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Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies

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Published Separately:

OECD Guidance for Country Data Review Reports on Plant Protection Products and their Active Substances - Monograph Guidance (1998, revised 1999)

OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substances - Dossier Guidance (1998, revised 1999)

Report of the Pesticide Aquatic Risk Indicators Expert Group (2000)

ABOUT THE OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation composed of 30 industrialised countries in North America, Europe and the Pacific. The OECD works to co-ordinate and harmonize government policies, address issues of mutual concern, and respond to international problems.

The work of the OECD related to chemical safety is carried out in the **Environment, Health and Safety Programme**. As part of its work on chemical testing, the OECD has issued several Council Decisions and Recommendations (the former legally binding on Member countries), as well as numerous Guidance Documents and technical reports. The best known of these publications, the **OECD Test Guidelines**, is a collection of methods used to assess the hazards of chemicals and of chemical preparations. These methods cover tests for physical and chemical properties, effects on human health and wildlife, and accumulation and degradation in the environment. The OECD Test Guidelines are recognised world-wide as the standard reference tool for chemical testing.

The Pesticide Programme was created in 1992 within the OECD's Environment, Health and Safety Division to help OECD countries: 1) harmonize their pesticide review procedures, 2) share the work of evaluating pesticides, and 3) reduce risks associated with pesticide use.

The Pesticide Programme is directed by a body called the Working Group on Pesticides, composed primarily of delegates from OECD Member countries, but also including representatives from the European Commission and other international organisations (e.g. United Nations Food and Agriculture Organization, United Nations Environment Programme, World Health Organization, Council of Europe), and observers from the pesticide industry and public interest organisations (NGO's).

In addition to the **Series on Testing and Assessment** and **the Series on Pesticides**, the Environment, Health and Safety (EHS) Division publishes documents in six other series: **Good Laboratory Practice and Compliance Monitoring**; **Risk Management**; **Harmonization of Regulatory Oversight in Biotechnology**; **Chemical Accidents**; **Pollutant Release and Transfer Registers**; **and Emission Scenario Documents**. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (see next page).

This publication was produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC). It was approved for derestriction by the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, the governing body of the Environment, Health and Safety Division.

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. UNITAR joined the IOMC in 1997 to become the seventh Participating Organization. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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PREFACE

The initial aim of this OECD project was to develop harmonised guidance on conducting independent evaluations and writing reviews of subchronic oral toxicity tests. Since most available evaluation guidelines indicate that they are applicable to all routes of administration (not just the oral route), and since such indications are, to a significant extent, applicable to repeat-dose toxicity studies other than just those classified as subchronic (90-day), these "guidance notes" should be more generally applicable (e.g. to chronic toxicity, carcinogenicity, reproductive toxicity, neurotoxicity and immunotoxicity studies). Furthermore, whilst these "guidance notes" have been prepared for the purpose of assisting in the interpretation and transparent reporting of toxicological data on pesticides, they may have some use as guidance on evaluating studies in other programs. If used as such, these "guidance notes" would enable repeat-dose studies for different groups of chemicals (e.g. pesticides, biocides and industrial chemicals) to be assessed in the same way.

The objective of these "guidance notes" is to outline core concepts in order to obviate the need to make reference to large numbers of text books, but to refer the reader to other useful sources when more detailed and specific information is required. They are intended to complement OECD Test Guidelines and other publications by the OECD, including the *Guidance for Industry Data Submissions ("Dossier Guidance")* and *Guidance for Country Data Review Reports ("Monograph Guidance") on Plant Protection Products and their Active Substances* (OECD, 1998a, b). However, whereas the latter publication provides guidance on the format and presentation of entire review reports (monographs), [including acceptability criteria for industry data submissions (dossiers), terminology, and structure], these "guidance notes" place emphasis on data interpretation, scientific judgement, and report writing in the context of regulatory toxicology evaluations.

Although information on experimental design is already provided in OECD Test Guidelines 407 – 413 (1998c), the subject is also discussed in these "guidance notes". This is because toxicologists may have to assess studies that 1) pre-date the development of OECD Test Guidelines, 2) were designed according to other test guidelines, or 3) do not conform to any test guidelines.

It should be noted that OECD Test Guidelines 407, 408 and 409 (adopted May 1981) have been updated (adopted July 1995 and September 1998). Test Guideline 408 (*Repeated Dose 90-day Oral Toxicity Study in Rodents*) places more emphasis on neurological effects as a specific end-point and may give an indication of immunological effects and reproductive organ toxicity. The revisions of the guidelines reflect scientific progress in toxicology testing[1].

The initial version of this OECD document was based in part on the US EPA SEP document (EPA-540/9-85-020) on *Toxicity Potential (Guidance for Analysis and Evaluation of Subchronic and Chronic Exposure Studies),* which also covers repeat-dose studies in general terms.

With respect to harmonisation of test guidelines, those used by US Office of Pesticide Programs (OPP) and the Office of Pollution Prevention and Toxics (OPPTS) have been harmonised with those of the OECD (see *Federal Register*, June 20, 1996).

The first draft of this document was prepared by the Chemicals Unit, Department of Health & Ageing, Canberra, Australia, as part of an OECD project to develop harmonised guidance on the preparation of data reviews for toxicity studies. Subsequent drafts incorporated useful amendments and additions suggested by agencies and organisations in a large number of OECD countries. In particular, staff from the US EPA, the Canadian Pest Management Regulatory Agency (PMRA) and several German Federal Ministries made very significant contributions to the development of the final manuscript.

[1] At the time of preparing these "guidance notes", test guideline work was focussed on establishing appropriate end-points for assessing the potential endocrine disruptor activity of chemicals.

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1. INTRODUCTION

Of all the chemicals to which humans might be exposed, pesticides are unique by reason of their deliberate introduction into the environment to kill or otherwise control life forms considered detrimental to human welfare. Experimental animals have served as useful models for detection of potential human responses to these hazardous or potentially hazardous substances. Regulations relating to acceptable practices for conducting and reporting toxicology studies in animals, and guidelines that suggest acceptable and useful experimental designs (protocols) for evaluating the possible adverse health effects (hazards) of pesticidal agents have been published by a number of national agencies and international bodies.

Subchronic toxicology studies have been designed to permit determination of toxic effects associated with repeated exposure for a period of at least 90 days (OECD, 1998c; US EPA, 1998). This type of study can provide information relating to toxic effects and potential health hazards likely to arise from repeated exposures over a limited time period. Data from this type of study are also useful in predicting potentially important toxicity end points, identifying potential target organs and systems, and in establishing the dose regimen in chronic exposure studies.

The purpose of this document is twofold:

(1) to present a general guide to the analysis and evaluation of data from studies involving repeated exposures of toxicity test species to pesticides and other chemicals; and
(2) to outline the kind of information which should be included in an independent assessment of toxicity studies. It does **not** take the place of many excellent texts on the subjects of toxicology, clinical chemistry and pathology, nor does it attempt to consider the multiplicity of effects likely to be encountered in subchronic or chronic exposure studies.

This document should be used together with other relevant national guidance and requirements documents. A broad level of guidance is provided on approaches to hazard assessment and on some of the problems and pitfalls which may arise during an assessment of possible compound-related changes in parameters measured in toxicity studies. The text reflects scientific understanding and standards as at the date of issue. Over time, the scientific community will gain a better understanding of the mechanisms of toxicity and this may lead to changes in both methodology and interpretation of results; analysis and evaluation of toxicity studies should reflect scientific consensus at the time the data are reviewed. ***It has been prepared to provide guidance on the process of evaluating toxicology data i.e. the process of hazard assessment. It does not attempt to provide more than minimal comment on the process of risk assessment, a process which may vary according to national preferences and priorities. Furthermore, whilst these notes have been prepared for the purpose of conducting assessments on pesticides, they may have some applicability in evaluating studies in other programs e.g. biocides and industrial chemicals.***

Below are given some definitions and comment on terms and concepts used in this document.

1.1 Definitions

- **Toxicity** means the intrinsic capacity of a chemical substance or a mixture of substances to induce injury.

- **Hazard** means the observed toxic manifestation(s) induced by a known quantity of a substance under known exposure conditions[2]. The term is frequently used interchangeably with "intrinsic toxicity".

[2] A more general definition of a 'hazard' is any threat to people and what they value.

- **Risk** means the probability that the identified hazard(s) will or will not be encountered under anticipated exposure conditions[3]. The identification of hazard and assessment of the risk potential of a given substance are informed judgments. Such judgments are usually based on data relating to toxicity, proposed uses, and anticipated exposure conditions. Use of a pesticide product and expected exposure conditions define the type, probable extent (duration and degree) of exposure, as well as the size and composition of the exposed population. A particular pesticide product may have one or more potential risks depending on use(s) and attendant exposure conditions.

  Paracelsus (1493-1541) stated that "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy." (Klaassen, 1996). This concept is a fundamental principle of toxicology and hazard assessment. The risk of a pesticide to humans and the environment is related to exposure conditions and **cannot** be directly equated to the intrinsic toxicity of the substance. This may be illustrated by the following: imagine a perfect containment system which absolutely prevents any exposure of humans and the environment to a toxic substance. Since exposure is zero, the risk to humans and the environment is also zero, although the toxicity of the substance remains unchanged.

- **Dose** refers to a stated quantity or concentration of a substance to which an organism is exposed. It is most commonly expressed as the amount of test substance per unit weight of test animal (e.g. mg/kg body weight).

- **Dosage** is a general term comprising the dose, its frequency and the duration of dosing. Dosage is properly applied to any rate or ratio involving a dose. Dosages often involve the dimension of time (e.g. mg/kg/day), but the meaning is not restricted to this relationship (Hayes, 1991).

- **Dose-Response Relationship** means the correlative association existing between the dose administered and the response (effect) or spectrum of responses that is obtained. The concept expressed by this term is indispensable to the identification, evaluation, and interpretation of most pharmacological and toxicological responses to chemicals. The basic assumptions which underlie and support the concept are: (a) the observed response is a function of the concentration at a site, (b) the concentration at a site is a function of the dose, and (c) response and dose are causally related (Eaton & Klaassen, 1996). The existence of a dose-response relationship for a particular biological or toxicological response (effect) provides a defensible conclusion that the response is a result of exposure to a known substance.

- **Subchronic Toxicity Studies** are most commonly, repeat-dose studies of 13 weeks (90 days) duration. The term, **short-term repeat-dose studies**, may be used to describe those studies involving administration of multiple doses for periods less than this, whilst the term, **subchronic studies**, can be taken to encompass studies of at least 90-days duration. A short-term study has been defined (WHO, 1990) as "having a duration lasting up to 10% of the animal's lifespan, 90 days in rats and mice, or 1 year in dogs", although the US EPA considers a 1-year dog study to be a chronic study. Whilst there may not be complete agreement on these terms with respect to study duration, within the context of pesticide regulation it is accepted that the main purpose of subchronic testing is to identify any target organs and to establish dose levels for chronic exposure studies. Repeat-dose studies are generally conducted in at least two species, one a rodent (usually rats) and the other a non-rodent species (usually dogs). Other than its use in dermal toxicity testing and in developmental studies, the rabbit is

[3] More broadly defined, 'risks' are measures of the likelihood of harm or loss from hazards i.e. the word 'risk' implies both the existence of a threat and its potential for happening.

not generally accepted as a non-rodent for general toxicology testing purposes, unless satisfactory evidence of its particular suitability is provided.

- **Chronic Toxicity Studies**, or long-term studies, are defined as studies lasting for the greater part of the lifespan of the test animals, usually 18 months in mice, 2 years in rats (WHO, 1987; 1990). The protocol for these studies may cover the investigation of chronic toxicity or carcinogenicity, or both.

1.2 Concepts

1.2.1 Dosing Regimen

The purpose of repeat-dose exposure studies is the detection of valid biological evidence for any toxic and/or oncogenic potential of the substance being investigated. Therefore, protocols should maximise the sensitivity of the test without significantly altering the accuracy and interpretability of the biological data obtained. The dose regimen has an extremely important bearing on these two critical elements.

Since the determination of dose-responses for any observed effects is one of the objectives of short-term studies, at least 3 dose levels are normally required, as well as controls. However, OECD Test Guidelines 408 and 409 specify that if a test at one dose level equivalent to at least 1000 mg/kg bw/day produces no observed adverse effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. Test Guidelines 408 and 409 further state that the limit test applies except when human exposure indicates the need for a higher dose level to be used.

The choice of the highest dose to be used in repeat-dose studies needs to be carefully considered and **the reasons for the final choice clearly defined**. Ideally, the dose selection for these studies should maximise the detection of potential dose-response relationships and facilitate the extrapolation of these to potential hazards for other species including humans. The largest administered dose should **not** compromise biological interpretability of the observed responses. For example, it is generally considered that the upper dose should not: (a) cause a body weight decrement from concurrent control values of greater than 10-12%; (b) in a dietary study, exceed 5% of the total diet because of potential nutritional imbalances caused at higher levels or; (c) produce severe toxic, pharmacological or physiological effects that might shorten duration of the study or otherwise compromise the study results; (d) in a carcinogenicity study, alter survival in a significant manner due to effects other than tumour production. Data on pharmacokinetics or metabolism may be helpful in determining dose levels, particularly if there is evidence of bioaccumulation of the test compound or metabolites, or evidence of dose-dependent changes in detoxification.

The International Life Sciences Institute (ILSI) 'Risk Sciences Working Group on Dose Selection' has published the outcomes of its deliberations on the principles to be taken into consideration in the selection of doses in chronic rodent bioassays (Foran JA and the ILSI Risk Sciences Working Group on Dose Selection, 1997); this provides a useful discussion of issues to be taken into account in selecting doses for repeat-dose toxicity testing.

Although it may be argued that responses observed at doses far in excess of levels experienced under real or potential exposure conditions legitimately fall within the classical dose-response concept, there are valid scientific concerns that such doses introduce biases of considerable importance into the already difficult task of evaluating animal dose responses and the assessment of their relevance to human hazard identification and risk (Paynter, 1984). High doses which overwhelm normal mechanisms for

metabolism, detoxification and/or excretion, or produce severe tissue damage (i.e. necrosis, demyelination) can make interpretation difficult or lead to inappropriate conclusions.

With respect to the selection of the low dose, it is commonly accepted that the lowest dose should not produce any evidence of toxicity (i.e. allows the establishment of a NOAEL).

1.2.2 Dosing Route

For repeat-dose studies, the oral route is most commonly used, with administration by dietary admixture, gavage, or in capsules (for non-rodents). However, depending on the possible route of exposure of occupationally-exposed workers or the public to a chemical, it may need to be investigated in short-term or subchronic toxicity studies by the dermal and/or inhalational route. OECD Test Guidelines (OECD, 1998c) give further information about protocols and conduct of 14-day and 28-day oral studies in rodents (Guideline no. 407), 90-day oral studies in rodents (no. 408) and non-rodents (no. 409), 21/28-day dermal studies (no. 410), 90-day dermal studies (no. 411), 14-day or 28-day inhalational studies (no. 412), and 90-day inhalational studies (no. 413).

For dermal exposure the material, in a suitable vehicle, is applied to the clipped skin of rats, rabbits or guinea-pigs; OECD Test Guidelines recommend even application to an area representing about 10% of the total body surface area. The site is generally occluded with polyethylene sheeting and gauze patches, or semi-occluded, in order to prevent dislodgement of material and oral ingestion, which could affect the validity or usefulness of the study. For volatile or semi-volatile materials, application and covering procedures should minimise the possibility of evaporation. Useful chapters or sections on dermal toxicity testing may be found in textbooks on toxicology e.g. Derelanko & Hollinger (1995) and Hayes (1994).

The surface area of the respiratory membrane is large, estimated at approximately 50 - 100 square metres in the normal adult cf. the estimated area of the small intestine at 250 square metres (Guyton, 1991) and much more air (about 5000 times, by volume) is inhaled each day than food or water is ingested (McClellan & Henderson, 1989). Thus, exposure to airborne material is potentially greater than via dermal or oral exposure. Airborne material can be gases or vapours, liquid droplets or solutions, aerosols (solid and vapour components), or dry fibres or powders. As a consequence, to conduct inhalational toxicity studies, mechanisms needed to deliver chemicals to a test chamber in a form that can be inhaled are complex, particularly when coupled with the need to include measuring devices which can establish particle size, concentration and form of the material in the exposure chamber. Furthermore, many factors can influence the inhalation, deposition and retention of inhaled materials in the respiratory tract. Of critical importance, in both the conduct and assessment of such studies, is the need to establish what portion of the material delivered to the exposure chamber was in a respirable form. In addition to standard toxicology texts, some useful specific references on inhalation toxicology include McClellan & Henderson (1989), Mohr et al (1988) and Salem (1987).

1.2.3 Treatment-related Responses

Responses produced by chemicals in humans and experimental animals may differ according to the quantity of the substance received and the duration and frequency of exposure e.g. responses to acute exposures (a single exposure or multiple exposures occurring within twenty four hours or less) may be different from those produced by subchronic and chronic exposures. Not all observed responses within a study, irrespective of exposure duration or frequency, will represent toxicity *per se*. They may encompass a range of effects from physiological through to toxic manifestations. It sometimes may be difficult to make a clear distinction between these responses. If an evaluator is uncertain of the type or the biological significance of a response, he/she should not hesitate to obtain competent advice for resolving the

uncertainty. It is essential that all relevant toxicity end points (statistically and/or biologically significant) be identified for consideration when evaluating data to determine non-toxic dose levels.

Classification of treatment-related responses

Contrasting approaches are available to the classification of the types of responses that a living organism can manifest during or after exposure to a xenobiotic. **The way in which treatment-related responses are described or classified may differ between regulatory agencies, depending on national policy considerations.**

- **Adverse vs Non-adverse Responses.** Some agencies prefer to classify responses as either **adverse** or **non-adverse**, which has the perceived advantage of simplicity and direct relevance to hazard and risk assessment of pesticides. In this paradigm, an adverse response is defined as "*any treatment-related response that results in change in the morphology, physiology, growth, development or life span of an organism, which results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other environmental influences*" (OECD/IPCS 1998). The decision on how to classify effects as adverse or non-adverse is made on a case-by-case basis by reference to the overall weight of evidence. The definition of adverse would cover any toxic response, but would also encompass an event such as increased activity of the hepatic cytochrome-P450-containing monooxygenase system (enzyme induction), *if* it altered hormonal homeostasis and caused tumour production, for example, or increased the organism's susceptibility to injury by other chemicals.

However, an alternative conceptual scheme may be preferred. This second scheme is based on a more general biological science viewpoint, which recognises **physiological responses**, **toxic responses**, and under some circumstances, **pharmacological responses**.

- **Physiological** responses are non-adverse. They vary within limits which are in accord with the normal functioning of a living organism; examples of such response are the usual respiratory and pulse rate increases associated with increased physical activity, systemic changes associated with normal pregnancy, and those associated with homeostatic mechanisms. These variable factors are not important toxicity end points in subchronic and chronic exposure studies unless their fluctuations are abnormally altered by a dose regimen. If such alterations occur at a particular dose or are part of a dose-response relationship, they should be correlated with other toxicity end points which may be present. Altered physiological functions arising from interaction of a xenobiotic with a cellular receptor site, are often referred to as **pharmacological** responses if they are reversible and of limited duration. Whilst some of these responses may be undesirable under certain circumstances, they are distinguished from toxic (adverse) responses by generally not causing injury. Provided there were no adverse consequences, enzyme induction could be considered to be an example of a pharmacological response. But if enzyme induction caused tumourigenesis, it would have to be regarded as an adverse response and be accounted for in the hazard and risk assessment process.

