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DRAFT GUIDANCE FOR A SINGLE DOSE STUDY FOR THE DERIVATION OF AN ACUTE REFERENCE DOSE

A. Background

Regulatory requirements or legislation s relating to the protection of human health have lead to the need to consider the establishment of an Acute Reference Value for all potentially acutely toxic substances with relevant acute human exposure scenarios. This applies mainly to pesticide, biocide, veterinary drug residues in food and drinking water for which an **Acute Reference Dose** (ARfD) has to be considered (1, 2, 3, 4). Regulatory authorities are required to protect the general population against effects induced by acute exposure to hazardous substances, if the **Tolerable Daily Intake** (TDI) is substantially exceeded for short periods of time (7). However, the derivation of **Acceptable Exposure Limits** (AEL) (5, 6) is also relevant for human health risk assessment of pesticides, biocides, and other chemicals with regard to potential acute effects that may occur as a result of primary and secondary exposure of professional and non-professional users and bystanders.

In the context of this guidance document, the term Acute Reference Value will be used as a synonym or more generic term for ARfD, AEL_{acute}, acuteAOEL, acute POD or comparable other external reference values referring to acute exposure.

The Acute Reference Value is an estimate of the amount of a substance, normally expressed on a body weight basis, that can be ingested from food and/or drinking water, or that can be tolerated by dermal or inhalational exposure to environmental media (e.g. air, water, soil) in a period of 24 hours or less, without appreciable health risk to human beings, on the basis of all the known facts at the time of the evaluation (2). It can be transformed into an internal value considering the extent of absorption of the substance along the respective route of application. This internal value is equivalent to the acute systemic Acceptable Exposure Limit (AEL) applied in different regulatory frameworks.

The decision to set an Acute Reference Value as a toxicological limit value should be based solely on toxicological reasons.

A numerical cut-off for setting Acute Reference Values is proposed. For pesticides, such a cut-off should be around 5 mg/kg bw. If calculations indicate that an Acute Reference Value is going to be higher than 5 mg/kg bw, it would not be necessary for practical reasons to set an Acute Reference Value, as food residue or environmental levels necessary to achieve this intake would be highly unlikely to occur in practice.

If cut-off criteria do not preclude the setting of an Acute Reference Value, the value should be established by using the most appropriate endpoint, based on the relevant guidance documents for setting Acute Reference Values (1, 2, 3, 4, 5).

However, there will be cases where effects which are normally considered relevant for setting Acute Reference Values cannot be identified from the acute toxicological database, even though the need for setting such a value is evident. In such cases, a conservative Acute Reference Value is frequently derived from the NOAELs for non-acute effects seen in repeated dose studies. The appropriateness of using doses and endpoints from subchronic and chronic studies to establish Acute Reference Values needs to be carefully considered. Particular weight should be given to findings noted at the beginning of repeated dose studies.

Nevertheless, in the absence of information to the contrary, all adverse effects seen in repeated dose studies should be evaluated for their relevance. The resulting Acute Reference Values may be overly conservative and a risk assessment conducted on this basis may indicate an unacceptable risk even after, as a first step in the refinement of the risk assessment, exposure assessment has been re-addressed.
In such a situation, as a further step of refinement, an additional animal study specifically elucidating toxicity after single dose might be justified under certain circumstances to avoid an over-conservative risk assessment and to meet the common needs for best science, appropriate health protection measures and a reduced/refined animal testing.

The results of such a study should (i) clarify whether a substance poses an unacceptable acute risk and (ii) allow the derivation of a refined Acute Reference Value for acute intake of residues in food and drinking water or for acute exposure of users and bystanders.

This approach should contribute to moving away from paradigms that involve extensive animal testing for "every possible adverse outcome" to a more science-based approach in which existing knowledge combined with estimates of exposure are used to determine what specific *in vivo* testing is appropriate.

At the 13th Meeting of the OECD Working Group on Pesticides in 2002, the JMPR presented a proposal for an OECD Test Guideline animal study, designed to establish an Acute Reference Dose for dietary risk assessment for human health (8). The EC, Spain and CropLife International supported this proposal, suggested improvements and recommended that JMPR should proceed by approaching the Working Group of National Co-ordinators to the Test Guidelines Programme (WNT).

The 19th WNT meeting then agreed, that a harmonised guidance should be developed for those cases in which more data from a appropriately designed single dose study were needed for derivation of Acute Reference Values. The WNT agreed to include the project in the work plan for 2007.

