

Residues of veterinary drugs in food

Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives

Geneva, August 1996

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PREFACE

This edition of the guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives (JECFA) replaces the guidelines issued in 1989 (ICS/89.41).

These guidelines are intended primarily for WHO Temporary Advisers who prepare working papers for JECFA and for Members who have been assigned to peer review them and propose evaluations. The guidelines are also useful to manufacturers who wish to prepare summaries of data that they provide. The WHO Secretariat encourages the submission of such summaries by manufacturers.

Major changes to the previous edition of the guidelines are the following:

1. Modification of headings for toxicological studies. It should be noted in particular that multigeneration reproductive toxicity and developmental toxicity studies should be grouped under **reproductive toxicity**.
2. A description of the procedure for the preparation of consolidated working papers by Temporary Advisers and Members is provided (section 1, paragraph 2).
3. Temporary Advisers are requested to prepare a *table of contents* for each working paper (section 2.2).
4. Temporary Advisers are requested to include biochemical transformation schemes (section 2.4.1.2)
5. Temporary Advisers are requested to indicate the GLP status of studies and to indicate when studies are in compliance with recognized testing guidelines (section 2.4.2).
6. A sample report item has been included as an appendix, which should serve as a model for the preparation of the **Comments** and **Evaluation** sections (Appendix E).
7. Temporary Advisers are requested to include in a **Notes** section a listing of all the no-observed-effect levels (NOELS) from pivotal studies that were reviewed (section 2.7 and Appendix G).
8. Expanded and revised guidance on organization and punctuation of references for citation in the text and listing at the end of the working paper (section 2.8).

These guidelines are available from the WHO Joint Secretary, Joint FAO/WHO Expert Committee on Food Additives, International Programme on Chemical Safety, World Health Organization, 1211 Geneva 27, Switzerland. Comments on these guidelines and suggestions for future editions are gladly accepted.

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GUIDELINES FOR THE PREPARATION OF TOXICOLOGICAL WORKING PAPERS ON RESIDUES OF VETERINARY DRUGS IN FOOD FOR THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. Introduction

These notes are designed to guide WHO Temporary Advisers in the preparation of toxicological working papers for consideration at meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). These working papers summarize the toxicological and related data that are used for making quantitative determinations of safety of residues of veterinary drugs in food. When possible, acceptable daily intakes (ADIs) are established on the basis of these data.

Temporary Advisers are requested to prepare working papers using these guidelines. Their working papers should include all relevant sections except for the ***Evaluation*** section. The draft working paper is then transmitted to the Member who has been assigned the compound for comments and a proposed evaluation. The Temporary Adviser and Member should work together to prepare a consolidated working paper that contains all relevant sections, which is submitted to the WHO Secretariat in sufficient time before the meeting for reproduction and distribution to other Members.

Most working papers are published after meetings of the Expert Committee as toxicological monographs in the ***WHO Food Additives Series***. To facilitate their editing and to avoid delays in their publication, the WHO Secretariat would appreciate close adherence by Temporary Advisers to the standard style described in these guidelines.

The working paper pattern is outlined in Appendix A, which shows the order in which items appear and numbering style. Boldfacing should be used with all titles and headings.

Working papers should be submitted in ***single spacing***. They should be provided in electronic format on diskettes at the time of the meeting. If a Macintosh computer is used, the file should be converted to a format that can be used in DOS or Windows.

Two types of toxicological monographs are published after the meeting, full monographs and monograph addenda. Full monographs are published on veterinary drugs that are reviewed by the Committee for the first time. When re-evaluations are performed, monograph addenda are often prepared, which summarize the relevant safety data that have become available since the most recent evaluation; they do not contain the summaries included in earlier monographs. The same pattern is followed with both, although addenda usually contain fewer sections than do full monographs.

2. Working paper structure and content

This section summarizes the types of information that are usually included in toxicological working papers and the way that this information should be organized. The studies that are listed do not comprise a checklist of required studies. Rather, they are included to provide guidance on ways the usual types of data should be summarized and organized (see Appendix A).