- **Toxic** responses are, by definition, adverse. They may be reversible or irreversible but are distinguished from other types of responses by being injurious and therefore adverse and harmful to living organisms or tissues. A chemical which causes a physiological or pharmacological effect may produce a toxic response if the exposure is prolonged and/or if the dose is increased beyond a certain level. The reversibility or otherwise of such responses may also depend on these two factors. The reversibility or irreversibility of an histopathological

change will depend on the ability of the injured organ or tissue to regenerate. For example, liver has a relatively great ability to regenerate and many types of injury to this organ are reversible. By contrast, differentiated cells of the central nervous system are not replaced and many injuries to the CNS are irreversible.

1.2.4 Effect and No-effect Levels

- **NOEL.** An important concept is the 'No-Observed-Effect Level' or 'No-Observable-Effect Level' (NOEL). It is the highest dose of a substance administered to a group of experimental animals at which there is an absence of observable effects on morphology, functional capacity, growth, development or life span, which are observed or measured at higher dose levels used in the study. Thus, dosing animals at the NOEL should not produce any biologically significant differences between the group of chemically exposed animals and an unexposed control group of animals maintained under identical conditions. The NOEL is expressed in milligrams of chemical per kilogram of body weight per day (mg/kg bw/day) or, in a feeding study, in ppm in food (converted to mg/kg bw of compound intake by measured or estimated food intake over the period of the study).

  The NOEL has been simply defined (WHO, 1990) as the highest dose of a substance which causes no changes distinguishable from those observed in normal (control) animals.

- **NOAEL.** The No-Observed-Adverse-Effect Level is the highest dose of a substance at which no toxic (i.e. adverse) effects are observed (WHO, 1990). It may also be worded in more detail thus: The NOAEL is defined as the highest exposure at which there is no statistically- or biologically-significant increase in the frequency of an adverse effect when compared to a control group (National Academy of Sciences/National Research Council, 1994). The definition of NOEL is equivalent, but with the removal of the term, 'adverse'.

Often the issue in the use of the terms NOEL or NOAEL is in deciding whether a compound-related effect noted in a particular study is necessarily an 'adverse' effect. For example, some toxicologists would consider liver enlargement associated with cytochrome P-450 induction as being an adaptive pharmacological response. Others may consider it to be an adverse effect, based on the potential to enhance the toxicity of other xenobiotics, to disrupt hormonal homeostasis, or to cause hyperplastic or neoplastic responses through enhanced cellular turnover. Judgement may also be influenced by national policy considerations. For example, some agencies consider plasma cholinesterase inhibition to be an adverse effect, whereas others do not. Therefore, while these "Guidance Notes" use both NOEL and NOAEL, the two terms may not be equivalent[4].

- **LOEL.** The Lowest-Observed-Effect Level is the lowest dose of a substance which causes changes distinguishable from those observed in normal (control) animals (WHO, 1990).

[4] Consider the following hypothetical case. In a 90-day study using 3 doses, the test chemical has no observed effects at the bottom dose, causes enzyme induction, liver enlargement and plasma cholinesterase inhibition at and above the mid dose, and erythrocyte cholinesterase inhibition at the top dose. The bottom dose would be designated as the NOEL. If the effects at the mid dose were regarded as non-adverse, this dose could be termed the NOAEL or the LOEL. If the effects at the mid dose were regarded as adverse, this dose would be termed the LOAEL.

- **LOAEL.** The Lowest-Observed-Adverse-Effect Level is the lowest dose of a substance which causes adverse changes distinguishable from those observed in normal (control) animals; in all these definitions, the meaning of the term 'adverse' may often be a point of contention.

It should always be borne in mind that in any study, the NOEL/NOAEL and LOEL/LOAEL will be determined by the doses selected in that particular study.

- **Threshold Dose.** The acceptability and usefulness of the concept of the experimental NOEL/NOAEL depends on the scientific rationale supporting the existence and demonstrability of a **threshold** for responses produced by biologically active agents. As used here, the term "threshold" designates that level of a stimulus which comes just within the limits of perception, and below which level a recognisable response is **not** elicited.

1.2.5 Acceptable Daily Intake for Humans (ADI)

It is accepted that the absolute safety of chemicals to humans cannot be established because, whilst one can prove that a chemical can produce a toxicological effect, it is not possible to determine the absolute absence of a toxicological effect.

The acceptable daily intake of a chemical is defined as the daily intake which, during an entire lifetime, appears to be without appreciable risk on the basis of the available information at the time. It is expressed in milligrams of the chemical per kilogram of body weight (mg/kg bw). For this purpose, "without appreciable risk" is taken to mean that adverse effects will not result even after a lifetime of exposure. The ADI most commonly relates to intakes from food and water, and hence is derived as far as possible from feeding studies in animals.

The determination of an acceptable daily intake involves the establishment of an overall NOEL or NOAEL for a chemical which is generally the lowest NOEL or NOAEL in the most sensitive species. (As discussed above, the definition of 'adverse' may be agency-specific, and the NOEL and NOAEL are not necessarily the same. Note also that in occupational risk assessment, the NOEL/NOAEL may be influenced by a consideration of the relevant route of exposure.) This approach is justified unless there is evidence (1) from pharmacokinetic/metabolic studies that the most sensitive species shows a different toxicokinetic behaviour than humans and is therefore less relevant as a predictor of human toxicity than another toxicity test species; or (2) that the toxic effect which has the lowest NOEL/NOAEL is not relevant for humans; or (3) that the lowest NOEL/NOAEL is derived from an inadequate or invalid study. Thus it is emphasised that the full database must be used and all relevant findings correlated, when determining the most appropriate health endpoint.

An ADI is then derived from the NOEL or NOAEL; the qualitative approach taken follows the principles outlined in the IPCS Environmental Health Criteria Monograph Nos. 104 and 210 (WHO, 1990 and 1999). The uncertainty inherent in extrapolation between and within species has generally been dealt with by the use of safety (uncertainty) factors. The size of the safety factor is most often 100, but it may range from 10 to 5000, depending on the source and quality of data, the biological relevance of the endpoint, and the hazard assessment (carried out on a case-by-case basis).

Safety factors are not necessarily rigidly applied. When based on studies in animals, the usual safety factor is 100, derived by multiplying a factor of 10 for species extrapolation with a factor of 10 for individual variation in human populations. In general terms only, a safety factor of 10 would apply when appropriate human data were available. By contrast, further safety factors may have to be incorporated to provide additional protection for special risk groups (e.g. infants), or where the toxicological database is of poor quality. Further safety factors may also be used when the toxicology database is incomplete (e.g.

field trial of a new chemical where it is proposed that produce from treated plants or animals are to be consumed) or the nature of the potential hazards indicates the need for additional caution. Utilising further safety factors that may range up to 10, 20 or even 50, an overall safety factor of 1000-5000 may be applied.[5] The ADI is calculated by dividing the NOEL or NOAEL by the safety factor. This approach assumes that exposure at less than the ADI is without appreciable risk but there is no attempt to quantify the level of risk.

1.2.6 Reference Dose (RfD)

Although regarded by many as being synonymous with "ADI", the RfD is in fact distinctly defined. It was developed by a US EPA work group for assessment of risks associated with systemic toxicity, but not carcinogenicity. The RfD is, in general, an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. Usually, doses less than the RfD are not likely to be associated with adverse health risks, and are therefore less likely to be of regulatory concern. As the frequency and/or magnitude of the exposures exceeding the RfD increase, the probability of adverse effects in a human population increases. However, all doses below the RfD are not assumed to be "acceptable" (or risk-free), and nor are all doses that exceed the RfD necessarily "unacceptable" (ie, result in adverse effects).

The RfD is derived by dividing the NOAEL or LOAEL by an Uncertainty Factor (UF) that reflects the data set upon which the RfD is based. In practice, the standard UFs used in determining RfDs for pesticides are 10 to account for interspecies extrapolation and 10 for intraspecies variation. Additional UFs may be applied for the use of a LOAEL instead of a NOAEL, and when extrapolating from shorter than chronic animal studies. In addition, a Modifying Factor (MF) of greater than zero but less than or equal to 10 is sometimes also applied, based on a professional judgement of the *entire* database of the chemical. The equation is RfD = NOAEL or LOAEL / (UF x MF), expressed in mg/kg bw/day.

For a detailed explanation of the RfD concept, how it varies from the ADI, and its role in risk management, the reader is referred to the relevant Background Document (US EPA, 1993; see: http://www.epa.gov/ngispgm3/iris/rfd.htm).

Where datasets allow appropriate analysis, alternative concepts such as the Benchmark Dose or Effective Dose (EDx) are under consideration by regulatory agencies in calculating health endpoints. For a discussion of the Benchmark Dose (BMD) concept, and the methods used, the reader is referred to e.g. Barnes et al (1995). Commonly, a threshold dose for an adverse effect is established and a NOEL derived. The BMD method has been developed to take into account the shape of the dose-response curve and the group sample size; it aims to quantify the risk associated with doses at or above the NOEL. It is not a risk extrapolation method i.e. it does not make numerical extrapolations to extremely low doses/levels of risk.

[5] The European Commission Co-ordination (ECCO) peer review team has recently advocated the use of a stepwise approach. The steps 100 (usual safety factor), 250, 500 and 1000 should be preferred, but clear written rules for the justification of safety factors higher than 100 must be defined (eg a safety factor of 1000 might be applied to the LOEL for carcinogenic and teratogenic effects.) [1174/ECCO/BBA/97, 5 January 1999: "Documentation, clarification and updating information with respect to Section 4 of Part B of the ECCO-Manual"]

1.2.7 Risk Management

Risk management is the process by which the risk from an identified hazard is controlled and exposure is maintained at a level which provides an acceptably low risk. Risk management activities are concerned generally with establishing air, water and food exposure standards, identifying sub-populations at risk and taking remedial action following excessive exposure.

1.2.8 Regulatory Decision Making

Regulatory actions may be based on some or all of a number of distinct processes, summarised as follows:

- **Hazard Assessment.** Assessment of the inherent properties of chemicals and their capacity to harm humans and the environment.

- **Risk Assessment.** Estimation of the probability of any harm occurring and its likely extent. Risk assessments contain some or all of the following steps:

    - Hazard Identification. The determination of whether a particular chemical is or is not causally linked to particular health effects.

    - Dose-response Assessment. The determination of the relation between the magnitude of exposure and the probability of occurrence of the health effects in question.

    - Exposure Assessment. The determination of the extent of human exposure before or after application of regulatory controls. For agricultural/veterinary chemicals, this is often calculated from national food consumption data and market basket surveys of pesticide residue in foodstuffs); daily intake calculations are based on the procedures as outlined in *Guidelines for Predicting Dietary Intake of Pesticide Residues (revised)* (1997) prepared by the Global Environment Monitoring - Food Contamination Monitoring and Assessment Programme in collaboration with the Codex Committee on Pesticide Residues, and published by the WHO (WHO, 1997). See also Chapter 5 of IPCS Environmental Health Criteria Monograph No. 210.

- **Risk Characterisation.** The description of the nature and often the magnitude of human risk, including attendant uncertainty, in which the exposure and dose-response assessments are combined to produce a risk estimate and in which the strengths and weaknesses, major assumptions, judgements and estimates of uncertainties, are discussed.

- **Benefit Assessment.** Analysis of possible advantages with a certain use of a chemical product.

- **Assessment/Analysis of Consequences.** Prediction of the consequences of choosing a certain decision alternative.

- **Risk-Benefit Assessment.** Assessment based on an acceptable risk, from the standpoint of society at the time of the decision.

The different steps in the process, leading to the management of the identified risks, are made independently of each other and in a manner in which facts and scientific observations are to be distinguished from general viewpoints. Thus, the process of risk assessment uses the factual base (the hazard assessment and exposure assessment database) to define the likely health effects in individuals or

populations under given exposure conditions to hazardous materials and situations. Risk management is the process of weighing policy alternatives and selecting the most appropriate regulatory action, integrating the result of risk assessment with social, economic, and political concerns to reach a decision (National Academy of Sciences/National Research Council, 1983; see also Chapter 6 of IPCS Environmental Health Criteria Monograph No. 210).

The concept of separating risk assessment and risk management functions, to the maximum extent feasible, allows evaluators to concentrate on analysis, evaluation, and interpretation of toxicological data according to sound scientific principles and without regard for subsequent regulatory decisions and risk management actions. At least some of the controversy surrounding regulatory actions has resulted from a blurring of the distinction between the risk assessment process and risk management policy decision (National Academy of Sciences/National Research Council, 1983).

2. DOCUMENTATION AND DATA ACCEPTANCE

The quality, integrity, and completeness of the reporting of experimental data from toxicity studies are essential to the subsequent independent analysis and evaluation of submitted studies. In essence, quality evaluations expected of regulatory agencies have their foundations in the submitted evidential documentation. Therefore, a qualitative assessment of the acceptability of study reports is an important part of the process of independent evaluation.

In order to be acceptable to a regulatory agency, studies must be of an adequate standard. The EC Technical Guidance Document on risk assessment for new and existing substances (EC, 1996) defines the adequacy of an experiment in terms of its *reliability* and *relevance*.

2.1 Reliability

Reliability covers the inherent quality of the study, relating to test methods and the way that the conduct and results of the study are described.

Parameters included here are: the observational and experimental methods; frequency and duration of exposure; the species, strain, sex and age of the animals used; the numbers of animals used per dosage group; dose, route and frequency of dosing; and the conditions under which the substance was tested.

There are many guidelines to the generation of scientifically valid data which concern good experimental design, laboratory practice and reporting e.g. OECD and US EPA guidelines, and accepted codes of Good Laboratory Practice, or GLP (OECD, 1982; US EPA, 1983). They can be useful as aids in determining report and data acceptability. However, the evaluator needs to make a judgment about how well the study, in toto, facilitates the identification of potential adverse effects, or lack thereof, of the substance being evaluated, rather than how precisely it fits a prescribed test guideline or "recipe". The experience of senior evaluators can be helpful in resolving concerns about acceptability of study conduct and/or reporting.

The evaluator should read through the report, including supporting data presentations, and make a judgement as to whether the study was well conducted and reported or whether significant deficiencies exist. If there are obvious major deficiencies which would lead the reviewing toxicologist to consider the study invalid, further evaluation may be a waste of resources. Individual countries' procedures to be adopted in cases in which studies are deficient are likely to be different.

The evaluator should also consider any effects of modifying factors which may result in major inequalities between control and treated animals. This qualitative consideration has more to do with the evaluation and interpretation of data than with acceptability of documentation. It is placed here because determination of the factors which may have a major influence on toxicological data needs to be made prior to the analysis of the data. There are many factors influencing the responses of experimental animals to chemical substances; some of these are discussed by Doull (l980). Some influences may be quite subtle, as exemplified by studies performed by Thompson et al. (1982), in which it was noted that the onset of acute pulmonary oedema in rats being used in immune hypersensitivity studies was sudden and seasonal. Subsequent studies demonstrated the reasons for this finding. Circadian rhythms and seasonal physiological variations can subtly influence experimental results. Such factors influencing

animal responses can be troublesome when their effects are confused with or misinterpreted as toxic responses to treatment.

Occasionally, subsequent detailed analysis of the data will indicate deficiencies which were not obvious during the initial perusal of the report. Such deficiencies should be noted and the analysis completed as far as possible.

Doubts on the part of the evaluator regarding the completeness and/or competency of the conduct and reporting of the study should be discussed with the evaluator's supervisor, and appropriate action taken, according to national/regional procedures in place.

2.2 Relevance

Relevance covers the extent to which a study is appropriate for a particular hazard or risk assessment.

To assess the relevance of data, it is necessary to judge if an appropriate species has been studied, if the route of exposure is relevant to the population and exposure scenario under consideration, and if the test chemical is representative of the chemical to which the population is or will be exposed. The test chemical should therefore be properly identified and any significant impurities described (EC, 1996).

If the test chemical is a pesticide, most studies will have been performed on animals, and there will often be no data on its metabolism, toxicokinetics or toxicity in humans. Under these circumstances, adverse effects observed in animal studies will normally be assumed to occur in humans, even if the threshold level of exposure is unknown. Clear and well documented evidence of a species-specific effect / response would therefore be required before an animal study was deemed irrelevant to humans. (The EC Technical Guidance Document cites the example of light hydrocarbon-induced renal nephropathy in male rats.)

In certain cases (e.g. parasiticides, antibiotics or some organophosphates) where human data are available on the test chemical or a close structural analogue, it may be possible to judge the relevance of animal data on the basis of comparative metabolism and toxicokinetics, or clinical experience. An example is abamectin, an agricultural acaricide and veterinary parasiticide having enhanced developmental toxicity in P-glycoprotein deficient CF-1 strain mice. The relevance of studies in the CF-1 mouse has been dismissed by JMPR following comparative studies with abamectin in P-glycoprotein normal CD-1 mice, and veterinary and human clinical experience obtained with its close structural relative, ivermectin.

These qualitative considerations establish the acceptability not only of specific reports but also the acceptability of the eventual evaluation, interpretation, judgments, and risk assessments made by toxicologists.

The acceptability of reports and other technical information submitted to regulatory agencies is primarily a scientific judgment. The submitters of the information deserve to know the rationale for any rejection of data. Therefore, the rationale for rejecting a study should be succinctly stated in the evaluation document.

Whilst directed at laboratories conducting toxicology studies, a useful reference in regard to issues of quality control is the IPCS EHC monograph no. 141 on *Quality Management for Chemical Safety Testing* (WHO, 1992).

The Canadian PMRA data screening pro-forma for 90-day studies is reproduced at Appendix II. This was subsequently incorporated into the OECD Working Group on Pesticides *Forms for Screening Test and Study Reports and Summaries for Completeness* (OECD, 1998d).

Further guidance on completeness checks is provided in the OECD *Guidance for Industry Data Submissions* and *Guidance for Country Data Review Reports on Plant Protection Products* (OECD, 1998a, b).

3. ANALYSIS AND EVALUATION OF ADVERSE EFFECTS IN REPEAT-DOSE TOXICITY STUDIES

It is important that all toxicity data and the methods by which they are obtained be subjected to critical and independent scientific assessment by the regulatory evaluator. As the primary emphasis is on independent assessment, it is the evaluator's responsibility to ensure evaluation reports, including any company summaries and company-sponsored "expert reports" which may be used, comprehensively document study results, interpretations and conclusions in an accurate, clear and transparent manner.

A valuable guidance document for evaluating data and conducting assessments is the IPCS monograph on *Principles for the Toxicological Assessment of Pesticide Residues in Food* (EHC 104) (WHO, 1990) and related monographs i.e. EHC 6, 70 and 141(WHO, 1978, 1987, 1992). Furthermore, evaluators may refer to the *OECD Guidelines for Testing of Chemicals* to check the adequacy of certain studies. Another useful document is *Guidelines for the Preparation of Toxicological Working Papers for the Joint FAO/WHO Expert Committee on Food Additives*, Geneva, July 1987 (ICS/89.41) (FAO/WHO, 1987).

Regardless of the route of administration, both subchronic and chronic exposure studies share many common toxicity end points.

The revision of OECD Test Guidelines for 90-day oral toxicity studies in rodents and non-rodents (Test Guidelines 408 and 409; adopted 21st September 1998) has placed additional emphasis on neurological, immunological and reproductive endpoints i.e. studies should allow for the identification of chemicals which may require further investigation in these areas. It is beyond the scope of this document to provide detailed guidance on the assessment of these parameters.

