of toxicokinetics:

- OECD Guidelines for the Testing of Chemicals. Test No. : 417 Toxicokinetics
- US EPA Health Effects Test Guidelines. OCSPP 870.7485: Metabolism and Pharmacokinetics
- EC Testing Method B.36. Toxicokinetics. Council Regulation (EC) No. 440/2008

## 7.5 HUMAN TOXICOLOGY DATA

All information relating to human experience with the chemical must be provided if available. The information may arise as a result of voluntary intake, occupational exposure during the manufacture of the chemical, worker exposure during field use, or reports of accidental poisoning.

## 7.6 TOXICOLOGICAL INFORMATION OF MAJOR OR IMPORTANT IMPURITY OF PESTICIDES AND SYNERGIST

**i. Toxicity of major or important impurity of pesticides**

Although it is recognised that toxicity studies usually examine the toxicity of the active ingredients, impurities or metabolites may contribute significantly to the toxicity of the compound. In general, studies employing the active ingredient provide an overall estimate of toxicity of the parent compound and its metabolites.

However, where metabolites produced in target animals are significantly different from those produced in laboratory animals, toxicity studies on those metabolites are required. Submitted data should allow an assessment to be made on what metabolites should be included in the residue definition for risk assessment purposes.

All impurities with concentrations of 1 g/kg or greater (or those with concentrations of less than 1 g/kg that may be toxicologically significant) should be identified and where necessary, subjected to appropriate toxicological studies.

**ii. Toxicity of synergist**

Where two or more active constituents are formulated together, toxicity studies must be performed with the formulated product to investigate the possibility of purpose.

Where synergism or potentiation is found, further studies to clarify their toxicological significance may be required.

## APPENDIX I: TOXICOLOGICAL DATA REQUIREMENTS FOR PESTICIDE REGISTRATION (COMMODITY & PROPRIETARY)

| DATA REQUIREMENTS | COMMODITY | PROPRIETARY |
|---|---|---|
| Acute toxicological data | | |
| Acute oral toxicity study | √ | √ |
| Acute dermal toxicity study | √ | √ |
| Acute inhalation toxicity study | √ | √ |
| Skin irritation study | √ | √ |
| Eye irritation study | √ | √ |
| Dermal/skin sensitisation study | √ | √ |
| Acute delayed neurotoxicity in hens (for organophosphates and carbamates) | √ | √ |
| Sub-acute toxicological data | | |
| Repeated dose 21 or 28 days dermal toxicity study | - | √ |
| Repeated dose 28 days oral delayed neurotoxicity in hens (organophosphates and carbamates if triggered by findings of acute delayed neurotoxicity) | - | √ |
| Sub-acute 90 days dietary feeding study | - | √ |
| Chronic toxicological data | | |
| Chronic dietary feeding study | - | √ |
| Carcinogenicity study | - | √ |
| Supplemental toxicological data | | |
| Developmental study | - | √ |
| Reproductive study | - | √ |
| Genotoxicity study | - | √ |
| Metabolic study | - | √ |
| Human toxicological data | | |
| • Industrial exposure data<br>• Accidental data<br>• Suicidal data<br>• Volunteers data<br>• Poisoning symptoms<br>• Antidote statements<br>• Protective clothing | √* | √* |
| Toxicological information of every ingredient, synergist and major or important impurity of pesticides | √* | √* |

[KEY : (√ ) = Required; ( √* ) = Conditionally required; (-) = Not required]