B. Purpose

This guidance document is expected to harmonise the derivation of Acute Reference Values suitable for refined acute risk assessment in a range of acute human exposure scenarios. This proposed guidance aims to:

- reduce the need to conduct unnecessary tests on animals,
- reduce the need to repeat animal tests which have not been performed in a way which adequately satisfy the requirements of different regulatory agencies,
- harmonised test procedures for determining acute toxic effects of a compound in situations where available data are not able to adequately characterise the acute hazard.

This document proposes a test protocol for an acute single dose study which allows the derivation of a NOAEL/LOAEL or benchmark dose for the most relevant acute effect(s) in the most appropriate species.

This protocol covers investigations of a comprehensive range of relevant endpoints which may arise after a single exposure, or during one day of dietary exposure to a test substance. In particular, it is tailored to determine the most appropriate NOAEL to derive a refined Acute Reference Value with special emphasis on evaluating whether toxic effects observed in the standard package of repeated dose studies may also occur after single doses, to address additional parameters not usually examined in repeated dose studies as well as to provide

further information on the dose-response curve and time to peak of acute toxic effects after single dose exposure.

The aim of the single dose study is not:

- to identify lethal doses or provide data on mortality or morbidity after acute exposure to a chemical,
- to investigate the reversibility of acute effects, or
- to investigate developmental effects or corrosive/irritation properties.

C. Basic Considerations

It is important to note that a specific study aimed at a refinement of Acute Reference Values can only be considered in a **first step** after the toxicology profile of an active substance has been appropriately evaluated by a detailed consideration of the standard toxicological data package. The relevant toxicological endpoints should be documented and reasonably well understood.

After reviewing the available toxicological database and possible exposure scenarios, the **second step** is to perform a tiered human health risk assessment which includes a comparison of the Acute Reference Value with the total internal body burden, based on a worst- case assumption for the potential acute exposure. If this does not indicate unacceptable health risks, no further refinement of risk characterisation is required.

If there is a borderline situation or, alternatively, a clear concern, then the refinement of the risk characterisation should first focus on a refinement of the exposure assessment (from potential exposure to actual exposure). If this does not indicate unacceptable health risks, then no further refinement of risk characterisation is required.

However, if the refined risk assessment has shown clear indications of unacceptable health risks, and if conservative assumptions were used in setting the Acute Reference Value, then the default approach is to proceed to step three and consider the need for a single dose study in order to establish a refined Acute Reference Value. This will be necessary only for a limited number of substances. It is not intended that this *In-vivo* test becomes a routine data requirement.

The **third step** is to perform a single dose toxicity study in the most appropriate species, according to the protocol outlined in the annex to this guidance document. The selection of the most appropriate species should be based on relevant effects noted in the repeated dose studies.

D. Principle of the Single Dose Test

If the performance of a single dose study can be justified, then all available information on the substance (such as toxicokinetic and toxicodynamic properties, relevant information on structural analogues, results of previous acute and repeated dose toxicity studies etc.) should be taken into consideration before starting the experiment. These data will aid in the decisions on the route of administration, the choice of vehicle, the selection of the most suitable animal species, the selection of dosage levels and any modification to the observation and measurement schedules.

Observations on the experimental animals are mainly based on those listed in the revised OECD Test Guideline 407. Therefore, additional validation of these test parameters in experimental studies is not considered necessary.

Different doses of the test substance are administered as a single dose to several groups of experimental animals, one dose level per group. A vehicle control group is also included. The animals are closely monitored for signs of toxicity until termination of subgroups at one of two time points post-treatment (at 24 hours and between 48 and 120 hours).

E. Animal Welfare Consideration

For reasons of animal welfare, the request for additional experimental animal data should always be a last resort in risk assessment process; additional single dose study should not be performed:

- if the derivation of an Acute Reference Value is considered unnecessary on toxicological reasons (e.g. see criteria recommended by the 2004 JMPR),
- if adequate acute toxicity studies are available which indicate relevant effects after single exposure, i.e. developmental toxicity and acute neurotoxicity studies,
- if adequate repeated dose studies are available which indicate acute effects shortly after exposure,
- if a compound has negligible residues such that refined dietary exposure estimates indicate an adequate margin of safety even if measured against a conservative Acute Reference Value derived from a repeated dose study, and
- if exposure estimates indicate levels of exposure which provide an adequate margin of safety even when measured against a conservative Acute Reference Value derived from a repeated dose study.