2.1 Title - The main title should be the name usually given to the veterinary drug.

2.2 Table of contents - A table of contents consisting of section headings and page numbers should be included on the first page of the working paper (see a recently-published monograph, from WHO Food Additives Series 35 onward, for an example).

2.3 Explanation - If the veterinary drug has not been evaluated previously by the Expert Committee, it should be so stated, along with a brief description of the substance and its primary uses. If it has been evaluated before, previous evaluations should be referenced by number using the standardized reference list of JECFA publications, which may be found in annexes to recent JECFA reports (WHO Technical Report Series) and evaluations (WHO Food Additives Series). Thus, the report of the forty-third meeting would be referenced as (Annex 1, reference 113) and the toxicological monographs prepared after the forty-third meeting would be referenced as (Annex 1, reference 114). Reference to previous Committees should be made by number (such as the thirty-sixth meeting of the Committee), rather than by year, because in some cases reports have not been published in the same year as the meeting and in some years more than one meeting has been held, which creates confusion. Reasons for the present re-evaluation should be given and, if a full monograph on a substance that has been evaluated previously is being prepared, a statement should be made to the effect that the previously published monograph has been expanded and is reproduced in its entirety below. The structure of the veterinary drug should be included in a full monograph.

2.4 Biological data - This section contains summarized descriptions of studies that are important for assessing the safety of the substance. Studies that provide the basis for the evaluation should be summarized in greater detail than other studies. Single paragraphs composed of one-sentence summaries may be sufficient for reporting the results of studies of limited design or minor relevance for the evaluation.

The author's conclusions should be summarized in this section. If the person who arrived at the conclusion is not identified, it is assumed that it is the author of the study. When the Temporary Adviser disagrees with the conclusions of the author, he or she should discuss the controversial issues and present his/her own conclusions as a separate paragraph. This paragraph should conclude with the Temporary Adviser's determination of the no-observed-effect level (NOEL).

If studies on more than one animal species are summarized under one heading, the studies should be subgrouped in such a way that studies in smaller rodent species are listed first, with larger species listed last.

When adjacent paragraphs summarize different studies under the same heading, an extra space should be left between them. However, an extra space should not be left between paragraphs when they both summarize the same study.

Biological data should be subgrouped under three headings, **Biochemical aspects**, **Toxicological studies**, and **Observations in humans**. In a full monograph, but not in a monograph addendum, if no data are available under any of these headings or under subheadings under **Toxicological studies**, except for **Special studies**, the heading should be included in the working paper with the statement "No information available."

2.4.1 Biochemical aspects - These studies are designed to measure the concentration/time profiles of the ingested substance and its metabolites in the various organs and tissues of the body. The Committee uses them for interpreting the toxicological studies, including elucidating the mechanism of toxicity, which facilitates the establishment of an ADI. Comparisons of biochemical data between experimental animals and humans helps determine the relevance of toxicity observed in animals, which is likely to result in a more rational assessment of risk to humans; such comparisons should be summarized at the end of this section.

In the request for data that is issued before the meeting, the Secretariat asks that pharmacokinetic, metabolic, and pharmacodynamic studies in both experimental and food-producing animals be submitted to both the authors of the toxicological monographs (prepared by WHO Temporary Advisers) and residues monographs (prepared by FAO Consultants). However, the WHO Temporary Adviser is responsible only for summarizing the data in experimental animals, while the

FAO Consultant is responsible only for summarizing studies in food-producing animals. Even though the author of the toxicological working paper does not summarize the data in food-producing animals, he/she should perform a cursory review of these data so that the significance of qualitative and quantitative differences in metabolic patterns can be assessed. Such differences should be noted and their possible significance to the safety assessment should be discussed in the working paper. If data from food-producing animals are not submitted, the WHO Temporary Adviser should request them.