3.1 Analysis and Evaluation of Major Study Parameters

It needs to be borne in mind that not all observed effects of test substances are necessarily toxic effects. Rather, they may be adaptive (e.g. liver enzyme induction leading to some hepatic enlargement, but see Section 1.2.3 under 'Treatment-related Responses') or may be a manifestation of a pharmacological effect (e.g. in an animal colony suffering from various low-grade infections, an antibiotic will lower leucocyte counts in treated animals relative to controls; obviously it is not appropriate to describe this as a leukopenic effect of the chemical).

Concurrent control groups should always be used; notwithstanding the value of historical control ranges, it is generally not appropriate to rely on statistical comparisons with historical controls since some biological parameters can vary significantly over time (and even between concurrent randomised control groups). Controls must be age-matched because some forms of toxicity represent no more than acceleration and/or enhancement of age-related changes. Examples of pathological changes in aged rodents which may be affected by compound administration include chronic progressive glomerulonephropathy, peripheral nerve degeneration, amyloidosis and various neoplasms.

Historical control data may be useful when evaluating the acceptability of the "normal" data obtained from control groups (Haseman et al, 1984; Paynter, 1984; Sumi et al, 1976; Tarone, 1982). Any departure from the norm by the control groups should be discussed in the evaluation document and taken into consideration, especially during the conduct of any statistical analysis. The finding of consistent departures from the norm in control groups could necessitate investigation of the source of the animals.

Ideally, historical control data which is submitted for consideration should be taken from the same laboratory, utilising the same strain, age and sex of animals obtained from the same supplier, and only include those studies conducted within a 2 to 3-year span on either side of the study under review, with identification of study methodology (e.g. pre-sampling conditions such as fasting or non-fasting, assay methodology for study parameters, histopathological criteria for lesion identification, time of terminal sacrifice etc.) which could have affected the results. Where historical data is used in an assessment, it should be clearly identified (see Section 5.2).

The use of non-treated and vehicle-control groups aids assessment of effects due to vehicle or excipients. When a vehicle is used, the need for vehicle-treated controls is paramount. Since some parameters can be affected by animal handling (e.g. serum ALT was raised in mice which were grasped around the body cf. unhandled or tail-handled mice; Swaim et al, 1985), control animals should be treated in the same way as test animals.

Control animals must receive as much attention during the analysis and evaluation process as do the treated ones. Any untreated animal or group may exhibit some signs of abnormality or drift from the norm for that species or strain. Because of the possibility that statistically significant differences between treated and control groups are the result of abnormal values among the controls, such differences should usually be dose-related and should delineate a trend away from the norm for that stock of animals, if they are to be indicative of a true compound-related effect.

Basic parameters (eg, bodyweight gain, food consumption or conversion efficiency) are important and delineate the LOEL in a high proportion of studies. It is not uncommon for such parameters to be affected earlier in a study and at a lower dose than many other markers. Weil and McCollister (l963) analysed toxicity end points, other than oncogenicity, from short- and long-term tests and concluded that only a relatively small number of end points were effective in delineating the lowest dosage producing an effect in such tests. Body weight, liver weight, kidney weight, and liver pathology delineated this dosage level in 92% of test chemicals in short-term (subchronic) studies and 100% in long-term (chronic) studies. To reach 100% efficiency in short-term studies, renal and testicular histopathology had to be included. Heywood (l981) surveyed the toxicological profiles of fifty compounds in rodent and non-rodent species and confirmed these observations. For this reason, these criteria should receive careful attention in the analysis and evaluation process.

However, there is no implication that these criteria delineate all of the stress markers or toxicity end points particularly since toxicology testing has undergone significant development and refinement since those study reviews were reported, particularly with respect to serum biochemistry and neurotoxicology. Evaluators therefore should be aware that effects on any endpoint may be important.

As noted above (Sections 1.1 and 1.2), the existence of a dose-response relationship for a particular biological or toxicological effect provides strong evidence that the response is a result of exposure to the agent being tested. In conducting a hazard assessment and reporting studies, the correlation between external dose and the incidence and/or intensity of toxicological endpoints need to be considered and reported.

3.1.1 Mortality/Survival

Death is a highly definitive end point! Therefore, reasonable efforts should be made to determine the cause or likely cause of individual deaths. The evaluation of pathological lesions or morphological changes in belatedly-observed deaths are frequently complicated by postmortem autolysis. The separation of deaths caused by factors unrelated to exposure to the test agent (e.g. acute or chronic infections, age or disease-related degenerative processes, anatomical abnormalities, negligent handling or accident) from

toxicity-induced deaths is important. All data relating to moribund or dead animals during their study life, as well as the results of postmortem examinations, should be scrutinised in an attempt to make this distinction. Note that OECD Test Guidelines 408 and 409 state that, unless limited by the physical-chemical nature or biological effects of the test substance, the highest dose level is to be chosen with the aim to induce toxicity but not death or severe suffering.

Analysis of mortality requires more than a statistical treatment of incidence at termination of a study. Survival/mortality data can be influenced by factors other than the test substance. Changes in the protocol during the course of a study can complicate the analysis e.g. alterations in dosage levels can produce a confusing mortality pattern.

Any unusual mortality pattern should be explained by the data submitter on biological or toxicological grounds. If overall mortality is high (i.e. significantly greater than expected for the particular colony and strain) for any repeat-dose study, or for a particular group within a study, a credible explanation should be provided. (If this is not the case, national agencies may consider conducting a laboratory and data base audit.)

An evaluation of mortality patterns within each group is important. Such patterns may indicate that mortality is clustered early or late in the course of the study, is intermittent and scattered throughout the duration of the study, or has a higher incidence in one sex than in the other. The analysis of the cause of individual deaths will aid in determining the toxicological significance of these various patterns. Early deaths within treated groups may just reflect deaths of the more susceptible animals in the test population. Alternatively, it may indicate changes in compound intake per unit body weight, in those experiments in which the quantity of test substance in the diet is kept constant. Relative to body weight, young rats ingest more food than older rats and hence, young rats ingest relatively more of the test substance than do older rats. Early deaths may therefore be the result of the higher exposure, on a mg/kg/d basis, of young animals compared to older animals.

Deaths which are clustered at a specific time period may reflect a spontaneous epidemic disease situation of limited duration. High mortality associated with infectious agents in treated groups, in the absence of such evidence in the concurrent control group, could indicate an immuno-suppressive action of the chemical being tested.

The effect of dietary intake on mortality needs to be considered. A compound administered in the diet may make the laboratory chow more or less palatable, may have a pharmacological stimulant or depressant effect on appetite, or may affect the partitioning of the nutrients in the food. Likewise, decreased water consumption (e.g. in the case of an unpalatable compound administered in the water) will lead to reduced food consumption. These effects may significantly influence longevity since it has been clearly shown in animals species that long-term dietary restriction very significantly increases lifespan (e.g. Tucker, 1979). Conversely, excessive *ad libitum* intake of highly nutritious diets can reduce lifespan cf. the expected average lifespan for an animal species/strain (see e.g. Table 3; reduced lifespan of bred *vs* wild rats). To date, regulatory authorities have not come to any decision on recommending restricted diets *vs*. *ad libitum* feeding in toxicity study guidelines; some useful references on this topic include Keenan et al (1998; see also other related articles by this author), Klinger et al (1996), Masoro (1992), and Thurman et al (1995).

3.1.2 Clinical Observations

Adverse clinical signs (gross observations) noted during the exposure period may correlate with toxicity end points or disease processes. These can be used as supportive evidence for dose-response relationships and may play a role in the determination of the NOEL/NOAEL. However, not all adverse

clinical signs will correlate with pathological or morphological changes in organs or tissues. Some will be caused by biochemical or physiological effects i.e. incoordination, muscle twitching, tremor, or diarrhoea may indicate acetylcholinesterase inhibition without any morphological changes being evident in nervous tissue.

Most clinical signs observed during physical examination of individual animals are determined without the aid of instruments. Therefore, it is important that all deviations from the "normal" observed in control and treatment groups be adequately described and recorded during the study and presented in the study report.

Many of these qualitative signs can be counted, scored for intensity, and tabulated as incidences. However, statistical analysis is of limited value. The evaluator must rely on the number of individuals per group exhibiting signs of a particular type, as well as the intensity of the responses, to gain an impression of a dose response-relationship.

Clinical observations such as palpable tumours or those which might be associated with neoplasia (e.g. haematuria, abdominal distension, or impaired respiration) may be useful in defining the time a tumour was first suspected as being present. Such signs might aid in the evaluation of decreased tumour latency in long-term rodent studies. They may also aid in determining cause of death. **A statement of the correlations, or the lack thereof, between clinical signs and specific toxicity end points should be made in the evaluation.**

Ophthalmoscopic examinations prior to test substance administration and at study termination should be performed on at least the control and high-dose animals. The relatively limited usefulness of gross examination of the eye by ophthalmoscope should be borne in mind, particularly in studies on compounds with potential visual system toxicity; other investigations should be considered in conjunction with ophthalmoscopy e.g. histopathological data on the eye and optic nerve, and, if available, any electroretinographic data (e.g. for organophosphorus compounds).

Useful information on gross behavioural observations in laboratory animals and abnormal behaviour patterns can be found in Bayne (1996).

The revised OECD Test Guidelines for 90-day oral toxicity studies in rodents and non-rodents (Test Guidelines 408 and 409; adopted 21st September 1998) have placed additional emphasis on neurological endpoints i.e. studies should allow for the identification of chemicals with the potential to cause neurotoxic effects, which may warrant further in-depth investigation. The reader is referred to the references cited in Test Guideline 408 relating to neurotoxicity assessment, including sensory reactivity to stimuli of different types (auditory, visual, proprioceptive), grip strength, and motor activity.

3.1.3 Body Weight Changes, Food and Water Consumption

Body weight changes (gains or losses) for individual animals and groups of animals when compared to concurrent control changes during the course of a study are a criterion of some importance (Heywood, 1981; Roubicek et al, 1964: Weil & McCollister, 1963). Such changes are usually related to food intake, and analysis of one without an analysis of the other is of limited value. Weight decrement may not always be related to toxicity *per se* (Seefeld & Petersen, 1984). Occasionally the incorporation of the test substance into the diet will reduce the palatability of the diet to many individuals in all treatment groups or to the majority of individuals in the higher dietary level groups. Food spillage needs to be considered in the evaluation of food palatability and compound intake. The same considerations apply if the compound is administered in drinking water.

This effect is often evidenced during the first two or three weeks of the study. Sometimes animals in the affected groups(s) are able to accommodate to the diet and a gradual increase in group weight gain will occur (Nolen, 1972). In subchronic studies, the lag in group weight gain may persist, even though the individual animal gains per gram of food consumed (food utilisation efficiency) are favourable after the accommodation, and produce a statistically significant difference between the affected group and the concurrent controls which is not related to toxicity of the test substance (McLean & McLean, 1969). Sometimes the addition of the test substance will interact with one or more essential nutritional elements in the diet thereby producing weight gain decrements or alterations of toxic responses (Casterline & Williams, 1969; Conner & Newbern, 1984; Rogers et al, 1974). This phenomenon may be encountered in subchronic studies and when identified, can usually be overcome by acceptable means before a chronic study is initiated. Infrequently, control values for weight gain (at one or more time points) can be low, causing the other value to appear unusually high.

Diet composition, food and water consumption, and body weight gains *per se* can also have an important influence on many aspects of animal responses including shifts in metabolic, hormonal, and homeostatic mechanisms (Kennedy, 1969) as well as disease processes (Berg & Simms, 1960; Paynter, 1984; Ross & Bras, 1965; Tannenbaum, 1940) and maturation (Innami et al, 1973), and should be considered when unusual effects are observed in the absence of any indication of injury to organs and other vital systems.

The evaluation of body weight changes and attendant effects is significantly aided by the graphical presentation of group mean body weights and food consumption *vs* compound consumption (on a mg/kg body weight basis). This allows quick identification of any unusual or sudden changes in gain or loss by any group.

3.1.4 Haematological, Clinical Chemistry, and Urinary Measurements

Regulatory guidelines generally suggest that haematological, clinical chemistry, and urinary parameters be routinely measured in subchronic and chronic toxicity studies. (Urinalyses are not routinely required for subchronic studies in rodents submitted under OECD guidelines or to the US EPA, unless there is an indication for doing so.)

Normal biological variation in inter-animal values and their alteration in response to a variety of inputs means that evaluators will have to contend with much “noise” in this area, and will frequently be presented with scattered, statistically significant effects, in the absence of any evidence of clinically significant relationships to specific toxicity end points. For example, Pearl et al (1966) restrained rats for 6 hours and followed aspartate transaminase (AST, or SGOT) and alanine transaminase (ALT, or SGPT) changes. These transaminases were very much elevated and the AST did not return to basal level within a period of six days, indicating an apparent susceptibility of these enzymes, particularly AST, to stress factors. To deal with “noise” there is a need to examine whether the effect noted is within the normal range of variation (concurrent and historical controls). Note that some of these parameters can vary significantly with no clinical manifestations but others (e.g. serum potassium) have a very narrow normal clinical range and small differences can be important.

Frequently these data show apparently ‘random’ changes in individual group(s) or, less commonly, non dose-related trends in changes across several groups. If using historical control data as an aid to evaluation, it must be kept in mind that “normal values” in haematological and clinical chemical measurements depend on the specific methods used to generate the data. Therefore, only values produced by the identical methods from the same laboratory are valid in such comparisons. Literature values for normal ranges which do not specify the method by which they were obtained should be used with caution.

These comments underline the importance of concurrent control data for clinical chemistry, haematology and urinalysis parameters.

An example of differences in several clinical parameters measured in the one animal is as follows: Dameron et al (1992) sought to determine if there were any differences in the results of clinical laboratory tests between blood samples collected from the orbital venous plexus (OVP) and the posterior vena cava (PVC) of adult male Sprague-Dawley rats. The coagulation times (prothrombin and partial thromboplastin) and serum Mg and P showed biologically significant differences between samples collected from the OVP and PVC. A good review of factors (physiological, environmental etc.) which can complicate the interpretation of findings in a toxicity study may be found in the *Handbook of Toxicologic Pathology* (Bucci, 1991).

In a recent overview of the usefulness of clinical chemistry data in subchronic toxicity studies, the relative sensitivities of 8 commonly used clinical chemistry parameters to detect potential toxic liver and kidney effects were evaluated for a series of 61 subchronic rat studies conducted by the US National Toxicology Program (Travlos et al, 1996). Liver and kidney lesions were reported in 31% and 41% of the studies respectively. There was an association between treatment-related increases in ALT and succinate dehydrogenase (SDH) activities and histopathological changes in the liver; SDH had greater positive and negative predictive value than similar changes in ALT. There was an association between treatment-related increases in BUN and creatinine and morphological kidney changes; creatinine concentration had a greater positive predictive value than similar changes in BUN.

Blood cytology, blood biochemistry, and urinalyses can provide useful information. Heywood (1981), in surveying the correlation of sensitive criteria of target organ toxicity across species, found that reduction of haematology values was a common effect recorded in all species in his survey when the haemopoietic system was affected. To gain maximum information from enzyme determinations it is important to consider the most appropriate enzymes, together with their distribution between and within organs and their sub-cellular location. ALT is found in greatest concentration in hepatocytes in rats, even more so in dogs. AP is present within biliary and canalicular membranes, kidney, intestine and bone (Tyson & Sawhney, 1985; Evans & Lake, 1998). Isozyme analysis is often used to differentiate bone injury from organ tissue damage as the form of AP from these sources is slightly different. CPK is mainly located in skeletal and heart muscle, whilst AST is found in various concentrations in most organs. It is clear that CPK is the most appropriate enzyme to detect muscle damage, while increases in ALT would probably reflect liver necrosis. Although AST is not organ-specific, it serves to confirm organ damage, especially for muscle and liver, if its activity changes in parallel with other enzymes. For hepatocellular evaluation, ALT, AST, SDH and GDH are the most appropriate, while for hepatobiliary evaluation, AP, 5'-nucleotidase, GGT and total bilirubin are the most appropriate measurements.

Sensitivity and specificity of the enzyme changes as diagnostic of organ pathology are greatly influenced by the species selected for testing (see e.g. Clampitt, 1978; Tyson & Sawhney, 1985). ALT is relatively specific to the liver in the cat, dog, ferret, mouse and rat, whereas in primates, ALT is present in heart, skeletal muscle and liver. In dogs, AP is a sensitive test for biliary function but in the rat it is of little diagnostic value since in the latter species its serum levels are relatively high and subject to variation with diet, being principally derived from the intestines (Tyson & Sawhney, 1985; Evans & Lake, 1998). It is evident that species differences are of great importance when specific clinical chemistries are selected for inclusion in toxicity studies.

In a recent overview of the usefulness of clinical chemistry data in subchronic toxicity studies, the relative sensitivities of 8 commonly used clinical chemistry parameters to detect potential toxic liver and kidney effects were evaluated for a series of 61 subchronic rat studies conducted by the US National Toxicology Program (Travlos et al, 1996). Liver and kidney lesions were reported in 31% and 41% of the

studies respectively. There was an association between treatment-related increases in ALT and succinate dehydrogenase (SDH) activities and histopathological changes in the liver; SDH had greater positive and negative predictive value than similar changes in ALT. There was an association between treatment-related increases in BUN and creatinine and morphological kidney changes; creatinine concentration had a greater positive predictive value than similar changes in BUN.

It is important to understand that many of these types of serum enzyme tests and urinalysis fail to detect minor injury or may reflect only transient or reversible changes. Therefore, evaluation and interpretation of the test results must be performed carefully and correlated with histopathological findings.

When analysis and evaluation of clinical data indicate a dose response relationship or a biologically important drift from concurrent control values, the evaluator should attempt to correlate the effect(s) with other manifestations of toxicity, and should indicate whether or not a correlation could be made.

Standard veterinary (e.g. Bush, 1991; Duncan et al, 1994; Evans, 1996; Fox et al, 1984; Jain, 1993) and human clinical manuals (e.g. Fischbach, 1996; Henry, 1984; Tyson & Sawhney, 1985; Walach, 1996) should be consulted for information about laboratory diagnostic tests and to assist in the evaluation of potential correlations between clinical chemistry, haematological, urinary data, and adverse effects.

Applicable regulatory guidelines often specify which laboratory parameters should be measured; those most commonly measured are included in the Table at Appendix III.

3.1.5 Absolute and Relative Organ Weights

It has been stated that the most efficient criteria for evaluation of the lowest dosage producing an effect in subchronic exposure studies are changes in liver, kidney, and body weights (Weil & McCollister, l963; Heywood, l981). However, it is generally considered that histopathology is more sensitive for establishing the lowest dose producing an effect than organ or body weight changes. Organ weights are usually reported as absolute organ weights and as relative organ weights (relative to body weight and/or brain weight).

Experimentally controllable and uncontrollable factors (i.e. circadian rhythms, food intake, dehydration, nature of the diet, age of animals, organ workload, stress, and method of killing) have an influence on organ and body weights and the variability of such data. A review of this subject by Weil (1970) should be consulted. The most important influencing factor appears to be the method of killing and the timing of necropsy. The killing method used not only affects the appearance of the tissue, important in describing gross necropsy observations, but also, in conjunction with the timing of necropsies, may cause postmortem shifts in organ weights (Boyd & Knight, 1963; Pfeiffer & Muller, 1967). A uniform exsanguination technique has been described and evaluated by Kanerva et al (1982) which significantly ($P<0.05$) reduced the absolute and relative liver and kidney weights with respect to these weights from animals that were not exsanguinated. The standard deviations of the mean absolute and relative liver weights were also significantly ($P<0.05$) reduced. In this study, exsanguination did not appear to affect the absolute or relative weights nor the standard deviations for heart, brain, and spleen. Additionally, the use of fasted animal body weights can reduce the variability of organ/body weight ratios. Adkins et al (1982) discuss the standardisation of the technique for determination of testes weights to reduce variability.