In the refined approach, a minimum, but sufficient number of animals of the most appropriate species should be utilised to produce the required additional data.

F. Consideration of the Route and Levels of Exposure

This acute single dose study should be performed only after a detailed consideration of the most likely exposure route for humans so that the study itself can be designed for this route (oral, dermal, or inhalation) and cover relevant levels of exposure. In general, oral administration would be the preferred route with route-to-route extrapolation performed, if necessary.

G. Consideration of Dose Selection

Three dose groups plus vehicle control group should be the minimum for deriving a NOAEL/LOAEL.

If a benchmark approach is intended, more than three dose groups should be considered. In this case, the number of animals per group could be reduced, as long as the necessary statistical requirements are fulfilled.

H. Consideration of Toxicokinetic Parameters to be Co-evaluated

The main objective of toxicokinetic data collection in the acute single dose study is to determine the systemic exposure and its relationship to the administered doses and the time course of any toxic effects seen in the study.

J. Consideration of Different Subpopulations

In principle, a single Acute Reference Value should be set to protect the whole population. However, it is also important to ensure that the Acute Reference Value is adequate to protect the embryo/foetus from possible *in utero* effects. Therefore, the consideration of developmental studies for the derivation of Acute Reference Values is recommended, as a more conservative approach. Because of critical windows of sensitivity for developmental effects, it should be assumed that most developmental endpoints from repeated dosing studies are relevant for setting acute dietary doses, unless there is evidence to the contrary (1, 2).
While an Acute Reference Value based on developmental (embryo/foetal) effects would be appropriate for women of child-bearing age, it is recognised that the same value may be overly conservative with respect to other subgroups in the population. For children aged 1 to 6 years, the use of a refined Acute Reference Value based on *in utero* effects could lead to an unreasonably conservative acute dietary risk assessment due to a generally higher intake of food commodities per unit bodyweight in children than in adults. For this age group, which is unlikely to be at risk for the developmental toxicity observed, separate modelling with respect to acute dietary intake of residues can be performed taking in account age-specific acute consumption data. On the other hand, it might be necessary to exclude a higher sensitivity of children to other forms of acute toxicity by testing during early life-stages

Therefore, in some situations it may be necessary to set a second Acute Reference Value based on another, non-developmental endpoint based on a single dose study in non-pregnant animals.

K. References

(1) Solecki et al. 2006

(2) JMPR, 2004: Report 2004

(3) JECFA, 2006: Evaluation of certain food contaminants 64. Report; WHO Technical report Series 930, Geneva 2006

(4) WHO, 2006: Expert Consultation for 2nd Addendum to the 3rd Edition of the Guidelines for Drinking Water Quality, Geneva 2006

(5) TNsG Biocides RC-Paper

(6) EC, 2006: Technical Guidance Document (reference p-TGD) Chapter 3, Human health hazard assessment

(7) WHO, 2006: Guidelines for Drinking Water Quality

(8) OECD Document ENV/JM/PEST(2002)11

(9) Draft Summary Record of the 19th Meeting of the Working Group of National Coordinators of the Test Guideline Programme, 28-20 March 2007, Doc. ENV/JM/TG/M(2007)1, No.: 55

Annex

DRAFT TEST PROTOCOL FOR A SINGLE DOSE ORAL TOXICITY STUDY

PRINCIPLE OF THE TEST

The test substance is administered orally as a single dose in graduated dose levels to several groups of experimental animals, one dose being used per group. A vehicle control group is also included. Subgroups are terminated at 24 hours and between 48-120 hours after treatment. The investigation of two time points is only necessary if the time peak of toxic effects is not observed within or at 24 h. The single dose study protocol as proposed is not intended to examine reversibility of acute effects. Appropriate justification should be submitted if a second time point is not considered necessary. The selection of the second time point should also be justified.

The animals are observed closely for signs of toxicity throughout the test. Animals that die or are killed during the test and the survivors that are killed at the end of their respective test periods are subjected to necropsy.

DESCRIPTION OF THE METHOD

Selection of Animal Species

The selection of animal species should be based on the results of the repeated dose studies, which usually restricts the choice to the rat or the dog. It should not be necessary to perform the study in both species.

Occasionally, mice may be more sensitive than rats or a better model for humans. If the mouse is the preferred rodent species, the principles described for the rat should be adapted accordingly.

A justification should be given for the selection of the species. It should be demonstrated that the species selected will respond to the relevant parameters with a higher sensitivity than other species or be more relevant to human exposure. Preferably, the animals used in this study should be from the same strain and source as the animals used in the key studies of the existing toxicological database for the test substance.