The types of biochemical studies that should be summarized under each heading are given below. Human biochemical studies that fall into these categories should be included in this section. Other human studies should be included under **Observations in humans**.

2.4.1.1 Absorption, distribution, and excretion

- Hydrolysis/digestion of the parent compound and its products in the mammalian gastrointestinal tract.
- The bioavailability of the unchanged compound and its hydrolysis or digestion products.
- The pattern and rate of distribution of absorbed substances within the animal.
- Mode and rate of excretion or elimination of the parent compound and its identified metabolites.

2.4.1.2 Biotransformation

- Metabolism of the parent compound, if absorbed as such, and of its products if they are not normal dietary or body constituents. The toxicological importance of the identified metabolites, whether stored or excreted, should be discussed (if known). A biochemical transformation scheme should be included along with an identification of the species to which it applies. Such schemes are usually provided by the manufacturer and can be photocopied for inclusion in the working paper. If a metabolic scheme is not provided by the data sponsor, it should be requested.

2.4.1.3 Effects on enzymes and other biochemical parameters

- The effects of absorbed substances and/or their metabolites on cellular and tissue enzyme production and morphology, chemical constitution, enzyme activity, or physicochemical state.

2.4.2 Toxicological studies - Summaries of toxicological studies generally comprise the bulk of a working paper. Five categories of studies on veterinary drugs should be routine; these are **acute toxicity, short-term toxicity, long-term toxicity/carcinogenicity, genotoxicity, and reproductive toxicity**. Sometimes these routine studies point toward the need for looking at particular target organs or tissues; such studies are classified as **special studies**. Appendix B provides guidance on the structure of toxicological summaries.

When the necessary information is provided, dose levels administered during the study should be given in terms of "mg of test substance/kg of body weight per day". If the author of the study presents administration levels in terms of "mg/animal per day", these values should be converted to mg/kg of body weight per day using animal weights if they are included in the report. With substances mixed in the diet, authors often express dosage levels in terms of ppm (parts per million), mg/kg feed, or percent of the substance in the diet. When feeding levels are presented in this way, they should be expressed in terms of mg/kg feed (when the lowest dose is 1000 ppm or more, it may be more appropriate to express the concentration as percent of the diet). However,

when determining the NOEL, these values should be converted to equivalent mg/kg of body weight per day figures using appropriate conversion factors (see section 2.6).

The Good Laboratory Practice (GLP) status of the study, along with the relevant authority, should be indicated. If there is no GLP certification, the Temporary Adviser should at least note whether the study was inspected by a Quality Assurance unit, as noted by the presence of a signed QA statement, and make some comment on the apparent quality of the protocol and adequacy of the methods used. In addition, whenever the author provides information on the test guideline or protocol that was followed, it should be so indicated. This information has not been provided in working papers in the past, so the format and placement of this information has not been standardized.

Studies performed by Industrial Bio-Test Laboratories should not be relied upon unless either (1) the study was performed before problems had been identified with the laboratory or (2) the study has been validated by the U.S. Environmental Protection Agency or Health and Welfare Canada (if not clear, the data sponsor should be queried). See section 2.8.2.2 for an example of the method for referencing validated studies.

Appendix B outlines the general information that should be included in summaries of toxicological studies.

2.4.2.1 Acute toxicity - Such studies, when properly designed and performed, identify extremely toxic compounds and provide useful information regarding target tissues and species and sex differences. When several species of both sexes are tested, information is obtained that may be useful for predicting whether toxicity is mediated through hormonal activity or whether one species should be investigated more extensively than others.

The results of acute toxicity studies that are expressed in terms of the LD₅₀ (lethal dose, median; oral, used for intramuscular intraperitoneal, or dermal administration) and/or LC₅₀ (lethal concentration, median; used for administration by inhalation) should be presented in tabular form as shown in Appendix C. When 3 or more LD₅₀ or LC₅₀ determinations by the same route in the same animal are available, the results may be expressed in a range in which the lowest to the highest values are recorded.