A not uncommon problem in interpretation of study findings is the misinterpretation of relative organ weight changes e.g. there is no sense in reporting an increase in relative brain weight in a toxicity study in which the chemical is having a significant effect in causing bodyweight loss or reducing body

weight gain - because the brain will be spared under conditions leading to reduced bodyweight, the relative brain weight will obviously increase! Similarly, other organs may change in relative weight in a manner dependent upon body weight rather than as a result of a specific compound effect - useful Tables of the relationship of relative organs weights to various levels of reduced bodyweights (produced by dietary restriction) may be found for rats in Shärer (1977) and related references; some data are given in Table 2. Thus, it must be borne in mind that when growth is markedly affected in a toxicity experiment, alterations of organ wt:body wt ratios have to be expected as a physiological response of the organism to decreased nutrient intake; such changes must be differentiated from organ weight changes resulting from primary toxic effects of the compound being tested.

Furthermore, the interpretation of organ weight changes must not be made solely on the determination of a statistically significant difference between the concurrent control value and a treatment group value. A proper evaluation will also include consideration of any correlation between organ weights (absolute and relative), histopathological and metabolic/pharmacodynamic data. Such correlations if they exist should be discussed in the evaluation documentation. Appendix IV contains a list of organs that should be weighed and / or examined histopathologically.

3.1.6 Postmortem Observations

Pathologists have an important role in toxicology since they provide information on the differences in tissue and organ morphology which will establish the presence or absence of lesions and whether there are dose effect relationships for such lesions. This data is critical to establishment of toxic and other effects produced by a substance. Zbinden (1976) discussed the role of the pathologist in some detail, as well as the use of semi-quantitative methods, as well as more accurate morphometric methods, for rating the severity of lesions. He cautioned that even with the use of such methods, care must be taken in evaluating tissue and organ pathology/lesions, because of the lack, at that time, of generally and internationally accepted nomenclature in toxicological pathology. The problems created by different nomenclature were also discussed by Haseman et al (l984). Although much progress has been made in the standardisation of nomenclature, to minimise any difficulties in this area, an experienced pathologist will describe each significant lesion type, at least once, in such detail that another competent pathologist can perceive a mental picture of the lesion and form a judgment as to its relevance to the histopathology induced by the chemical being tested.

The nomenclature used to describe pathology findings in experimental animal studies suffers from the lack of uniformity. To assist in the uniform description of pathologies, several initiatives have been undertaken:

- A series of *Guides for Toxicologic Pathology* has been published by the US Society of Toxicologic Pathologists (STP), in cooperation with the Armed Forces Institute of Pathology (AFIP) and the American Registry of Pathology (ARP), introducing the 'Standardized System of Nomenclature and Diagnostic Criteria' used by toxicologic pathologists around the world. These monographs are used to diagnose proliferative and non-proliferative lesions in laboratory animals. Divided by organ system, the Guides include morphologic descriptions and colour photomicrographs of spontaneous and induced lesions seen in laboratory animal safety and efficacy evaluations. Available monographs include guides to non-proliferative lesions of bone, cartilage, tooth and synovium, the alimentary canal, soft tissue and skeletal muscle, the central nervous system, and kidney and lower urinary tract in rats. Further details, including information about additional guides as they become available, may be found at: http://www3.afip.org/cgi-bin/bookstore.cgi.

- The Registry Nomenclature Information system (RENI), developed at Fraunhofer Institute of Toxicology and Aerosol Research. This comprehensive electronic system presents the WHO/IARC *International Classification of Rodent Tumours* (also available as a CD-ROM and in hard copy format as *IARC Scientific Publication 122*), together with additional information (Mohr & Moraweitz, 1995). RENI can be accessed online at: http://www.ita.fhg.de/reni.

- The Registry of Industrial Toxicology Animal-data (RITA), also maintained by the Fraunhofer Institute, containing validated data on tumours and other proliferative lesions from over 11000 control mice and rats, using standardised nomenclature and diagnostic criteria (Moraweitz et al., 1992; Mohr & Moraweitz, 1995; Bahnemann et al, 1995 and Mohr, 1999). Further information about RITA can be obtained from the RENI Web site above.

- The North American Control Animal Database (NACAD), which has the same purpose, structure and diagnostic criteria as RITA, but uses a slightly modified nomenclature aimed at amalgamating the STP and RITA systems. See: http://www.ita.fhg.de/reni/nacad_d.html.

- International Harmonization of Rat Nomenclature, which is a project intended to reconcile nomenclature of proliferative lesions in rats. This may be accessed at the Fraunhofer ITA Web server: http://www.ita.fhg.de/reni/rat_nomenclature/index.htm, or the STP Web site: http://www.toxpath.org/nomen/index.htm.

Pathology data can facilitate the interpretation of other data such as organ weight changes or haematology findings (e.g. Krinke et al, 1991).

Age-associated, especially geriatric, changes can have an extremely important effect on histopathology, as well as clinical chemistry, metabolic and pharmacokinetic parameters (Grice & Burek, 1983; Krinke et al, 1991; Mohr et al, 1992, 1994, 1996) and therefore, important overt, and frequently subtle, influences on observed physiological, pharmacological, and toxic responses during the latter part of any long-term study. As indicated earlier, spontaneous degenerative lesions, especially when misinterpreted as toxic effects, can cause major difficulty in hazard evaluation. It is essential in all cases where spontaneous and/or age associated lesions are present, to differentiate between such lesions and treatment induced lesions. References such as Grice and Burek (1983) and Benirschke et al (1978) (containing detailed descriptions of potential histopathological changes caused by toxic substances, spontaneous or degenerative and other diseases, and their incidences in experimental animals) are very helpful in this respect, as is advice from a competent and experienced pathologist.

An overview of factors (physiological, environmental etc.) which can complicate the interpretation of morphological findings in a toxicity study may be found in the *Handbook of Toxicologic Pathology* (Bucci, 1991).

3.2 Toxicokinetic and Metabolism Data

Toxicokinetic (absorption, distribution and elimination) and metabolic data on the handling of the substance in the test species, can be very useful in the evaluation and interpretation of subchronic and chronic exposure study data, as discussed by Paynter (1984) and references cited therein. References in this paper also discuss dose-dependent effects in the absorption process and in biotransformation interactions (Levy, 1968), the potential difficulties presented by impurities, the overloading of detoxification mechanisms (Munro, 1977) and other important experimental considerations (Dayton & Sanders, 1983).

The following serves as an example of a correlation between toxicokinetics and toxicology findings; a pesticide produced a particular target organ pathology in a repeat-dose study which did not show any significant dose response. Subsequent analysis of the toxicokinetic data showed that the test substance, which was highly lipophilic and metabolically very stable, reached a saturated plateau level in the target organ after only a few doses, even at the lowest dose.

A number of toxicology textbooks include chapters on pharmacokinetics and toxicology assessment e.g. Sharma & Coulombe (1996). The publication, *Science and Judgement in Risk Assessment (*National Academy of Sciences/National Research Council, 1994), has useful sections on the impact of pharmacokinetic information in risk assessment.

3.3 Statistical Tests

It must be borne in mind that the objective of a toxicology study is to demonstrate responses of biological importance. Where statistical analyses are used in the judgement process, an awareness of the validity of the test and the degree of certainty (confidence) pertaining within the context of the study should be demonstrated.

There are limitations associated with the use of statistics in toxicology (Gad & Weil, 1986): (1) statistics cannot make poor data better; (2) statistical significance may not imply biological significance; (3) an effect that may have biological significance may not be statistically significant; (4) the lack of statistical significance does not prove safety. The importance and relevance of any effect observed in a study must be assessed within the limitations imposed by the study design and the species being studied.

Appendix V lists some commonly used statistical tests. If statistical tests have not been used, if inappropriate tests appear to have been used, or if tests not commonly employed have been used, then it is appropriate for this to be noted in the assessment report (or other action taken e.g. data re-analysis by the sponsor or the assessor).

A number of textbooks and papers on the application of statistics in experimental toxicology and the life sciences are available; these include Dickens & Robinson (1996), Gad & Weil (1986), Gad & Weil (1989), Lee (1993), Salsburg (1986), Tallarida & Murray (1987) and Waner (1992).

3.4 Completion of Analysis

At this point an evaluator should have formulated judgments and supporting rationale concerning: (a) the acceptability of the study and its database; (b) the existence of biologically important toxic effects; (c) the relevance of any factors noted during the evaluation which might have had some bearing on the outcome of the study and modified the findings in some way; and (d) the likelihood that any of the observed effects were induced by the administered substance.

The evaluator should succinctly summarise the critical toxicokinetic and toxicological data, together with any modifying factors for the study under review. The lowest, or most appropriate NOEL/NOAEL, or the absence thereof [see discussion at 1.2.5 and 1.2.6 under ‘Acceptable Daily Intake (or Reference Dose) for Humans’], should be stated, with a clear indication of the effect(s) on which it was based (i.e. the lowest-observed effect level or LOEL should be apparent). It is important to correlate findings seen in different studies; whilst this is done within the final summary of all toxicity studies, it will often be appropriate to make some mention of cross-study correlations (or the unexpected/unexplained absence of them) within individual study summaries. Possible or proven mechanisms of toxicity should also be discussed and included in the summary.

4. EVALUATION OF THE WEIGHT-OF-EVIDENCE

The essential purpose of repeat-dose studies is the detection of valid biological evidence of the hazard potential of the substance being investigated. The evaluation of the weight of evidence[6] produced by toxicity studies is that process which considers the cumulative data pertinent to arriving at a level of concern about the potential adverse effects of a substance. It is composed of a series of judgments concerning the adequacy, validity, and appropriateness of the methods used to produce the database (i.e. the relevance and reliability of studies, as discussed under Section 2, 'Documentation and Data Acceptance'), and those judgments which bring into causal, complementary, parallel, or reciprocal relationships, all the data considered. Because knowledge about mechanisms of toxicity is still developing, because good epidemiological evidence is seldom available, and because animal studies are not always conclusive, the information available at a given time may provide only "persuasive" rather than "hard" evidence of a defensible presumption, one way or the other, about the potential health effects of a substance under given conditions of exposure. Therefore, it is necessary to succinctly discuss the rationale for judgments and conclusions contained in risk assessments, together with any associated uncertainties. This becomes important when new data or new scientific knowledge requires re-evaluation of the database or a change in a previous risk assessment or regulatory action.

At present, there is no acceptable substitute for informed judgment, based on sound scientific principles, in the analysis, evaluation, interpretation, and weighting of biological and toxicological data derived from animal toxicity studies conducted according to currently available protocols.

[6] In contrast, 'strength of evidence' is commonly taken to mean the degree of conviction regarding the outcome of a particular experiment e.g. the US National Toxicology Program's 'clear evidence', 'some evidence', 'equivocal evidence' and 'no evidence' of carcinogenicity. 'Weight of evidence' involves integration of **all** available data, not just one study.

5. THE EVALUATION REPORT- STRUCTURE AND FORMAT

This chapter should be read in conjunction with OECD Test Guidelines 407 - 413 (OECD, 1998c), and the OECD guidance documents for country data review reports and for industry data submissions (OECD, 1998a, b).

Where there are a number of studies in different species within a study classification, reports on each particular species should be grouped, preferably in order of increasing species size, in both Summaries and the Main Body of Report.

5.1 Study Identification

Evaluations of toxicity studies should include the following information to enable clear identification of the study. This information is important for the identification of the study, in the event that the report is referred to or resubmitted by the sponsor company at a later date, or submitted by another company. It can be incorporated into the heading and/or the first paragraph of the evaluation:

a.	Title of study (should identify species/dose-route/study duration)
b.	Report/study number
c.	Laboratory report/project number
d.	Study sponsor (usually the registrant)
e.	Testing laboratory and brief address
f.	Authors' names (if available/appropriate)
g.	Date of report
h.	Period over which the study was conducted
i.	Test guideline/protocol followed
j.	GLP status (or QA statement) and relevant authority
k.	Indication of whether the report is published or unpublished

Items a - g and k should also be included in the bibliography to the evaluation report / monograph.

5.2 Level of Study Reporting

The methodology used in the investigation and the study findings should be presented in sufficient detail for a reader to form an independent conclusion. Ideally, reports should obviate the need, during a subsequent review of the chemical, to have to refer back to the original study data.

Note: Until OECD countries have had the opportunity to exchange a number of reviews prepared under these notes, it is considered that evaluators, if in doubt about the level of detail or explanation of findings which need to be reported, should err on the side of inclusion of more, rather than less, information.

The importance of assessing whether observed changes/differences are treatment related must be stressed - if they are very clearly unrelated to the compound, coincidental findings should not be mentioned. However, if there is any concern that an effect could possibly be related to dosing, then it should be mentioned, with a comment about the lack of any dose-relationship or other unequivocal evidence. Tabulation of the data in question is useful in this situation - it enables a peer reviewer to examine relevant data to determine a level of concern, without the need to return to the raw data in the original study report.

Within the body of the evaluation report the following (minimum) information should be recorded:

1. A brief statement of the objective of the test or study (if there is a special or unusual reason for conducting the study)

2. The identity (including batch no.) and purity of the test material, including its common (generic) name.

   Note: The chemical names of the compound (IUPAC, CAS and common names) and synonyms, as well as the CAS number, company code names/numbers, any trade names, the empirical formula, the structural formula, the molecular weight and all available physicochemical data should be included at some point in the evaluation report. Information about identified impurities, isomer ratios and stability of the pure compound should also be included.

3. Details of the composition of solvents or dosing excipients, or, for compounds included in the diet, a brief description of the diet preparation (including information on any vehicle used and frequency of preparation). Analyses for stability, homogeneity and concentration of compound in the dietary admix should be reported. Whilst mixing, determination of the stability of the test material and mixtures, storage conditions, and administration to the animals are issues covered by Standard Operating Procedures (SOPs) and quality control procedures (e.g. see EHC 141; WHO, 1992), this information is important in determining compound intake over the course of the study and should be briefly reported. If it appears that there may have been problems in any of these procedures, then they should be reported in more detail, with a discussion of how this might affect the veracity or conclusions of the study.

4. Species, strain, age, and source of test animal used. (Information about strain and source is necessary, in the event of the need to establish historical control incidences of pathological findings or to check baseline physiological or biochemical parameters.)

5. Number of animals per sex and per group, as well as numbers of animals in any additional subgroups or recovery groups

6. For a non-GLP study or if possible problems or protocol deviations are identified by an evaluator, information on animal housing, environmental conditions and the animal acclimation period should be included. Since these issues should be covered by SOPs and quality control procedures in a GLP study (e.g. see EHC 141), minimal detail is appropriate (see sample assessments at Appendices VI - IX).

7. Dosage route and doses used (including the vehicle used for negative controls). Dietary levels should be quoted in ppm with measured or estimated daily intakes of the substance in

mg/kg bw/d (see Table 1) also reported. Details about dosing methods, especially for dermal and inhalational studies, should be recorded. For dermal studies, descriptions of the application procedure including site, manner in which the skin was treated (shaved, abraded, or non-abraded), whether the site was occluded (and method of occlusion), and the amount of body surface covered should be reported. For inhalational studies, the following information should be recorded: (1) methods for generation of the test atmosphere and description of the test chamber, including whether whole-body or nose-only exposure; (2) time to equilibration of the test atmosphere; (3) test atmosphere concentration; (4) particle size determination, size distribution and consistency over the course of the study.

8. Duration and timing of dosing

9. Sacrifice times

10. The observations made, parameters measured, and the frequency of their investigation.

11. Treatment-related effects on:

- mortalities	(with examination for cause of death)
- gross observations for behaviour and appearance	("clinical signs")
- food and water consumption	(water consumption is not specifically requested under OECD guidelines 408 and 409, unless the substance is administered in the drinking water)
- body weights/body weight changes	
- functional investigations	(e.g. ECG, motor activity, neurological tests, ophthalmology, blood coagulation)
- blood biochemistry (*)	
- haematology (*)	
- urinalyses (*)	(not specifically required for subchronic rodent studies in US EPA or OECD Test Guidelines)
- serum chemical concentrations	(if measured)
- macroscopic pathology	
- organ weights	(absolute and relative)
- microscopic histopathology	
- any other special investigations	

(*Depending on the regulatory guideline, at intervals during the study and at term)

12. In reporting findings, the percentage (and absolute) change relative to controls, the dose relatedness of the changes, the biological and statistical significance of findings, historical control ranges (if available, with information on the source of the historical data and how closely or otherwise it matches the study being evaluated), and suspected mechanisms of action should be covered. Tabulation of data is useful and incidences of findings should be given in sufficient detail to allow independent assessment from the report. Narrative accompanying such tabular data should address the toxicological significance of the results

and not repeat what is presented in the table. The report should identify the statistical method used to evaluate each parameter; Appendix V lists many of the statistical tests used in toxicology studies; if there is a paucity of statistical testing, or if tests are considered inappropriate, the assessor may consider it necessary to conduct a re-analysis or request the study sponsor/data owner to provide further comment and/or analysis.

13. If possible, target organs and mechanism(s) of action should be identified and discussed. The NOEL/NOAEL for each study (as appropriate) should be recorded, with a statement for its basis provided (so that the LOEL is clearly apparent).

14. Comment should be made on the adequacy of the study. Any deficiencies should be discussed in detail and comment made on the regulatory relevance of the study. Reasons for rejecting a study as part of a regulatory package should be clearly identified in the assessment report; for regulatory purposes some countries may also append a one-word descriptor indicating whether a study is acceptable or not.

The first paragraph should give a brief description of the experimental design, incorporating all essential details i.e. species and strain, number of animals/sex/group, doses used, route, duration of compound administration, the frequency of dosing, the vehicle and, if applicable, the duration of any recovery period and the number of recovery animals/group.

Dietary levels should be quoted as ppm in the food, together with measured and/or estimated mean intake (mg/kg/day) over the course of the study. Where no calculation based on food intake has been made, the conversion factors in Table 1 should be used. As outlined in IPCS Environmental Health Criteria Monograph No. 104 (WHO, 1990), if dietary intake is measured, then JMPR evaluations indicate that X ppm in the food is **equal** to Y mg/kg bw but if there is inadequate food intake data and the tabulated conversion factors are used, then it is reported that X ppm in the food is **equivalent** to Y mg/kg bw.

For dermal studies, descriptions of the application procedure (skin site preparation, vehicle(s) used, and covering method used to prevent oral ingestion and/or dislodgement of the dose) and the amount of body surface area covered, need to be reported. For such studies, the test substance should be applied evenly over an area approximately 10% of the total body surface area (OECD Test Guidelines 410 and 411). Note that mice, rats, rabbits and guinea pigs are acceptable species for repeat-dose dermal studies.