Rats: Females should be nulliparous and non-pregnant. At the commencement of the study the weight variation of the animals used should not exceed $\pm$ 20 % of the mean weight of each sex. The test compound should be administered when the animals are between 8 and 10 weeks old. However, if children are considered to be more sensitive than adults, it might be appropriate to use younger animals (weanlings).

Dogs: Young adult animals should be used. The test compound should be administered preferably at 4-6 months of age and not later than 9 months of age.

Housing and Feeding Conditions

Rats: The temperature in the experimental animal room should be 22 °C ($\pm$ 3 °C). Although relative humidity should be at least 30 % and preferably not exceed 70 % other than during

room cleaning, the aim should be 50-60 %. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used, with an unlimited supply of drinking water. Animals may be housed individually, or be caged in small groups of the same sex. For group caging, no more than five animals should be housed per cage.

Dogs: It is recommended that animals are caged individually. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark.

The feed should be analysed for contaminants. A sample of the diet should be retained until finalisation of the report.

Preparation of Animals

Healthy young adult animals are randomly assigned to the control and treatment groups. Cages should be arranged in such a way that possible effects due to cage placement are minimised. The animals are identified uniquely and kept in their cages for at least 5 days prior to the start of the study to allow for acclimatisation to the laboratory conditions.

Preparation of Doses

Based on the definition of the Acute Reference Dose, the acute intake is generally assessed on a per day basis. A worst-case exposure scenario would be to assume that daily intake occurs in a single meal. Therefore, the most appropriate animal dosing would be by gavage in rodents and by capsule in dogs. This dosing regimen would be particularly relevant when effects are C_{max}-dependent and rapidly reversible (e.g. inhibition of acetylcholinesterase by carbamates). However, other means of dosing are also appropriate. Dogs in particular will consume a daily ration completely within one hour. Data on the palatability of the intended dose levels in diet have to be available in this case.

Where necessary, the test substance is dissolved or suspended in a suitable vehicle. For vehicles other than water the toxic characteristics of the vehicle must be known. The homogeneity of the test substance in the vehicle should be assured.

PROCEDURE

Number and Sex of Animals

The numbers of experimental animals used should be based on statistical power calculations and the variability of the specific end-points noted in the repeated dose studies as being especially relevant. For each dose, equal numbers of animals should be sacrificed at both termination time points.

At least 10 rats (5 rats per sex and per group) should be used at each dose level, including the vehicle control group.

A minimum of four dogs per sex and per dose group should be used for the 24-hour evaluation. Additional subgroups of the same size should be used for the evaluation at the second time point. However, if identification of the toxic effects is possible in live animals at the 24 hour time point, it may be sufficient to use only 4 dogs per sex and dose group which are then sacrificed at the 48-120 hour time point for pathomorphological examinations.

If existing data on the chemical show that one sex is clearly and consistently much more sensitive than the other for the endpoint(s) identified as being relevant for acute toxicity, then the study design may be modified to include only the more sensitive sex.

If it is intended to establish a benchmark dose level rather than a NOAEL/LOAEL, it may be sensible to increase the number of dose groups. A reduced spacing of dose levels may allow the study to be conducted with fewer animals per subgroup, depending on the statistical requirements for this approach.

Considerations relating to the substance under consideration

All available information on the substance, i.e. physico-chemical, toxicokinetic and toxicodynamic properties of the test substance, available relevant information on structural analogues of the substance (structure-activity relationships (SARs), results of previously conducted toxicity studies of the test substance (e.g. acute toxicity including lethal dose levels and acute effects, toxicity after repeated application should be taken into consideration before testing in order to conduct the study in the most appropriate way).

Some information on ADME may be able to be derived from chemical structure and physico-chemical data and results from toxicity studies (e.g. on NOAEL, indications of induction of metabolism).

The collection of all available information is important for a decision on the route of administration, the choice of the vehicle, the selection of animal species, the selection of dose levels and possibly for modifications of the dosing schedule (e.g. during the last week of pregnancy).

Dose Selection

At least three dose levels and a concurrent vehicle control should be used. Dose levels should be selected taking into account any existing toxicity and ADME data available for the test compound. The dose levels should be spaced to produce a gradation of toxic effects, ranging from recognisable toxicity but not death or severe suffering at the highest dose to no or only very slight effects at the low dose.

Possible starting points for setting dose levels are known LD_{50} values in animals and expected exposure levels in humans.