Other acute toxicity data important to the evaluation, such as the nature of toxicity and target tissues, may be presented in summary form below the table.

2.4.2.2 Short-term toxicity - Toxicological studies in which substances are administered in regularly-repeated doses over periods ranging up to one year to most small-animal species and up to two years to dogs and primates should be summarized in this section.

These studies, when properly performed, provide important information regarding the major toxic effect(s) of the test substance and its dose/response relationship. Short-term toxicity studies are often performed to ensure proper dose selection in long-term toxicity studies, and they can point toward target tissues and organs. In some cases, short-term toxicity studies can help clarify lowest-effect dose levels for effects observed in long-term toxicity studies and they can provide retrospective information that is useful for the interpretation of long-term toxicity/carcinogenicity studies, e.g., early signs of toxicity in the kidney or liver when tumours appear in these organs after long-term exposure.

The same general format and type of information as outlined under **Long-term toxicity/carcinogenicity** studies (section 2.4.2.3) should be included in summaries of short-term studies.

2.4.2.3 Long-term toxicity/carcinogenicity - Toxicological studies in which substances are administered in regularly-repeated doses over the greater part of the normal lifespan of the animal

species are summarized in this section. These studies are used for detecting chronic effects that are not observed in shorter studies. Long-term studies that are designed to investigate specific effects, such as carcinogenicity, should be included in this section.

Toxicological summaries of short-term toxicity, long-term toxicity, and carcinogenicity studies should include the following information (see Appendix B):

- \$ Species, strain, and number of each sex per dose level, including controls.
- \$ Mode of administration.
- \$ Duration of administration (and duration of observation period if different).
- \$ Brief listing of the biological parameters that were studied, the techniques employed, and any other information about the design of the study considered to be noteworthy. When histopathological examinations were performed, the tissues that were examined should be indicated along with the identification of tissues that were of particular interest to the evaluation.
- \$ Toxicological findings and levels at which observed. Results of relevant statistical analyses may be presented here.
- \$ No-observed-effect levels (NOELs) for specific toxic effects.

Negative findings should be limited to general statements on survival, growth, reproduction, organ weights, tumour incidence, organ function tests, and gross and microscopic appearance of tissues.

2.4.2.4 Genotoxicity - Data from an appropriate battery of short-term *in vitro* and *in vivo* genotoxicity tests can be useful in elucidating the mechanism of toxicity of certain compounds. Results of these studies may also be considered when evaluating the results of rodent carcinogenicity bioassays or determining whether an *in vivo* carcinogenicity bioassay is necessary to adequately assess the carcinogenic potential of a substance. To present the data in a more understandable form and to conserve space, the results of short-term genotoxicity tests should be tabulated. Appendix D provides an example of tabular representation of such data.

2.4.2.5 Reproductive toxicity - These studies are designed to evaluate effects on the sexuality and fertility of males and females and on developmental toxicity. **Multigeneration reproductive toxicity studies** provide general information on the effects of the test substance on gonadal function, estrous cycles, mating behavior, conception, parturition, lactation, and growth and development of the offspring until the age of weaning. **Developmental toxicity studies** are used for assessing effects on the developing organism, which may include (1) death of the developing organism, (2) a structural abnormality, (3) altered growth, and (4) a functional deficiency.

2.4.2.6 Special studies - Special studies are those designed to test for specific effects, such as neurotoxicity, immunotoxicity, and allergenicity. Studies to evaluate potential adverse effects on the microbial ecology of the human intestinal tract should be performed on compounds with antimicrobial activity. Appendix A lists, in alphabetical order, most of the special studies that will be encountered. Special studies should be listed alphabetically.

2.4.3 Observations in humans - Results of observations in humans are useful for assessing the relevance of the results of animal studies and for confirming ADIs. All studies dealing with humans (except for those summarized under **Biochemical aspects** or **Special studies** on effects on gastrointestinal microflora) should be included in this section, including epidemiological surveys, clinical experience, anecdotal observations, health effect studies relating to occupational exposure, reports of abuse, and volunteer studies measuring intolerance. Details such as sex, age, and general statements of physical condition should be given if important to the evaluation.