For inhalational studies (see OECD Test Guidelines 412 and 413), the following information should be recorded: (1) methods for generation of the test atmosphere and description of the test chamber, including whether whole-body or nose-only exposure was used; (2) time to equilibration of the test atmosphere; (3) test atmosphere concentration; (4) particle size determination, size distribution and consistency over the course of the study. The latter information is particularly important, since if the bulk of particles are larger than the respirable limit, then exposure by the inhalational route will be inadequate. The interim criteria of the US EPA accepts acute studies if particles have MMADs in the range 1 - 4 µm and subchronic studies if MMADs are in the range 1 - 3 µm (Whalan & Redden, 1994); this latter document contains useful information on the inhalation of particulate material in toxicology studies. Other useful information is contained in Chapter 5 of the CRC *Handbook of Toxicology* (Derelanko & Hollinger, 1995).

An example heading and first paragraph is as follows (superscripts refer to the alphabetic and numeric points listed in 5.1 and 5.2 above):

Subchronic (13-week) Dietary Toxicity Study in Dogs[a]. Burke ED & Wills HO[f] ,Pesticide Corp.[d], Burton Labs Inc., Rocky River, NJ[e], Project No. 2174-112[c]. Report no. 5638[b]. Expt Date 15 Sept. - 20 Dec. 1983[h], Report date 26 Jan. 1984[g] (GLP; US and Japan)[j]

Purebred beagle dogs obtained from AnimaLabs Inc., NY[4] (5/group/sex)[5] were dosed with chlortoxane[2] (Batch no. #34-CD; 98.9% purity) in the feed[6] at doses of 0, 200, 2500 and 50000 ppm for 13 weeks[7]. Mean compound consumption[6] ranged between 6.9 to 9.6, 79.9 to 114.8 and 1698 to 2494 mg/kg bw/day for males and 7.3 to 10.1, 66.8 to 117.5 and 1801 to 2500 mg/kg bw/day for females. The study was conducted in accord with OECD Test Guideline No. 452[i] and was a standard subchronic study conducted prior to a chronic one-year study in the same species[1].

If there is no GLP certification, the evaluator should at least note whether the study was inspected by a Quality Assurance Unit (presence of a signed Q/A statement) and make some comment on the apparent quality of the protocol and adequacy of the methods used (see Section 2.1 “Reliability”).

Evaluators should determine the NOEL/NOAEL based on their independent assessment of the data as opposed to the conclusion of the company scientist(s), although this value, if different, should be quoted, with an explanation as to why a different conclusion was reached.

5.3 Layout and Formatting

It is not the intention to specify a standard format and layout for reports since this will to some extent depend upon the needs of national agencies (in terms of presentation of data to advisory or other committees etc.) and, to a lesser extent, on styles and preferences of individual evaluators. **However, the report should be structured to allow for ready access to all significant and relevant points arising out of the assessment.** The *OECD Guidance for Country Data Review Reports on Plant Protection Products and their Active Substances* (OECD, 1998b) should be consulted as a guide to overall report organisation.

Sample evaluations, with comments on their compliance with these general guidance notes, are attached at Appendices VI to IX.

An evaluation of a repeat-dose study should include comment on the effects of treatment on mortality and morbidity, clinical signs, food and water consumption, body weights, ophthalmoscopy, clinical chemistry, haematology, urinalysis, organ weights, gross and microscopic histopathology, and any other measured parameters.

Study findings would best be grouped into paragraphs e.g. Mortality/Clinical Signs; Body Weights; Food Consumption; Ophthalmoscopic Findings; Haematology; Clinical Chemistry; Urinalysis; Organ Weights; Gross Pathology; Histopathology; Conclusions; plus any other paragraphs for extra investigations (e.g. Neurobehavioural Assessment) as necessary. Some of these investigations could be combined into a single paragraph (e.g. Body Weights and Food Consumption; Haematology, Clinical Chemistry and Urinalysis; Gross and Microscopic Pathology), particularly if a lack of effects means individual paragraphs may only be a sentence or two long.

The use of large blocks of text without paragraph breaks makes it hard to locate specific data on a page. The use of sub-headings for the above paragraphs may be useful for clarity, particularly for evaluations of studies in which there are a large number of positive findings and there needs to be extensive reporting of the findings.

It often happens that parameters in some of the above areas of investigation do not change - such negative results should be briefly reported as a way of indicating that such measurements were performed. An alternative way of reporting the study is to list, at the beginning of the study evaluation, all the types of observations that were conducted, and then only report on the compound-related (or possibly compound-related) findings.

Within an evaluation report on a package of toxicology studies, repeat-dose toxicity studies should be presented by species (in the order mouse, rat, rabbit, dog, monkey) and in order of study length and grouped by route of administration.

5.4 Terminology used in Evaluation Reports

To avoid losing important aspects of the independent assessment in an unnecessarily long document, reports should be as concise and precise as possible, consistent with adequate reporting as outlined above. The use of abbreviations is acceptable, provided that they are in widespread use; other abbreviations used need to be clearly defined in the evaluation document. Notwithstanding, excessive use of abbreviations can make a document tedious and sometimes difficult, especially in cases where language differences exist and translation can make intuitive abbreviations (in English) less clear.

Appendix I lists commonly accepted abbreviations of terms used in toxicology studies. (Reference is also made to a more detailed set of abbreviations in Appendix 1 of the OECD guidance documents on the preparation of dossiers and monographs.)

With respect to abbreviated terminology to be used in evaluation reports, the terms ‘clinical chemistry/haematology/urinalysis parameters’ are taken to refer to those clinical biochemistry/haematology/urinalysis measurements listed in the Table at Appendix III; in the event that there are no changes in any of the parameters in the particular test battery, it is considered sufficient to state that “there were no changes in any clinical chemistry/haematology/urinalysis parameters”, without specifying all the individual parameters measured. If one or several of the parameters listed (Appendix III) were not included in the particular assay battery, then these omissions should be noted and, if necessary, a statement included on the adequacy of the parameters assessed.

Whilst adequately reporting and interpreting all relevant compound-related findings, there is a need to make evaluations as concise and succinct as possible; some useful shorthand expressions which can be used in reporting toxicology studies are:

- n/sex/group = number of animals per sex in each dosage group
- dose-related = effects of the compound were dose related
- compound (or drug)-related = effects were compound-, but not necessarily dose related
- po, iv, ip, sc, im (or upper case) = oral, intravenous, intraperitoneal, subcutaneous and intramuscular

- x/y = x animals affected out of y animals examined i.e. incidences of the finding in respective groups were 1/10, 2/8, 3/7 and 5/10.

Standard abbreviations for haematology and clinical chemistry parameters can also be used.

5.5 Bibliographic Citations

The following format should be used for the citation of company data and for data where the standard information is not available or unclear. The objective is to provide a unique identifier for each study. Note that content and order are the key points to note; minor changes in the format may be made, depending on individual country preferences.

Company Data

Author:	Surname, Initial
Date:	Year report written (not year submitted)
Title:	Full title as it appears in the report
Testing Laboratory:	(where different from Company name)
Report Number:	(and full date of the report)
Company Name, City and/or Country:	(data submitter/data owner)
Unpublished:	(if an unpublished report)
(Country Code):	(individual country/agency identifier, if applicable)

Examples:

Hartley M & Murray W (1994) S-1234 (Technical-grade) twenty-one day dermal study in rabbits. Happy Labs, United Kingdom. Report No. 007 Pesticide Company, Bilthoven, Netherlands. Unpublished. (Country/Agency identifier)

Ebert M & Leist A (1985) 21-Day dermal study in Wistar rats. Report No. 84.0223, Hoechst AG, Germany. (Country/Agency identifier)

Default entries

Where standard citation information is not available or is unclear, the following default entries should be used. Where authors are not identified, use the submitting company name.

Example:

Pesticide Company (1994) S-1234 (Technical-grade) twenty-one day dermal study in rabbits. Happy Labs, United Kingdom. Report No. 007, Pesticide Company, Bilthoven, Netherlands. Unpublished. (Country/Agency identifier)

Another possibility in the absence of authors' names would be to reference the name of the Study Director.

If the Report Number is not specified, state that the Report Number is not available. If another identifier is used (e.g. Study Number), it should be stated.

Example:

Hartley M & Murray W (1994) S-1234 (Technical-grade) twenty-one day dermal study in rabbits. Happy Labs, United Kingdom. Report No. not specified, Study No. 2468, Pesticide Company, Bilthoven, Netherlands. Unpublished. (Country/Agency identifier)

The full date of the report may be useful as a study identifier.

Example:

Hartley M & Murray W (1994) S-1234 (Technical-grade) twenty-one day dermal study in rabbits. Happy Labs, United Kingdom. Report Date 30th February, 1994. Pesticide Company, Bilthoven, Netherlands. Unpublished. (Country/Agency identifier)

[Note: A minor modification has been made to the example citations previously proposed at the November 1994 OECD meeting at Bilthoven by deleting the commas after the surnames and substituting '&' for 'and' between authors names.]

Note: In the main body of the evaluation report, the headings for individual studies may contain more detail than the above citation for unpublished reports i.e. headings should include all the above information plus the start and end dates of the experimental phase of the study, study numbers and/or any other report identifiers.

Literature References

It is suggested that references be listed in the order: author, year, title, journal; volume, and pagination.

Example:

White D, Ruehl KJ, Borman SA & Little J (1988) Effects of methylmercury on the microtubule system of mouse lymphocytes. Toxicol Appl Pharmacol **94**(1): 66-75.

This citation style is as used by the IPCS for its EHC monographs and is consistent with recommendations to reduce keystrokes by eliminating unnecessary full-stops, commas etc. Names of journals should be abbreviated according to the ISDS (International Serials Data System) list of Serial Title Word Abbreviations, or given in full.

5.6 General Comments

Detailed comments about the analysis and evaluation of toxicology studies have been made in Section 3 of this document. The following further general comments may be made.

If possible, compound-related changes in biochemical, haematological or urinalysis parameters should be linked with organ weight, gross pathology and/or histopathological changes. There are a number of useful reference books in this regard e.g. *Organ Function Tests in Toxicology Evaluation* (Tyson & Sawhney, 1985).

The following points also should be noted in evaluating repeat-dose toxicity data:

- Findings should be considered on the basis of both statistical significance and likely biological significance. The variability of biological data must be remembered in assessing a statistically-significant result. Conversely, a finding which is not statistically significant may have biological significance when considered in the light of the likely toxicological or pharmacological action of the compound, or when combined with results from other studies. Thus, evaluators should report trends or transient changes in parameters if there is an indication that these may be related to dosing with the compound in some way (see Section 4 for more detailed discussion). This information may be useful when comparing results across studies and in the consideration of the overall significance or relevance of an observed effect i.e. in one study an effect may be only a trend whilst in another study it may be very clearly treatment-related.

- A difficult problem for evaluators is the fact that some studies producing either clearly positive or negative results may have to be considered as flawed. In any long-term study there may be questionable components of the study and the experienced toxicologist must learn to recognise what is useful and discard what is not (see Section 2 for more detailed discussion on these points). The use of a seriously flawed negative study may provide only a false sense of security. On the other hand, a flawed positive study may be entitled to some weight; how much is a matter of judgement (Task Force of Past Presidents, 1982). Data obtained from studies carried out many years ago should not be dismissed out-of-hand simply because they do not meet today's standards; they may provide some useful information. Again, this is a matter for scientific interpretation and judgement on a case-by-case basis.

- With respect to plasma levels of the test chemical measured in toxicity studies, an important point to note is that in rats there is a marked influence of sex hormones on liver biotransformation processes (see e.g. Chhabra & Fouts, 1974). In general, male rats metabolise xenobiotics (as well as endogenous substrates) faster than females, a finding not generally seen in other species. Thus rat studies may exhibit sex differences in plasma kinetics and in clinical and toxicological effects of the test chemical. These findings may not be relevant to human exposure.

- In many cases issues arise which cannot be elucidated by the "standard" toxicity test battery. Evaluators should consider whether there is a need for any special studies e.g. studies to investigate in detail specific toxic effects (may include comparative studies with other chemicals of the class which are already marketed) e.g. ocular toxicity of a new organophosphorus compound, or effects on the immune system; immunosuppressant effects may help explain increased incidences of infections, mortalities and/or tumours in the standard long-term toxicity studies.

- If an evaluator believes that any of the above studies are necessary but they have not been provided, then it is appropriate to highlight this in the final summary and assessment; in some cases the sponsoring Company may be able to provide the relevant study prior to completion of the assessment report.

If an evaluator refers to data (e.g. historical control data) not included in the study report, that information should be referenced in the bibliography.

TABLE 1. APPROXIMATE RELATION OF PARTS PER MILLION IN DIET TO MG/KG BODY WEIGHT/DAY*

Animal	Weight (kg)	Grams Food Consumed Per Day (Liquids Omitted)	Type of Diet	1 ppm in food equivalent to, in mg/kg bw/day	1 mg/kg bw day equivalent to, in ppm of diet
Mouse	0.02	3		0.150	7
Chick	0.40	50	Dry	0.125	8
Rat, young	0.10	10	Laboratory	0.100	10
Rat, older	0.40	20	Chow	0.050	20
Guinea pig	0.75	30	Diets	0.040	25
Rabbit	2.0	60		0.030	33
Dog	10.0	250		0.025	40
Cat	2	100	Moist	0.050	20
Monkey	5	250	semi-solid	0.050	20
Dog	10	750	diets	0.075	13
Man	60	1500		0.025	40
Pig or Sheep	60	2400	Relatively	0.040	25
Cattle, maint'ce	500	7500	dry grain-	0.015	65
Cattle, fattening	500	15000	forage	0.030	33
Horse	500	10000	mixtures	0.020	50

* From Lehman (1954), as reproduced in IPCS Environmental Health Criteria No. 70, WHO (1987).

As outlined in IPCS Environmental Health Criteria Monograph No. 104 (WHO, 1990), if dietary intake is measured, then JMPR evaluations indicate that X ppm in the food is **equal** to Y mg/kg bw but if there is inadequate food intake data and the tabulated conversion factors are used, then it is reported that X ppm in the food is **equivalent** to Y mg/kg bw.

TABLE 2: EFFECT OF RESTRICTED FOOD CONSUMPTION IN RATS ON BODY WEIGHT, RELATIVE ORGAN WEIGHTS AND SOME LABORATORY PARAMETERS

Strain	**Ibm (RORO)**	**CD**		
Period of deprivation	4-13 weeks	14 days		
Wt at study commencement	ll0 g	l40-160 g		
Sex	male	female/male		
Food (% control)	65-38	83/62	53/42	30/25
Body wt (% control)	73-66	88/74	67/58	45/38
Relative Organ Weight (as % of control r.o.w.)*				
brain	127-140	112/130	143/148	202/230
stomach	-	118/162	140/162	157/188
lung	-	-	-	134/
kidney	nc	-/nc	108/nc	122/nc
submaxillary gland	-	-/123	116/126	157/188
thymus	-	-	126/82	19/25
thyroid	110-118	-	-	133/129
spleen	84-103	-	-/80	51/54
liver	64-77	90/75	78/68	64/60
uterus	-	-	76/-	67/-
large intestine	-	nc/nc	nc/nc	nc/nc
small intestine	-	nc/nc	nc/nc	nc/nc
heart	nc	nc/nc	nc/nc	nc/nc
adrenals	135/154	nc/nc	nc/nc	nc/nc
ovaries	-	nc/nc	nc/nc	nc/nc
pituitary	nc	-	-	-
testes	ll8-138	-/136	-/159	-/191
seminal vesicles/prostate	l04-71	-	-/72	-/32
Laboratory parameters (data, for males only, given as % of 'control' values)				
AST	-	-	-	533
ALT	-	62	-	393
BUN	-	54	71	215
SAP	-	70	64	52
Hb/Hct	-	"increasing"	"increasing"	"increasing"
WBC	-	63	55	53

r.o.w. = “relative organ weight”, organ weight expressed relative to bodyweight
nc = “no change”
- = “not determined”

Data for Ibm (RORO) strain taken from Shärer (l977) and for CD rats from Dairman (l978).
* Values are expressed as percentages of control r.o.w. eg. the 127-140% value for brain reflects the sparing of the brain in animals which have lost significant body weight (down to 66-73% of control animals).

TABLE 3. SOME INFORMATION ON *RATTUS NORVEGICUS*

Parameter		Value	Parameter	Value
Adult wt (g)	- male	200-500	Type of ovulation	spontaneous
	- female	250-350	Fertilization after ovulation (h)	7-10
Ave lifespan (yrs)	- lab.	2-3		
	- wild	4-5	No. of eggs shed	10+
Approx. dietary consumption/day (g)		15-20	Egg viability (h)	10-12
Water consumption (mL)		24-35	Gestation period (d)	21-22
Approx. urine vol./day (mL)		11-15	Usual litter size	9-11
Approx. faecal mass/day (g)		9-15		(range 6-15)
Body temp. (deg C)		37.3	Litter frequency/yr	7-9
Heart rate (/min)		300-375	Wt at birth (g)	4-5
		(range 260-600)	Optimum weaning age (d)	21
Ventilation rate (/min)		100		(range 18-23)
		(range 66-210)	Optimum weaning wt (g)	35-40
Tidal volume (mL)		0.86	Menopause (months)	15-18
		(range 0.60-1.25)	Chromosome no. (diploid)	42
O_2 consumption (mL/g/h)		2.0	Age at first oestrus (d)	36
Basal metab. rate (kcal./m^2/d) (300 g rat)		802	Age at first ovulatory oestrus cycle (d)	77
			(range 45-147)	
Arterial BP - systolic		116	Parturition length (h)	1-4
(mm Hg) - diastolic		90	Puberty (days)	50-60
Wt at maturity (g)	- male	170-210	RBC count (million/mL)	7.0-9.7
	- female	150-170	Hct (%)	46
Recomm. min. breeding age (weeks)		12	Cardiac output (mL/Min)	50
		(range 9-14)	Blood vol./100g body wt	5.6-7.1
Recomm. max. breeding age (mnths)		12-15	Mean blood pH	7.40
Time of ovulation after oestrus (h)		8-11		
Plasma osmol. (mOsm/kg)		288-336	Lymphocytes - % of WBCs	86
Oestrus cycle (days)		4-5	Platelets (million/cu.mm)	0.5-1
Oestrus (heat) duration (h)		12	Leucocytes (/µL)	9000
		(range 9-20)		(range 6000-18000)

Data from several authors collected in the 'Whole Rat Catalogue', l983, Harvard Bioscience, USA.
NB: Values will vary for different rat strains.
The following information may also be useful:
- Liver plasma flow in the rat is near 7 mL/min per 200 g body wt (Altman and Dittmer, l974).
- The first oestrous cycle in dams after delivery is 20-24 h postpartum, then following on after lactation.
- The tail of the rat may reach 85% of body length; it is longer in the female than the male.
- Hair growth is cyclic with a resting and growing phase lasting about l7 days each.
- Ossification is not complete until after the first year of life.

TABLE 4. INFORMATION ON DOGS - SOME PHYSICAL AND PHYSIOLOGICAL PARAMETERS

Parameter	Value
Lifespan	12-14 yr
Adult weight	6-25 kg
Birth weight	300-500 g
Adult food consumption	250-1,200 g/day
Adult water consumption	100-400 mL/day
Breeding age (males)	9-12 months
Breeding age (females)	10-12 months
Oestrous cycle	biannual, monoestrus
Gestation period	56-58 days
Weaning age	6-8 weeks
Litter size	4-8
Blood volume (adult)	8-9%, 75-110 mL/kg
Maximum safe bleed	8-10 mL/kg
Red cell count	5.5-8.5 x 10^6/mm^3
White cell count	6-14 x 10^3/mm^3
Haemoglobin	13-18 g/dL
Haematocrit	38-52%
Platelet count	200-600 x 10^3/mm^3
Heart rate	80-140 beats/min
Respiration rate	10-30 breaths/min
Rectal temperature	38.5^{o}C
Urine pH	7.0-7.8
Urine volume	25-45 mL/kg
Chromosome number	2n=78

This Table was adapted from Derelanko & Hollinger (1995), with data sources cited in that text. Whilst there are a great variety of breeds and strains of dogs, the pure-bred beagle is the most commonly used in toxicology studies, owing to its uniform and relatively small size, docile temperament, physiological similarity to humans, and ability to adapt to life in large cages or pens.