In addition, the highest/overall NOAEL from the repeated dose studies using the same animal species could be selected as the low dose and together with one or two of the effect doses from the repeated dose studies. The high dose may be limited to 1000 mg/kg bw/d, unless expected human exposure indicates the need for a higher dose level to be used.

Special consideration should be given if the NOAEL from the repeated dose studies is representative for provoking acute effects, e.g. clinical effects observed at the begin of a repeated dose study.

If the test substance is a pesticide and the results of the study will be used for the derivation of an Acute Reference Dose related to acute intake estimations, the highest dose level should not exceed 500 mg/kg bw/d. For other chemical substances or for regulatory purposes (e.g. bystander risk assessment for pesticides) a higher or lower cut-off dose level might be justified.

Administration of Doses

The most appropriate dosing would be by gavage in rodents and by capsule in dogs. Gavage should be done in a single dose to fasted animals using a stomach tube or a suitable intubation cannula.

The maximum volume of liquid that can be administered at one time depends upon the size of the test animal. The volume should not exceed 1 mL/100 g body weight, except for aqueous solutions, where 2 mL/100 g bw may be used. With the exception of irritating or corrosive substances, which are likely to cause exacerbated effects with higher concentrations, variability in volume should be minimised by adjusting the concentration to ensure a constant dosing volume at all dose levels.

Apart for treatment with vehicle instead of the test substance, the animals in the control group should be handled in an identical manner to those in the test group. If a vehicle is used to administer the test substance, the control group should receive the vehicle in the same volume used as total application volume (vehicle + test compound) in the treated groups. If different volumes are administered to the different treatment groups, the control should receive the vehicle at the highest volume used.

If administration is via feed in the dog, the single dose should be consumed completely in one meal within approximately one hour; a confirmation of this consumption time should be provided in the study report.

Inhalation and dermal exposure routes may be used in exceptional cases. Administration would be performed according to the corresponding guidelines on acute toxicity testing.

Clinical Observations

Clinical observations should be made in all animals at least once before exposure to the test substance (to allow for within-subject comparisons) and at least 0.5, 1, 2, 4 and 24 hours after dosing. The peak period of the anticipated effects should be considered.

In the 48-120-hour subgroups further observations should be made at least twice daily after the first 24 hours.

Observations should be carefully recorded, preferably using scoring systems, explicitly defined/reported by the testing laboratory. Effort should be made to ensure that variations in the test conditions are minimal and that observer bias is excluded.

Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern).

Changes in gait, posture, response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards) should also be recorded.

Body Weight and Food/Water Consumption

All animals should be weighed on the day of treatment and prior to sacrifice of the subgroup.

In addition, the animals of the 48-120-hour subgroups should also be weighed every 24 hours after treatment.

Measurements of food consumption and drinking water intake should be made daily.

Toxicokinetics

Information on toxicokinetics should be obtained before commencing this single dose study. However, frequently toxicokinetic data will only be available for the rat. If the dog is used as the more appropriate species in the single dose study, additional information on toxicokinetics may be necessary. Collection of samples for substance plasma levels at different time points can be incorporated into the design of the study if it does no interfere with other investigations. Blood samples should at least be taken at subgroup termination time points.

Functional Observations

The elements described in this guideline may be combined with the design of an acute neurotoxicity battery study, as long as none of the requirements of both guidelines are violated by the combination.

If the test species used is the rat, sensory reactivity to stimuli of different types (e.g. auditory, visual, and proprioceptive stimuli), grip strength and motor activity should be assessed unless existing data from repeated dose studies indicate that these parameters are not affected by the test substance.

This evaluation should be conducted in the peak period of the anticipated effect, e.g. 1, 2 or 4 hours, as well as just before sacrifice of the subgroups. If the peak effect is expected to be close to 24 hours then the 24 hour observation is sufficient.

Haematology

Unless existing data from repeated dose studies indicate that the blood cells and/or the haematopoietic system are not target sites, the following haematological examinations should be made just prior to or as part of the procedure for killing the animals at the end of the test period: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, and blood clotting time/potential. Justification should be given, if these parameters are not investigated.

Clinical Biochemistry

Clinical biochemistry determinations should be performed on blood samples of all animals taken just prior to or as part of the procedure for killing the animals at the end of the test period. The following investigations of plasma or serum should be included: glucose, total cholesterol, urea, creatinine, total protein, albumin, at least two enzymes indicative of hepatocellular effects (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase and sorbitol dehydrogenase). Measurements of additional enzymes and bile acids may provide useful information under certain circumstances.