Other useful metabolic, physiological and biochemical information on toxicology test species may be found in e.g. Siglin & Rutledge (1995) and Hollinger (1995).

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[Note: This reference is to an old edition of this well-known textbook but the latest edition (at page 22) still refers to this particular chapter in the 2nd edition for detailed discussion on factors influencing animal responses to toxic substances]

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APPENDIX I

List of commonly-used abbreviations/acronyms*

d	Day	**mL**	Millilitre
h	Hour	**ng**	Nanogram
kg	Kilogram	**ppm**	Parts per million
L	Litre	**s**	Second
m	Metre	**µg**	Microgram
mg	Milligram	**iv**	Intravenous
id	Intradermal	**ip**	Intraperitoneal
po	Oral	**im**	Intramuscular
sc	Subcutaneous	**bw**	Bodyweight
mg/kg bw/d	Mg/kg bodyweight/day		

AP	Alkaline phosphatase
BUN	Blood urea nitrogen
AST	Aspartate aminotransferase (SGOT)
ALT	Alanine aminotransferase (SGPT)
CPK	Creatine phosphokinase
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
PCV	Packed cell volume (Haematocrit)
RBC	Red blood cell/erythrocyte
WBC	White blood cell/leucocyte

ADI	Acceptable Daily Intake
GLP	Good Laboratory Practice
LOEL	Lowest Observable Effect Level
LOAEL	Lowest Observable Adverse Effect Level
MRL	Maximum Residue Limit or Level
NOEL	No Observable Effect Level
NOAEL	No Observable Adverse Effect Level
RfD	Reference Dose

JMPR	Joint Meeting on Pesticide Residues
IARC	International Agency for Research on Cancer
NTP	National Toxicology Program
IPCS	International Programme on Chemical Safety
WHO	World Health Organisation

* For a comprehensive list of standard terms and abbreviations, see Appendix 1 of the *OECD Guidance for Country Data Review Reports on Plant Protection Products and their Active Substances* (*Monograph Guidance*), July 1998.

APPENDIX II

TOXICOLOGY DATA SCREEN: SHORT TERM STUDY

This screening form is intended for use with the following types of studies: 4.3.1 (TGAI) Short-Term Oral (90 day) (rodent); 4.3.4 (TGAI) Short-Term Dermal (90 day); 4.3.6 (TGAI) Short-Term Inhalation (90 day); 4.3.8 (TGAI) Other Short-Term Studies if the study is a 90 day study; 4.7.1 (EP) Short-Term Oral (90 day); 4.7.3 (EP) Short-Term Dermal (90 day); 4.7.5 (EP) Short-Term Inhalation (90 day); 4.7.7 (EP) Other Short-Term Studies if the study is a 90 day study.

Following a "➢" provide details. Elsewhere, answer with yes or no. Provide page reference where indicated. Shaded areas are for internal use only.

STUDY INFORMATION

DACO ➢	
Submission No.➢	**TGAI or EP ➢**
Technical Active(s) ➢	
Trade Name ➢	
Vol. No(s) ➢	
Report Title ➢	
Co. Report No.➢	
Report Date (dd/mm/yy) ➢	
Conducting Laboratory ➢	
Author(s)➢	
Sponsor ➢	
Dose levels specified:	
GLP:	
QA:	
Summary provided:	
Study Code➢	**Species Code➢**

TEST MATERIAL

Lot No. of test material ➢
TGAI - Purity (%) or EP - test material identified ➢

ANIMALS		
Species➢		
Strain➢		
Source of animals➢		
Number/group/sex➢		
Control group present:		
Acclimation period➢		
Age at study initiation➢		
Body weight at study initiation➢		
Randomization:		
No. animals/cage➢		

DOSING INFORMATION - Oral rodent and non-rodent		
Dosing method➢		
Nominal dose levels (specify units)➢		
Actual dose levels (mg/kg bw/day)➢		
Concentration adjusted for bw (frequency)➢		
Vehicle➢		
Control group dosed with vehicle:		
Management of control group same as treatment groups:		
Frequency of dosing (days/week)➢		
Preparation of fresh dosing material (frequency)➢		
Assessment of concentration (provide range)➢		
Assessment of homogeneity:		
Assessment of stability:		

DOSING INFORMATION - Dermal		
Dose levels (mg/kg bw/day)➢		
Concentration adjusted for bw (frequency)➢		
Volume/site➢		
Vehicle➢		
Control group dosed with vehicle:		
Management of control group same as treatment groups:		
Length of dosing period➢		
Frequency of dosing (days/week)➢		

DOSING INFORMATION - Inhalation		
Nominal dose levels➢		
Actual dose levels➢		
Vehicle➢		
Control group dosed with vehicle:		
Management of control group same as treatment groups:		
Duration of exposure (h/day)➢		
Frequency of dosing (days/week)➢		
Exposure (Whole-body, Head-only, Nose-only)➢		
Rate of air flow➢		
Description of chamber design:		
Chamber environmental conditions:		
Method of particle generation:		
Particle size distribution:		

EXPERIMENTAL PROCEDURE/OBSERVATIONS		
FOOD CONSUMPTION		
Frequency of recording➢		
Mean data for each treatment group:		
Individual data for all animals:		
WATER CONSUMPTION (If test material is given in drinking water)		
Frequency of recording➢		
Mean data for each treatment group:		
Individual data for all animals:		
TEST MATERIAL INTAKE (If test material is given in food or in drinking water)		
Mean data for each treatment group:		
CLINICAL/BEHAVIORAL		
Frequency of recording➢		
Individual data for all animals:		
BODY WEIGHT		
Frequency of recording➢		
Mean data for each treatment group:		
Individual data for all animals:		
OPHTHALMOSCOPIC EXAMINATION		
Individual data prior to study initiation for control and high dose groups:		
Individual data at termination for control group and high dose groups:		
PROTOCOL		
Method of blood collection➢		
Fasting period prior to blood collection➢		
Method of urine collection (if performed)➢		
Fasting period prior to urine collection (if performed)➢		
Method of sacrifice➢		

HEMATOLOGY		
Mean data at termination for each treatment group:		
Individual data at termination for all animals:		
CLINICAL CHEMISTRY		
Mean data at termination for each treatment group:		
Individual data at termination for all animals:		
URINALYSIS (if performed)		
Mean data for each treatment group:		
Individual data for all animals:		
GROSS NECROPSY		
Individual data for all animals:		
ORGAN WEIGHTS		
Organs weighed➢		
Mean data for each treatment group:		
Individual data for all animals:		
HISTOPATHOLOGY		
Individual data for control and high dose groups:		
HISTOPATHOLOGY GRADING SYSTEM		
Were grading systems used by the pathologist provided:		
HISTORICAL CONTROL DATA (if applicable)		
Data provided within 5 years of study conduct:		
Data presented by study with dates of conduct indicated:		

NO(A)EL, basis for establishment and other significant findings (report summaries)➢

LO(A)EL and basis for establishment➢

Screening Officer ➢	**Date ➢**
Screening deficiencies noted ➢	
Comments to Preliminary Review Officer ➢	
Preliminary Review Officer ➢	**Date ➢**
Preliminary Review deficiencies noted ➢	
Comments to Reviewer ➢	

APPENDIX III

Recommendations for clinical chemistry, haematology & urinalysis parameters

Clinical Chemistry	Haematology	Urinalyses
albumin alkaline phosphatase ALT (serum alanine aminotransferase) AST (serum aspartate aminotransferase) bilirubin (total) calcium chloride cholesterol (total) creatinine (blood) CPK (creatine phosphokinase) GGT (gamma-glutamyl transpeptidase) globulin glucose (blood) LDH (lactate dehydrogenase) phosphorus potassium protein (total) sodium SDH (succinate dehydrogenase) triglycerides urea nitrogen (blood)	clotting parameters (clotting time, prothrombin time) erythrocyte count haematocrit (packed cell volume) haemoglobin leucocyte differential count leucocyte total count platelet count reticulocyte count MCH (mean corpuscular haemoglobin) MCHC (mean corpuscular haemoglobin concentration) MCV (mean corpuscular volume) blood smear	appearance specific gravity glucose ketones sediment (microscopic) occult blood pH protein volume bilirubin urobilinogen

The deliberations of a joint international committee, established to provide advice for clinical pathology testing of laboratory animal species used in regulated toxicity and safety studies, has published its recommendations, including those parameters which should be measured (Weingand et al, 1996). Whilst these recommendations have not been formally incorporated into national or international guidelines at this stage, they are noted, as follows:

For repeated-dose studies in rodent species, clinical pathology testing is necessary at study termination. Interim study testing may not be necessary in long-duration studies provided that it has been done in short-duration studies using dose levels not substantially lower than those used in the long-duration studies. For repeated-dose studies in non-rodent species, clinical pathology testing is recommended at study termination and at least once at an earlier interval. For studies of 2 to 6 weeks in duration in non-rodent species, testing is also recommended within 7 days of initiation of dosing, unless it compromises the health of the animals. If a study contains recovery groups, clinical pathology testing at study termination is recommended.

Clinical chemistry

The core clinical chemistry tests recommended are glucose, urea nitrogen, creatinine, total protein, albumin, calculated globulin, calcium, sodium, potassium, total cholesterol, and appropriate hepatocellular and hepatobiliary tests. For hepatocellular evaluation, measurement of a minimum of two scientifically appropriate blood tests is recommended, e.g. alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, glutamate dehydrogenase, or total bile acids. For hepatobiliary evaluation, measurement of a minimum of two appropriate blood tests is recommended, e.g. alkaline phosphatase, gamma-glutamyltransferase, 5'-nucleotidase, total bilirubin, or total bile acids.

[Note: Cholinesterase determinations in blood and red cells are required if there is evidence that the chemical is likely to have an effect on this enzyme (e.g. organophosphate and carbamate pesticides). Apart from JMHW (Japanese Ministry of Health & Welfare) guidelines (for pharmaceuticals) and MITI (Japanese Ministry of International Trade and Industry) guidelines (for industrial chemicals), blood/serum triglycerides are not routinely required in subchronic or chronic study guidelines.]

Haematology

The core hematology tests recommended are total leukocyte (white blood cell) count, absolute differential leukocyte count, erythrocyte (red blood cell) count, evaluation of red blood cell morphology, platelet (thrombocyte) count, hemoglobin concentration, hematocrit (or packed cell volume), mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. In the absence of automated reticulocyte counting capabilities, blood smears from each animal should be prepared for reticulocyte counts. Bone marrow cytology slides should be prepared from each animal at termination. Prothrombin time and activated partial thromboplastin time (or appropriate alternatives) and platelet count are the minimum recommended laboratory tests of hemostasis.

[Blood smears can provide information, especially in animals that show evidence of anaemia from other haematology evaluations. Although blood smears are not required for most of the 1998 US EPA OPPTS toxicology guidelines, blood cells are usually examined during WBC differential counts and abnormal findings are reported.]

Urinalysis

Urinalysis should be conducted at least once during a study. For routine urinalysis, an overnight collection (approximately 16 h) is recommended. It is recommended that the core tests should include an assessment of urine appearance (color and turbidity), volume, specific gravity or osmolality, pH, and either the quantitative or semi-quantitative determination of total protein and glucose.

[Note: A number of guidelines (e.g. OECD, US EPA) do not specify a requirement for the conduct of urinalysis in subchronic studies in rodents, whilst others do not specify parameters to be measured (e.g. EC). (Urinalysis is required by the US EPA for non-rodent studies.) The above comprehensively lists all parameters to be found in those guidelines which specify urinalysis parameters to be measured (see Table 17; Derelanko & Hollinger, 1995).]

APPENDIX IV

List of organs for organ weight determination and for histopathological examination

Organs Weighed	Tissues Examined		
adrenals*# brain*# epididymides*# heart*# kidneys*# liver*# ovaries*# spleen*# testes*# thymus*# thyroid (w/parathyroid) uterus*#	adrenals*# accessory sex organs# aorta*# blood smear bone* bone marrow*# brain (3 levels)*# cecum* colon* duodenum* epididymides* eyes# eyes (optic nerve)* gall bladder*# Harderian glands head - 3 sections (nasal cavity, para-nasal sinus, tongue, oral cavity, naso-pharynx, inner-ear)	heart*# ileum* intestines (small and large)# jejunum* kidneys*# lacrimal gland liver*# lungs*# lymph nodes*# mammary gland*# muscle (smooth) muscle (skeletal)*# nerve (peripheral)*# oesophagus*# ovaries*# pancreas*# pituitary*#	prostate*# rectum* salivary gland*# seminal vesicle* skin*# spinal cord (cervical thoracic, lumbar)*# spleen*# sternum stomach*# testes*# thymus*# thyroid (w/parathyroid)*# trachea*# urinary bladder*# uterus*# vagina Zymbal's gland gross lesions*#

List adapted from the Canadian *Toxicology Data Screen - Subchronic Study* proforma.

Minimum OECD requirements; thyroid (w/parathyroid) weights are also required if the study is done in a non-rodent species. Full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups. These examinations should be extended to animals of all other dosage groups, if treatment-related changes are observed in the high dose group.

* Minimum US EPA requirements; US EPA subchronic guidelines also require thyroid (w/parathyroid) weights if the study is done in a non-rodent species.

APPENDIX V

Listing of Some Applicable Statistical Tests

Tests for non-normality

- Chi-square
- Kolmogorov-Smirnov
- Shapiro-Wik

Tests for Homogeneity of Variance

- Bartlett's Test
- Levene's Test

Assumed Normally-distributed Data

1. Overall tests

- Analysis of Variance (ANOVA**,** *fixed effects model, Model 1 ANOVA most common, but other models may be encountered*)
- Analysis of covariance (ANCOVA)
- Pearson's Correlation Coefficient
- Linear regression to examine trends for dose-effects *(Test the relationship between the two parameters, dependent data. Test the significance of the regression slopes - e.g. are the responses between the sexes similar)*

2. Pairwise comparisons

- Duncan's Multiple Range Test
- Dunnett's t-Test (*compares control to each other group mean)*
- Scheffe's Test *(multiple comparison, less power than Newman-Keuls multiple range test)*
- Williams' t-Test
- Student's t-Test
- Fisher's Least Significant Difference (LSD) test

***Non-parametric Procedures (percentage values, ranks etc.*)**

- Kendall's Coefficient of Rank Correlation
- Mann-Whitney U-Test *(analogous to t-test)*
- Wilcoxon signed-rank test *(paired , matched paired data)*
- Kruskal-Wallis ANOVA
- Distribution-Free Multiple Comparisons tests (eg Dunn's test, Shirley's test)
- Jonckheere's test

Quantal Data (mortalities, pathology findings, etc)

- Fisher's Exact test
- R x C Chi Square test
- Litchfield & Wilcoxon - confidence limits of ED50 etc.

Multivariate Methods

- Hotellings T^2
- MANOVA

APPENDIX VI

Sample Evaluation - Example 1

The following is an actual assessment report, although the names of the chemical, the sponsor, conducting laboratory, study and laboratory codes have been altered to prevent identification of the study, the chemical or the data owner. Comments on particular issues in this example are made at various points throughout the text; additional comments on the format and level of reporting are to be found at the end of the example.

3-Month Dietary Study in Wistar Rats

Study on the oral toxicity of GD 85238 (Agveticide) in Wistar rats. Administration in the diet over 3 months. CASL, Toxicology Department, Ludwigsdorf, Germany. Reg. Doc. CASL 93/12179. Project No. A0779/94. Expt Date 15 Aug. 1993 - 18 Nov. 1994. Report Date 9 May 1995. (GLP: OECD)

Comment: The study authors were not included here because they were not clearly identified in the report. Reference to the study report being an unpublished document can be made in the bibliography; it is not necessary in the text of the evaluation.

Agveticide, GD 85238 (99.2% pure; batch no. AZ 123) was administered to SPF Wistar rats supplied by AnimalFarm Inc, UK (10/sex/group) at 0, 300, 1000 or 3000 ppm in the diet for 3 months, with calculated mean compound intakes of 0, 20, 66 or 200 (males) and 0, 24, 77 or 219 (females) mg/kg/day. [**Comment:** The term 'equal to' could be used instead of 'with calculated' - see Section 5.2.] Food consumption and body weights were determined once weekly. Water consumption was determined daily from the third week of study. Animals were checked daily and were subjected to a clinical examination once a week whilst being weighed. Two clinicochemical and two haematological examinations were done. [**Comment:** A statement as to time of analyses could be made here, but the following text, reporting results, makes it clear when these analyses were performed.] Ophthalmoscopic examinations were carried out before the beginning of the study and also on days 42, 52 and at the end of the study period, from the control and the high dose groups in both sexes. Rats in the intermediate dose groups underwent ophthalmoscopic examination on day 52 and at the end of the study only. At the end of the treatment, all rats were subjected to a gross- and microscopic histopathological examinations. Organs weighed and tissues undergoing histopathological examination are listed in a table attached to the study report. The test substance was analysed for stability and for homogeneity and stability in the diet. [**Comment:** This information needs to be checked by the assessor - more detailed information would need to be added if there were problems with the study in the area of dietary admixing and/or stability.]

There were no deaths. The body weight gain and the body weight at the end of the study were comparable in all groups. A decrease in food consumption was seen in the second week of the study in the high dose group of both sexes, but was comparable to control values thereafter. Increased water consumption of both sexes at 3000 ppm was recorded throughout the study. The only adverse clinical signs observed during the study includes a deteriorated general condition of rats at 3000 ppm on day 7 only.

Clinicochemical and haematological parameters measured in the study are listed in the Attachment. At 3000 ppm, the male group had a significantly reduced RBC count and increased mean cell volume (MCV) at 6 and 13 weeks after the beginning of the treatment and an increased mean corpuscular haemoglobin (MCH) after 6 weeks, but not after 13 weeks of treatment. Females at 3000 ppm at the end of the study had reduced erythrocyte levels, haemoglobin concentration (Hb) and haematocrit (Hct) values. These changes are indicative of haematotoxic potential of agveticide.

A significant reduction of white blood cells (WBC) in males of the 1000 and 3000 ppm groups at 13 weeks, was also observed to a lesser extent at 6 weeks. The parameters contributing to this included reduced neutrophilic polymorphonuclear granulocytes and lymphocytes at 3000 ppm and a reduction in lymphocytes at 1000 ppm. There was a significant change in serum electrolyte concentration at 13 weeks: sodium and potassium levels were reduced in males of the two highest dose groups; sodium and chloride levels were reduced in females in the 3000 ppm group. [The biological significance of these changes are discussed at end of evaluation.]

The only significant change in enzyme activities was a reduction of alkaline phosphatase activity in serum of both sexes at 3000 ppm at the end of the study; the significance of this change is unclear.

Females at 3000 ppm showed significantly increased globulin and cholesterol values at 6 and 13 weeks, significantly increased triglyceride values at 6 weeks (remaining elevated, but not statistically significant at 13 weeks), and increased total protein concentrations at 6 but not 13 weeks. These changes were not observed in males. [**Comment:** The biological significance of these changes are briefly discussed at end of evaluation.]

Table: 3-month rat study Project No. A0779/94

Doses (ppm)	0	300 male	1000	3000	0	300 female	1000	3000
Number of animals/group	10	10	10	10	10	10	10	10
Haematology (day 87)								
- RBC (TERA/L)	8.50	8.49	8.20	8.09*	7.87	7.88	7.71	7.42*
- MCV (FL)	47.98	47.69	48.82	49.65**	49.38	50.06	49.28	49.17
- HCT (L/L)	0.408	0.405	0.401	0.403	0.389	0.395	0.387	0.366**
- HGB (MMOL/L)	10.18	10.14	9.94	9.84	9.62	9.67	9.46	8.97**
- WBC (GIGA/L)	8.23	7.13	6.80*	5.97*	4.71	4.59	4.11	4.44
Blood chemistry (day 87)								
- sodium (MMOL/L)	143.06	142.23	140.13**	138.85**	140.86	140.64	140.99	137.42**
- potassium (MMOL/L)	7.11	6.78	6.54*	6.24**	6.34	6.35	6.23	5.80
- chloride (MMOL/L)	106.40	106.51	106.63	104.74	109.14	108.31	108.21	105.52**
- globulin (G/L)	33.57	32.58	30.65	32.68	28.47	29.18	30.46	30.99*
- cholesterol (MMOL/L)	2.29	2.52	2.26	2.54	1.96	2.17	2.16	3.01**
- triglyceride (MMOL/L)	4.73	5.53	5.27	2.62	2.77	3.30	4.11	4.14
Pathology								
Liver - focus	.	.	.	.	.	.	.	1
- cloudy swelling	.	.	2	10	.	.	4	10
- single cell necrosis	.	.	2	10	.	.	4	10
- hepatocellular alteration	.	.	.	1	.	.	2	1
Testes - Leydig cell hyperplasia	.	.	3	8				
Adrenal glands - enlargement	.	.	7	10	.	3	6	10
- discolouration	.	.	10	10	.	3	10	10
- hypertrophy z.fasc.	.	.	5	10	.	.	2	10
- vacuolation, z.fasciculata	.	.	7	10	.	.	6	10
Pituitary - cystic degeneration	5	4	9	10	1	1	2	1
Pancreas - acinar vacuolation	.	.	8	10	.	1	10	10
- islet cell hyperplasia	4	5	8	9	3	3	3	5
Ovary, luteal cell vacuolation					5	4	8	10
Eye - cataract	.	.	.	.	.	.	.	2

Statistics: Anova + Dunnetts tests (two sided): * P,0.05 ** P,0.01

Significantly increased absolute and relative liver weights were recorded in males at 3000 ppm and in females at 1000 and 3000 ppm. The liver was found to have cloudy swelling and centilobular single cell necrosis at the two highest doses in both sexes. This occurred in a dose related manner and was more pronounced in males.

Testicular weights were significantly increased at 3000 ppm (absolute and relative) and at 1000 ppm (absolute). Histological examination indicated a minimal diffuse hyperplasia of the interstitial Leydig cells at the two highest doses.

Significantly increased absolute and relative adrenal weights were seen at 3000 ppm in both sexes and at 1000 ppm in females, and increased absolute, but not relative, adrenal weights were observed in 1000 ppm males. Gross examination revealed dose related enlargement and discolouration of adrenal glands at the two highest dose levels in both sexes and at 300 ppm in females. Microscopic examination showed a hypertrophy of the zona fasciculata in all animals from the 3000 ppm groups, and in 5 males and 2 females from the 1000 ppm groups. Dose related cellular vacuolisation of the adrenal zona fasciculata and the intensity of lipoid deposition was significantly increased at 3000 and 1000 ppm. The deposition of lipoids occurred in all groups, but was more pronounced in males and was stated to be indicative of a deposition of cholesterol-containing compounds in the adrenal cortex.

There was an increase in the incidence of cystoid degeneration in the pituitary gland of males at the two highest doses. Some of these changes were ascribed to increased number of basophilic gonadotropin-forming cells and the ACTH cells. The pancreatic changes included an increased incidence of a minimal hyperplasia of the islets of Langerhans in males of the two highest dose groups and a dose related increase in the incidence and the severity of acinar vacuolation at the two highest doses in both sexes and in one female from the 300 ppm group. A dose dependent increase in the vacuolisation of the ovarian luteal cells was seen in the two highest dose groups.

Increased kidney weights were observed in males at 3000 ppm (absolute only) and in females at 1000 ppm (absolute and relative), but the changes were not dose related and were not accompanied by any histopathological changes and therefore, it was not possible to relate the kidney weight changes to the treatment with any confidence.

Ophthalmoscopical examinations at the end of the study indicated cataracts in two females of the 3000 ppm group. [**Comment:** No mention of incidence in controls would be taken to indicate a 'nil' finding.]

In summary, agveticide administration to rats for three months was associated with haematotoxicity in both sexes at 3000 ppm, lowering of white blood cell counts in males at 3000 and 1000 ppm, reduction of some electrolytes in both sexes and increases in cholesterol and triglyceride levels in females at 3000 ppm. Alterations of white blood cells, serum electrolytes, cholesterol and triglycerides levels are consistent with perturbed steroid levels. Liver and pancreas and organs associated with hormone homeostasis, i.e. testes, ovary, pituitary and adrenal gland were also the targets of agveticide-mediated toxicity. A NOEL was not achieved in this study because female rats had an increased incidence of enlarged and discoloured adrenal glands and of pancreatic vacuolation at the lowest dose tested in this study (300 ppm or 22 mg/kg/day).

Attachment to example assessment - Parameters Measured in Project No A0779/94

The study included measurement/investigation of the following standard parameters, with the exception of serum cholinesterase, LDH, ornithine decarboxylase, CPK and blood smears. Additionally, urinary nitrite was measured.

Clinical Chemistry	**Haematology**	**Urinalyses**
albumin alkaline phosphatase bilirubin (total) calcium chloride cholesterol (total) cholinesterase activity creatinine (blood) gamma-glutamyl transpeptidase globulin glucose (blood) LDH (serum lactate dehydrogenase) phosphorus potassium protein (total) ALT (serum alanine aminotransferase) AST (serum aspartate aminotransferase) sodium triglycerides urea nitrogen (blood) CPK (creatine phosphokinase)	clotting parameters (clotting time, prothrombin time) erythrocyte count haematocrit (packed cell volume) haemoglobin leucocyte differential count leucocyte total count platelet count reticulocyte count MCH MCHC MCV blood smear	appearance specific gravity glucose ketones sediment (microscopic) occult blood pH protein volume bilirubin urobilinogen

Attachment to example assessment (continued)

The study included measurement of the following standard parameters, with the exception of those in bold typeface.

Organs Weighed	**Tissues Examined**		
adrenals **brain** gonads **heart** kidneys liver **spleen** **thyroid (w/parathyroid)**	adrenals aorta **blood smear** bone bone marrow brain (3 levels) cecum colon duodenum epididymes eyes **eyes (optic nerve)** **gall bladder** **Harderian glands** head - 3 sections (nasal cavity, para-nasal sinus, tongue, oral cavity, naso-pharynx, inner-ear)	heart ileum jejunum kidneys lacrimal gland liver lungs lymph nodes mammary gland **muscle (smooth)** muscle (skeletal) nerve (peripheral) oesophagus ovaries pancreas pituitary	prostate rectum salivary gland seminal vesicle skin spinal cord (cervical thoracic, lumbar) spleen sternum stomach testes thymus thyroid (w/parathyroid) trachea urinary bladder uterus vagina **Zymbal's gland** **gross lesions**

Comments: The following comments are made on the above sample assessment:

The evaluation does not make use of paragraph headings as does the second example (see below).

For many of the parameters, the evaluation does not report the quantitative change relative to control (absolute and percentage change) as suggested in these Guidance Notes (Section 5.2, Point 11).

'Hepatocellur alteration' (noted in the Table) was probably not discussed in the text because of the low incidence of the finding.

Haematology changes at 6 weeks were mentioned in the text but not in the Table - this addition would add little to the evaluation as changes at term were generally present or more apparent than at 6 weeks (apart from MCH, which was mentioned in the text).

Regulatory standards are commonly based on findings in the most sensitive sex or species, although in their assessment reports, the US EPA would normally establish a NOEL in each sex.

The US EPA and the Canadian PMRA would require a Table of organ weight changes.

The term LOEL is not specifically stated above, but the last sentence makes clear the basis for establishing the NOEL i.e. the LOEL is clearly apparent.

Re the use of a study adequacy descriptor, Canadian and Australian evaluators would usually only comment if the study was inadequate. The US EPA includes a study adequacy descriptor.

APPENDIX VII

Sample Evaluation - Example 2

The following is an actual assessment report, although the names of the chemical, the sponsor, conducting laboratory, study and laboratory codes have been altered to prevent identification of the study, the chemical, or the data owner.

EXC-9928: 13-Week Dietary Toxicity Study in Rats. MR Tester & LA Goodwood. ToxSci Labs Washington Inc., Virginia. Lab. Project ID: TSL Study No. 47-634; Pesticide Chemicals Corp. Report No. 89DC-521; September 27, 1991 (GLP; US EPA & Japanese MAFF)

The in-life phase of the study was performed during the period 3rd Aug. - 6th Nov. 1989. Crl:CD BR strain rats from Charles River Labs, Raleigh, N. Carolina (10/sex/gp) were fed a diet containing 0, 20, 200, 2000 and 20000 ppm agveticide technical (Pesticide Chemicals Corp., Lot #AB-1172-A, Sample #89-43, purity 96.7%) for 13 weeks. The calculated mean daily intake of test compound in the respective groups was calculated as 1.30, 13.1, 133 and 1330 mg/kg/d (males) and 0, 1.55, 15.6, 155 and 1650 mg/kg/d (females). At the commencement of dosing, mean body weights were 225-235 g (males) and 167-173 g (females). The rats were housed individually in suspended stainless steel cages with wire-mesh floors. All animal rooms were environmentally-controlled, with temperature maintained at 66-72^{0}F, relative humidity at 34-75% and a 12-h light/dark cycle. Purina certified Laboratory Rodent Chow #5002 and tap water were available to all animals *ad libitum.*

Comment: Under GLP, housing etc. should closely controlled and monitored - thus it would be of limited value in an assessment report to include any more detail than the above text, unless of course there were problems that could affect the outcome of the study - as discussed in the above guidelines, there needs to be an assessment of study adequacy etc., which includes study conduct, during the process of review.

The appropriate amount of EXC-9928 technical was weighed, dissolved in acetone, mixed with 200 g of untreated feed and blended for approx. 2 min to form a premix. The premix was then brought to the appropriate final concentration with untreated feed and mixed for 30 min. The control diet contained an amount of acetone equivalent to the amount used in the highest dose group. Fresh diets were prepared and presented to the animals on a weekly basis. Concentrations of EXC-9928 in the final diet mixes were found to correspond well with the nominal dietary levels ($\pm$ 2%) throughout the study. The test substances was homogeneous and remained stable in the diet (stored at room temperature) for at least 10 days.

All rats were observed twice daily for mortality and clinical signs. Clinical examinations (including palpation for masses) were performed weekly. Body weights and food consumption were monitored weekly, starting 1 week prior to dosing. Ophthalmoscopic examinations were conducted prior to commencement of dosing and before terminal sacrifice. Neurobehavioural assessment was conducted in all animals prior to treatment and during week 13. Blood and urine were sampled from the overnight-fasted rats just prior to terminal sacrifice. Femoral bone marrow smears were prepared from all animals and evaluated for the myeloid:erythroid ratio. At termination, complete necropsies were performed on all animals. The brain, pituitary, adrenals, liver, kidneys, spleen and testes (+ epididymides) were weighed. Organs/tissues of interest were removed, fixed in 10% neutral-buffered formalin and prepared for sectioning; all samples from rats of the control 20 ppm, 2000 ppm and high-dose (20000 ppm) groups, the lung, liver, kidney, thyroid and spleen from rats of the 200 ppm group, and all gross lesions were examined microscopically.

Mortality/Clinical signs:

No treatment-related clinical signs of toxicity were observed and there were no deaths.

Body Weights:

Significantly ($p< 0.05$) decreased body weight gains (cf. controls) were observed in treated rats at 2000 ppm (males: -12.5%; females: -20%) and 20000 ppm (males: -15.5%; females: -29.2%) during dosing. Mean terminal body weights of the 2 000 ppm females (-11%) and 20000 ppm males (-9%) and females (-14%) was significantly ($p< 0.05$) lower than that of the controls.

Food Consumption:

Mean feed consumption was significantly ($p< 0.05$) decreased at 2000 ppm (males: -7%; females: -11%) and 20000 ppm (males: -7%; females: -10%) during the first 4 weeks of dosing but over the study showed only a slight decrease (males: - 3-4%; females: - 8-9%) which was not statistically significant. The calculated mean overall efficiency of food utilization was slightly lower in the 2000 and 20000 ppm groups cf. controls (values were 12.3%, 11.1% and 10.9% for males and 7.1%, 6.1% and 5.4% for females at 0, 2000 and 20000 ppm, respectively).

Neurobehavioural Assessment:

No treatment-related findings were observed for any of the battery of functional observation tests.

Ophthalmoscopic findings:

No treatment-related ophthalmoscopic abnormalities were noted at term.

Haematology, Clinical Chemistry and Urinalysis:

No treatment-related changes occurred in the 20 ppm or 200 ppm group. In the 2000 ppm and high-dose (20000 ppm) groups (both sexes), there was a slight but statistically significant ($p< 0.05$) reduction in RBC count, Hb and MCHC and a slight increase in mean cell volume. In 20000 ppm females, Hct and platelet count were significantly ($p< 0.05$) depressed and the MCHC and RBC count significantly ($p< 0.05$) increased above the concurrent control values. In addition, a decrease in the mean myeloid/erythroid ratio was observed at 2000 and 20000 ppm, but the change was not statistically significant except in 2000 ppm females. No other treatment-related haematological changes were observed.

Statistical evaluation of the blood chemistry parameters measured revealed slight increases in mean glucose and globulin values in 20000 ppm females. These changes were of low magnitude and were well within the limits of normal physiological variation and therefore, were not considered to be of toxicological significance.

No treatment-related abnormalities were detected in urinalysis parameters.

Organ Weights:

Statistically significant ($p< 0.05$) increases were observed in the absolute liver weights of 20000 ppm females (+18% cf. control group) and relative (organ/brain weight) liver weight of the 2000 and 20000 ppm females (+14 and +22%, respectively, cf. controls). Slightly elevated absolute (+ 9-10%) and relative (+13%, organ/brain weight) spleen weights were recorded for 20,000 ppm rats (both sexes), but the

increases were not statistically significant. Rats from all treated groups showed a non-dose related increase (+ 7-13%) in the relative (organ/brain weight) testis weight compared with the controls, which was statistically significant at 200, 2000 and 20000 ppm. In the absence of any supporting histopathology, the testicular weight change was not considered to be toxicologically relevant but rather could be reflective of a smaller than normal control value. Slightly lowered (statistically significant, $p < 0.05$) adrenal weights (both absolute and relative to brain weight) were noted in 2000 ppm males; the change was considered incidental to treatment due to its sporadic occurrence and the lack of supporting histopathological changes in the adrenals.

Gross Pathology:

Gross examination of specified organs/tissues revealed no changes attributable to the test material.

Histopathology:

No treatment-related histopathological lesions were observed in any organs of the 20 ppm or 200 ppm rats. There was an increased deposition of pigment in the spleen of males and females at 2000 ppm (10/10, slight to moderate) and high doses (20000 ppm) (10/l0, moderate to moderately severe). At the high dose (20000 ppm), tubular nephrosis of the kidney was also noted in 4/10 males, but not in any of the females.

Conclusions:

No treatment-related effects were observed in any of the rats administered agveticide at dose levels of 20 or 200 ppm in the diet for 13 weeks. Higher dietary levels of 2000 and 20000 ppm resulted in significant decreases in the overall body weight gain and mean food consumption during the first 4 weeks of dosing. The relative (organ/ brain weight) liver weights of female rats were increased. Slight haemolytic anaemia (reduction in RBC counts, Hb concentration and MCHC) and increased bone marrow erythropoiesis (decrease in mean myeloid/erythroid ratio) were observed. Histopathological findings revealed an increased deposition of pigment in the spleen. At the highest dose of 20000 ppm, additional treatment-related effects observed were slightly elevated absolute and relative (organ/brain weight) spleen weights in both sexes, depressed Hct and platelet count, increased MCH content and reticulocyte count and elevated absolute liver weight in females, and tubular nephrosis of the kidney in 4/10 males.

The NOEL for agveticide in rats was 200 ppm, equal to 13.1 mg/kg bw/day. Female rats appeared to be slightly more sensitive to the test substance than males.

Subchronic Toxicity [Summary Section]

Crl:CD BR strain rats (10/sax/gp) were administered RH-5992 technical (purity 96.4% or 98.6%) in their diet at dose levels of 0, 20, 200, 2000 or 20000 ppm (equal to 0, 1.30, 13.1, 133 and 1,330 mg/kg bw/day) for 13 weeks. The study NOEL was 200 ppm (equal to 13.1 mg/kg bw/day). At the next higher dose of 2000 ppm, there was a significant decrease in the overall body weight gain, and mean food consumption during the first 4 weeks of dosing and an increase in relative (organ/brain weight) liver weights (females only). Slight haemolytic anaemia (a reduction in the RBC count, Hb concentration and MCHC), increased bone marrow erythropoiesis (decrease in the mean myeloid/erythroid ratio) and an increased deposition of pigment in the spleen were observed. At the highest dose of 20000 ppm, additional treatment-related effects were s1ightly elevated absolute and relative (organ/brain weight) spleen weight in both sexes, depressed Hct and platelet count, increased MCH content and reticulocyte count and elevated absolute liver weight in females, and tubular nephrosis of the kidney in 4/10 males.

Comments: The US EPA Data Evaluation Report (DER) would normally require a Table of organ weight changes.

A LOEL is not specifically stated nor does the final sentence in the conclusion make clear the basis on which the NOEL was established.

APPENDIX VIII

Sample Evaluation - Example 3

The following sample assessment report is taken from the OECD dossier guidance.

Oral 90-day toxicity in the mouse

Report: IIA 5.3.2/01 White MW and Jones KL (1995) 90-day feeding study with chemx in CD-1 mice, Report No: CCC-14048

Guidelines: US EPA FIFRA Guideline § 82-1, equivalent to Directive 88/302/EEC (OJ No L133/8 of 30 May 1988)

GLP: Fully GLP compliant

Material and methods: The study was conducted during April to July 1993 by the Chemco Research Laboratory, New York. Chemx purity 99.1 % (Lot Number NPD-9209-4523-T) was administered in the diet for approximately 90 days to CD-1 strain mice (Charles River Laboratory, Portage, MI USA, initially aged approximately 8 wk and weighing 29-35 [M] or 24-28 [F] g), at the following doses - 0, 100, 1,000, 3,000 and x,000 mg/kg feed (10/sex/dose). Mice were housed individually in suspended steel cages and were allowed *ad libitum* access to water and feed. Animal housing and husbandry were in accordance with the provisions of the *Guide to the care and use of laboratory animals* (USPHS-NIH Publication No. 86-23). Each week, the test material was mixed into the diet at a concentration of x,000 mg/kg feed, and this mixture was used to prepare additional mixtures at 100, 1,000 and 3,000 mg/kg. The negative control group received plain diet. Prepared diets were stored under refrigeration or kept in the animal room until use. The stability and homogeneity of chemx in the dietary mixtures was checked by analysis using HPLC.