In addition, the investigation of serum markers of acute tissue damage should be considered. These need to be identified for chemicals in certain classes or on a case-by-case basis.

If a specific, potentially acute effect of the test substance has been observed using special techniques in repeated dose studies, then these techniques should also be used in this study.

- Cholinesterase inhibition in plasma, red blood cells, brain and peripheral nervous tissue should be measured for compounds known to inhibit these enzymes.

- Blood methaemoglobin should be measured for compounds known to increase methaemoglobin formation.
- For endocrine modulators, specific hormones, which could be affected after single exposure, should be measured.

Urinalysis determinations should be performed just prior to termination, if effects on these parameters are expected based on the results of repeated dose studies. Unless existing data clearly indicate that the parameter is not affected by the test substance, the following parameters should be evaluated: appearance, volume, osmolality or specific gravity, pH, protein, glucose, blood and blood cells, cell debris.

Pathology

Gross necropsy

All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, the cranial, thoracic and abdominal cavities and their contents.

The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination: all gross lesions, brain (representative regions including cerebrum, cerebellum, and pons), spinal cord, stomach, small and large intestines (including Peyer`s patches), liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea, and lungs (preserved by inflation with fixative and then immersion), gonads, accessory sex organs (e.g. uterus, prostate), urinary bladder, lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle, and a section of bone marrow (or, alternatively, a freshly mounted bone marrow aspirate). Specific attention should be paid to likely target organs based on the known properties of the test substance.

Organ weight

Unless existing data from repeated dose studies with the test substance indicate that an organ is not a target site, the following organs should be trimmed of any adherent tissue, as appropriate, and their wet weight should be measured as soon as possible after dissection to avoid drying: liver, kidneys, adrenals, testes, epididymides, thymus, spleen.

In addition, if relevant as target organ for acute effects of the test substance, the wet weight should be determined for the following organs as soon as possible after dissection to avoid drying: paired ovaries, uterus, seminal vesicles (including coagulating glands), and prostate (dorsolateral and ventral part combined). Alternatively, seminal vesicles and prostate may be trimmed after fixation. Clamp or ligature should be present during fixation as leakage of fluid provokes damage to fine structures in seminal vesicles.

The following organs should be weighed after fixation: thyroid (trimming should also be performed after fixation in order to avoid tissue damage) and dorsolateral and ventral parts of the prostate separately after separation.

Histopathology

Full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups unless existing data from repeated dose studies indicate that an organ is not a target site. These examinations should be extended to animals of all other dose groups, if treatment-related changes are observed in the high dose group.

All gross lesions shall be examined.

DATA AND REPORTING

Individual animal data should be provided. Additionally, all data should be summarised in tabular form showing, for each test group, the number of animals at the start of the test, the number of animals found dead during the test or sacrificed for humane reasons and their respective time of death, the number showing signs of toxicity, a description of the signs of toxicity observed, including time of onset, duration, and severity, the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.

When possible, numerical results should be evaluated by an appropriate and generally acceptable statistical method. The statistical method should be selected during the design of the study.

Test Report

The test report must include the following information:

Aim of the study:

- Justification for conducting such a single dose study
- Rationale for the specific design (e.g. choice of species and sex, dose selection, endpoint selection)

Guidelines and Quality Assurance:

- Test type (Guideline)
- GLP

Test substance:

- physical nature, purity and physicochemical properties
- identification data

Test animals:

- species and strain used
- number, age and sex of animals
- source, housing conditions, diet etc.
- individual weight of animals at the start of the test

Test conditions:

- rationale for dose level selection
- details of test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation
- details of the administration of the test substance
- conversion from diet test substance concentration (ppm) to the actual dose (mg/kg bw/d), if the test substance was administered via the diet
- details of food and water quality

Results:

- body weight/body weight changes
- food consumption, and water consumption, if applicable
- toxic response data by sex and dose level, including signs of toxicity
- nature, severity and duration of clinical signs
- functional observations (e.g., sensory reactivity, grip strength, motor activity assessments)
- haematological tests with relevant base-line values
- clinical biochemistry tests with relevant base-line values
- body weight at sacrifice and organ weight data
- gross necropsy findings
- a detailed description and tabulation of all histopathological findings
- statistical treatment of results

Summary and discussion of results

Conclusions, NO(A)EL, LO(A)EL (or benchmark dose, if applicable)

LITERATURE