Animals were observed twice daily for mortality and morbidity and examined weekly for clinical signs of toxicity. Feed consumption and body weight were also measured weekly. At termination, mice were asphyxiated with CO_2 and a fasted blood sample was obtained from the posterior vena cava. The following haematology and clinical chemistry parameters were measured: *haematology* - erythrocyte count (RBC), leukocyte count (WBC), neutrophil count, lymphocyte count, platelet count, haematocrit (HCT), haemoglobin (HGB), RBC indices (MCV, MCH, MCHC) and leukocyte differential; *clinical chemistry* - blood urea nitrogen (BUN), alanine aminotransferase activity (ALT), aspartate transaminase activity (AST), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGT). Gross pathological examination was performed on all animals and the kidneys, liver, spleen and testes were weighed. The following tissues examined histologically under light microscopy: gross lesions, kidneys, liver and lung from all animals and the spleen, testes and thyroids of control and high dose animals.

The following parameters were analysed statistically: body weight, body weight change, food consumption (2-tailed Dunnett's Multiple Comparison Test); incidence of histopathological lesions (1-tailed Fischer's Exact Test); haematology, clinical chemistry, absolute and relative organ weights (Bartlett's Test followed by either parametric [Dunnett's Test and linear regression] or non-parametric [Kruskall-Wallis, Jonckheere's &/or Mann-Whitney Tests] procedures); and outliers (Grubb's Test).

Findings: Chemx was found to be stable in the dietary preparation for 14 days at room temperature. The mean dietary concentrations throughout the study were 100, 1,040, 2,980 and x,xxx mg/kg feed. Homogeneity analyses revealed that the within-batch coefficient of variation in test article concentration,

was approximately 6 % at 100 and x,000 mg/kg. No data were presented for the intermediate concentrations. The mean achieved doses were [M/F] 18/32, 163/313, 550/887 and x,xxx/x,xxx mg/kg bw/d at the 100, 1,000, 3,000 and x,000 mg/kg feeding levels, respectively. There were no unscheduled deaths, no treatment-related clinical signs or no effects on feed consumption, body weight or body weight gain.

Haematology determinations revealed a significant ($p < 0.05$) linear dose-related trend towards decreasing neutrophil (NEU) count in treated females, which also displayed a tendency towards depressed lymphocyte (LYM) and elevated eosinophil (EOS) counts, compared with controls. Treated mice of both sexes showed decreased basophil (BAS) counts relative to controls (see Table). However, group standard deviations were large and statistical significance was not attained with respect to LYM, EOS and BAS counts. The haematological findings are considered to be of equivocal biological significance, as they may have arisen from among-individual variation.

Table: Selected haematology, clinical chemistry and pathology findings among mice (at termination)

FEEDING LEVEL (mg/kg feed)	0	100	1000	3000	x000	0	100	1000	3000	x000
			MALES					FEMALES		
NUMBER IN GROUP	10	10	10	9	9	10	10	10	9	9
HAEMATOLOGY (mean values)										
NEU (thousand / mm^3)*	2.154	1.555	1.594	1.617	1.962	1.256	1.101	0.767	0.731	0.959
LYM (thousand / mm^3)	5.723	5.774	4.092	4.488	5.251	4.372	4.280	3.147	3.257	3.269
BAS (thousand / mm^3)	0.039	0.031	0.024	0.029	0.030	0.024	0.020	0.017	0.019	0.019
EOS (thousand / mm^3)	0.112	0.101	0.114	0.146	0.140	0.058	0.083	0.073	0.040	0.073
CLINICAL CHEMISTRY (mean values)										
AP (IU/L)#	80	140~	152~~	148~~	104~	168	182	153	148	121~
AST (IU/L)	83	91	86	97	133	83	74	74	79	90
GROSS PATHOLOGY (incidence)										
Liver - discoloration	0	1	2	2	1	0	0	0	0	2
HISTOPATHOLOGY (incidence)										
Liver - haemorrhagic necrosis / fibrosis	0	0	0	0	0	0	0	0	0	1
- necrosis + inflammation	1	1	1	0	0	0	1	0	0	1
- mononuclear cell infiltrate	0	0	0	2	0	0	3	1	1	0
Spleen - excess haematopoiesis	0	-	-	-	2	0	-	-	-	0
Uterus - hydrometra	-	-	-	-	-	0	4~	0	0	0

* Linearly related to dose ($p < 0.05$) with a negative slope (females only).

~ ($p < 0.05$) and ~~($p < 0.01$) *vs* control

Linearly related to dose ($p < 0.05$) with a positive slope for males and a negative slope for females

Statistically significant increases in alkaline phosphatase (AP) activity occurred in treated males (see Table) but were attributed by the study authors to a low control mean value (historical control data were presented). The response was less marked at x,000 mg/kg than at lower doses. By contrast, AP activity was significantly depressed in x,000 mg/kg females. An elevated mean aspartate transaminase (AST)

activity was detected in x,000 mg/kg males, but was not statistically significant. This was caused by a high reading in a single animal, which also displayed elevated alanine aminotransferase (ALT) activity.

Mean terminal body weight, and absolute and relative organ weights, were unaffected by treatment. The only notable gross lesion was an increased incidence of hepatic discoloration among treated males and x,000 mg/kg females (see Table). However, histological findings in the liver were confined to sporadic, non-dose related instances of necrosis with inflammation or mononuclear cell infiltration, and haemorrhagic necrosis/fibrosis in a single x,000 mg/kg female. The only statistically significant microscopic finding was hydrometra, present in 4/10 females from the 100 mg/kg group. This is not considered to be treatment-related, in the absence of similar findings at higher doses. Excessive haematopoiesis was observed in the spleen of 2/10 males from the x,000 mg/kg group, but given that the finding was not replicated at the same dose in the 18-month dietary study in mice, it may be discounted.

Conclusion: The decreased AP activity in females at the highest dose of x,000 mg/kg is not considered to be toxicologically significant, in the absence of evidence that depression of serum AP activity is associated with tissue injury. As there were no treatment-related effects at up to and including the highest dose, the NOAEL can be set at x,000 mg/kg (x,xxx mg/kg bw/d).

APPENDIX IX

Sample Evaluation - Example 4

The following sample assessment report is taken from the OECD dossier guidance.

90-day toxicity in the rat

Report: IIA 5.3.2/02 White MW and Jones KL (1995) 90-day feeding study with chemx administered in feed to Sprague-Dawley rats, Report No: CCC-14049

Guidelines: US EPA FIFRA Guideline § 82-1, equivalent to Directive 88/302/EEC (OJ No L133/8 of 30 May 1988)

GLP: Fully GLP compliant

Material and methods: The study was conducted during February to May 1993 by the Chemco Research Laboratory, New York. Chemx purity 99.1 % (Lot Number NPD-9209-4523-T) was administered in the diet for a period of 3 months to Charles Rivers Sprague-Dawley rats approximately 7 weeks old with a weight range of 215.4- 286.7 g for males and 102.9-147.0 g for females were purchased from Charles River Raleigh, NC, at the following doses - 0, 20, 200, 2,000, 6,000 or xx,000 mg/kg feed (corresponding to 1.22, 12.1, 123.2, 370 and x,xxx mg/kg bw/day in males and 1.47, 14.6, 144.3, 448 and x,xxx mg/kg bw/day in females).

Animals were acclimatized for 27 days prior to study commencement. There were 10 rats/sex/dose for the main study and an additional 10 females/group for the concurrent reproductive study.

The test material was stable for at least 7 days at room temperature and was distributed uniformly in the feed. The concentration of the test substance in the feed was verified weekly. The required amount of test material was weighed and mixed with the diet using a Hobart HCM-450 mixer to produce the high-dose concentration. Further ground diet was added until the other required concentrations were achieved. Diets were prepared weekly and stored refrigerated or at room temperature before use. Samples of the 20 mg/kg and xx,000 mg/kg formulations were taken for analysis to check stability and homogeneity at the beginning of the study and to check the concentration in the feed on a weekly basis.

Animals were housed singly in suspended stainless steel cages. Husbandry conditions were in accordance with the USPHS-NIH publication *Guide to the Care and Use of Laboratory Animals*. Feed (Purina Mills Certified Rodent Chow) and tap water were provided *ad libitum*.

The animals were observed twice daily for mortality and weekly for clinical signs of toxicity. Animals were examined opthalmoscopically once at pre-test and once prior to terminal sacrifice (all at pre-test, control and high-dose prior to terminal sacrifice).

Food consumption was recorded weekly for all animals in the main study and weekly until mating for the reproduction study.

Body weights of each animal were determined one day pre-dosing and weekly thereafter. For the reproduction study, body weights were determined once weekly until mating, on Days 0, 7, 14 and 21 of gestation and Days 0 and 4 of lactation.

At study termination all animals were killed by CO_2 asphyxiation and exsanguination and blood was collected from all main study animals (animals fasted overnight) for haematological and clinical chemistry measurements. All appropriate tissues from the control and high-dose groups were removed, weighed, assessed for tumour incidence (grading system included in study attachment) embedded in paraffin, stained with haemotoxylin and eosin and submitted for histopathological examination where applicable.

For the reproduction section of the study, females were paired for a maximum of 7 days with sexually mature males at a 1:1 ratio. The day on which spermatozoa were found in the vaginal smear or a vaginal plug was observed was designated day 0. At the end of the study period mating, fertility, gestation length, litter weights and survival were determined. Animals were killed by CO_2 asphyxiation and exsanguination on or shortly after day 4 of lactation.

Dunnett's Multiple Comparison Test (two-tailed) was applied to the body weight, cumulative body weight change, food consumption and APTT data. Fishers Exact Test (one tailed) was applied to data on the incidence of histopathological lesions. Bartett's Test, Dunnett's Test, Linear Regression and non-parametric tests were used where appropriate.

Findings: The test material was homogeneous throughout the feed and was stable for up to 35 days (see Table).

Table: Homogeneity and stability of test material

Nominal concentration	Concentrat'n Analyzed (mg/kg)	% of nominal
20 mg/kg	T=22 M=22 B=22 S=19.7	110 110 113 89
xx,000 mg/kg	T = xx,xxx M = xx,xxx B = xx,xxx S = xx,xxx	102 104 98 94

T = top; M = middle; B = bottom of mixing bowl; S=35 d stability, mean % of nominal

Overall averages for consumption of test material at the 0, 20, 200, 2,000, 6,000 or xx,000 mg/kg dose groups corresponded to 1.22, 12.1, 123.2, 370 and x,xxx mg/kg bw/day in males and 1.47, 14.6, 144.3, 448 and x,xxx mg/kg bw/day in females respectively.

No mortalities occurred in the test animals at any dose level for either the main or reproduction studies. There were no treatment-related clinical signs of toxicity observed in animals at any dose for either the main or reproductive studies.

There were no statistically significant changes in food consumption in animals at any dose level in either the main or the reproductive studies. There were no statistically significant differences in food consumption in pre-mated females used in the reproduction study. Food consumption was not measured during gestation or lactation.

Cumulative weight gains in low and mid-dose males and all female dose-groups were not different from those of control animals (see Table). Cumulative weight gains in high-dose males were lower than those of control animals from days 31 - 92 (approx. 15 % less) reaching statistical significance between days 3 - 31 and 31 - 79.

Table: Cumulative mean body weight gain, males (selected time periods)

Dose (mg/kg)	Day 3 n=10	Day 31 n=10	Day 79 n=10	Day 92 n=10
control	23.16[1] (21.8)[2]	250.14 (22.9)	395.28 (60.5)	412.46 (64.6)
20	21.48 (17.8)	254.42 (18.6)	388.07 (37.8)	409.63 (42.7)
200	29.22 (7.1)	253.87 (27.3)	386.66 (61.1)	411.07 (73.4)
2,000	30.08 (7.7)	245.13 (18.7)	386.97 (49.8)	411.91 (62.4)
6,000	29.73 (6.7)	231.17 (25.7)	366.69 (53.9)	387.09 (58.4)
xx,000	28.20 (4.8)	219.79 *(16.3)	332.13* (39.9)	349.29 (47.1)

[1] weight in grams; [2] standard deviation

There was a dose-related lower terminal body weight in males (which was 10 % lower in the high-dose compared to control groups) (see Table). For the reproduction study phase, there were no statistically significant differences among treatment groups in maternal group mean body weights or weight changes during gestation except for an 8 % lower body weights in high-dose females by the end of gestation.

Table: Terminal Body Weights

Parameter	n=10/ sex	control	20 mg/kg	200 mg/kg	2,000 mg/kg	6,000 mg/kg	xx,0000 mg/kg
Terminal Body Weights	Main Study M	630.0[1] (71.1)[2]	628.9 (52.5) (100)[3]	629.2 (82.4) (100)	626.5 (67.6) (99)	602.1 (67.0) (96)	575.8 (61.5) (90)
	F	280.5 (31.7)	298.5 (30.7) (106)	283.2 (34.8) (101)	299.9 (26.1) (107)	304.2 (37.5) (108)	283.2 (32.9) (101)
	Repro. Study F	450.9 (41.6)	487.7 (38.8) (108)	465.8 (65.4) (103)	450.2 (62.5) (98)	441.4 (34.1) (98)	416.1 (52.2) (92)

[1] weight in grams; [2] standard deviation; [3] % of control group value

There were no statistically significant differences in measures of mating, fertility or gestational length at any dose level. There were no statistically significant differences in pup weights at birth or during the first four days of lactation at any dose group.

There were no statistically significant changes in litter weights or survival. There was an increase in numbers of pups found dead on lactation days 0 - 4 at 200 mg/kg (combined male/female survival as a % of control = 85.8); however, the reduction was not statistically significant or dose-related and was within historical control ranges for this strain of rat (historical control reference provided).

There were no treatment-related ocular abnormalities.

There were no statistically significant differences among treatment groups in any haematological parameter measured (total erythrocyte count, total leucocyte count, platelet count, haematocrit, haemoglobin concentration, red blood cell indices - mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular concentration).

Table: Clinical chemistry - selected parameters

Param.	**n=10/ sex**	**control**	**20 mg/kg**	**200 mg/kg**	**2,000 mg/kg**	**6,000 mg/kg**	**xx,000 mg/kg**
ALT/GPT (IU/L)	M	40.0 (9.2)	36.3 (9.32)	33.4 (8.9)	34.6 (9.3)	50.0 (18.6)	31.9* (3.8)
	F	42.8 (20.9)	46.7 (27.4)	38.2 (17.8)	35.1 (10.1)	37.2 (11.0)	28.5 (6.7)
GLU (mg/dL)	M	227.0 (20.1)	228.4 (25.2)	218.0 (27.3)	252.7 (39.4)	230.0 (44.3)	229.6 (26.7)
	F	160.7 (28.1)	214.2* (38.3)	188.8 (46.8)	205.00 (35.5)	227.7** (43.7)	181.9 (41.7)
TP (g/dL)	M	6.58 (0.4)	6.82 (0.4)	6.87 (0.3)	6.77 (0/3)	6.78 (0.4)	6.55 (0.3)
	F	6.91 (0.4)	7.61 **(0.5)	7.06 (0.3)	7.34 (0.4)	7.26 (0.2)	7.01 (0.5)
AP (g/dL)	M	4.27 (0.2)	4.17 (0.3)	4.38 (0.3)	4.31 (0.2)	4.42 (0.2)	4.13 (0.1)
	F	4.72 (0.5)	5.47** (0.5)	5.00 (0.3)	4.99 (0.4)	4.90 (0.3)	4.61 (0.5)
Ca (mg/dL)	M	11.46 (0.4)	11.68 (0.4)	11.60 (0.6)	11.71 (0.5)	11.61 (0.6)	11.51 (0.4)
	F	11.22 (0.4)	11.90 *(0.5)	11.44 (0.7)	11.76 (0.6)	11.52 (0.5)	11.34 (0.5)
Cl (meQ/L)	M	100.3 (1.9)	101.4 (1.7)	101.8 (1.1)	102.4 (2.5)	103.9** (1.9)	104.4** (2.0)
	F	100.9 (1.8)	100.5 (1.8)	101.8 (1.8)	101.2 (1.6)	101.9 (1.4)	101.9 (2.3)

* $p \leq 0.05$ ** $p \leq 0.01$ ALT/GPT - alanine amino transferase/glutamic pyruvic transaminase;
GLU - glucose; TP - total protein; AP - alkaline phosphatase; Ca - calcium; Cl - chloride

A statistically significant decrease in mean alanine amino transferase occurred in high-dose males however the level was within the normal range of values (see Table). Statistically significant increases in mean glucose, albumin, total protein and calcium levels occurred in females; however, these were not dose-related and since they fell within the normal range of values were not considered to be treatment-related. There was a dose-related increase in chloride levels in males which reached significance at 6,000 mg/kg and xx,000 mg/kg; however, these levels were within the normal range of values and are not considered toxicologically significant.

Table: Incidence of selected pathologies

Parameter	n=10 /sex	cntrl	20 mg/kg	200 mg/kg	2,000 mg/kg	6,000 mg/kg	xx,000 mg/kg
Kidney: pylonephritis	M	0	0	0	0	0	1
	F	0	0	0	0	0	2
Kidney: hydro-nephrosis (bilateral)	M	2	0	2	0	0	0
	F	0	0	0	0	0	2
Kidney: hyperplasia, pelvic epithelium	M	0	0	0	0	0	1
	F	0	0	0	0	0	1
Kidney: mineralization or protein accumulation	M	1	0	4	2	4	4
	F	3	0	4	3	2	2
Urinary and/or Kidney calculi	M	0	0	0	0	0	1
	F	0	0	0	0	0	2
Urinary bladder or ureter hyperplasia	M	0	0	0	0	0	1
	F	0	0	0	0	0	1
Urinary mineraliz'n or Protein accumulation	M	0	0	0	0	0	1
	F	-	-	-	-	-	-

There were no dose-related or statistically significant changes in the mean or absolute organ weights (data not shown in the Table). There were no dose-dependent or statistically significant increases in microscopic lesions noted. Lesions were noted in the mesenteric and submaxillary lymph nodes in males at 20 mg/kg feed, however, the incidence was not dose-related. At the high dose several lesion types (pyelonephritis, hydronephrosis and hyperplasia of the mucosal and/or pelvic epithelium in the kidneys) were seen which were in conjunction with kidney and/or bladder calculi. The incidence of lesions was not dose-related or statistically increased in comparison to the incidence levels in control animals. However, the incidence of calculi in animals of the age used in the study is unusual and may be a treatment-related effect.

Conclusion: At the high dose several lesion types were seen which were in conjunction with kidney and/or bladder calculi. The incidence of lesions was not dose-related or statistically increased in comparison to the incidence level in control animals. However, the incidence of calculi in animals of the age used in the study is unusual and therefore may be a treatment-related effect. There were no statistically significant differences among treatment groups in the reproduction part of the study: maternal group mean body weights or weight changes during gestation; measures of mating, fertility or gestational length; pup weights at birth or during the first four days of lactation; or litter weights or survival.

The NOAEL for the study was x,xxx mg/kg (xxx mg/kg bw/day) based on body weight reduction in males and the occurrence of calculi in both sexes at the next highest dose which may be treatment-related. The x,xxx mg/kg dose level is considered a NOAEL since there was a dose-related increase in chloride level in males which was within the normal range of values. The dose levels (0, x0, x00, x,000, and xx,000 mg/kg) were chosen for the long term study.