2.5 Comments - This section should contain short summaries of the biological findings in the studies of significance for the evaluation. The findings should be listed in the same general order

as they are summarized in the main body of the working paper. Short paragraphs that consist of relevant findings in a specific type of study or in only one study should be used instead of long paragraphs that include results from several studies. All relevant NOELs should be included. When the NOEL is the same dose level (in mg/kg feed) in males and females, only one value in terms of mg/kg of body weight per day (the sex with the lower value) should be given. Details such as sex, age, and general statements of physical condition should be given if important to the evaluation. Issues that require resolution at the meeting should be stated either here or in a **Notes** section (see section 2.7).

This and the **Evaluation** section, which is prepared by the Member (see section 1, paragraph 2), serve as the starting point for the evaluation. These sections usually serve as the first draft for the report. Therefore it greatly increases efficiency and ensures a more orderly process if the Temporary Adviser prepares the **Comments** section in a manner consistent with the way that it will be presented in the report.

Appendix E contains an example of a report item, and should serve as a model for the preparation of the **Comments** and **Evaluation** sections.

2.6 Evaluation - A proposal should be provided by the Member as to whether the Committee should allocate an ADI and, if so, on what basis. *Principles for the safety assessment of food additives and contaminants in food* (WHO Environmental Health Criteria, No. 70) and JECFA reports on residues of veterinary drugs should be consulted for guidance on selection of safety factors in establishing ADIs. If the Member proposes a temporary ADI, he or she should indicate the data required to resolve the outstanding issues to permit the establishment of an ADI. If no ADI can be established, the information that the Committee would wish to have before reviewing the compound again should be listed.

NOELs should be given in terms of mg/kg of body weight per day, and there are generally three ways to get there:

1. When the author of the study has presented data in these units (gavage studies) the NOEL should be reported in terms of " \bar{x} mg/kg of body weight per day".
2. When the author has presented data in terms of parts per million (ppm), mg/kg feed, or percent in the diet and food consumption and body-weight data are available, the intake in terms of mg/kg body weight per day can be calculated directly. The calculated values should ordinarily be rounded to two significant figures. The NOEL should be reported as " \bar{x} ppm (or mg/kg feed or percent in the diet), equal to \bar{y} mg/kg of body weight per day". Only one value should be reported, which is derived from the sex that gives the lower value based on food intake and body-weight data.
3. When the author has presented data in terms of ppm, mg/kg feed, or percent in the diet and food consumption data are not available, the table in Appendix F should be used for calculating intake. The NOEL should be presented as " \bar{x} ppm (or mg/kg feed or percent in the diet), equivalent to \bar{y} mg/kg of body weight per day".

The ADI is expressed in terms of mg/kg of body weight, and it should be rounded to one significant figure. The basis for the proposed ADI and safety factor should be provided.

If a temporary ADI is proposed, information required on the substance should be listed, along with a date by which time the results of the indicated studies should be submitted to WHO for evaluation.

2.7 Notes - This section is not included in the published monograph after the meeting. Information that the Temporary Adviser would like to bring to the attention of the Committee for resolution should be included here.

All of the NOELs and critical effects that have been identified in the relevant studies that are summarized in the working paper should be listed, in the order in which they are summarized in the working paper, as shown in Appendix G. Such a summary table, which will not be published in the toxicological monograph, is of great value at the meeting.

2.8 References

2.8.1 Citations in the text - References in the text should be in parenthesis following the relevant summaries (e.g. Williams, 1987a; Dalidowicz & Babbitt, 1986; Dalidowicz et al., 1986; Dalidowicz, 1987).

Where a report has more than two authors, the first author is followed by "et al.". It should be noted that "et al." is not underlined or italicized, "&" replaces "and", the punctuation must be correct, and several references to the same statement (including more than one by the same author(s)) are placed in chronological order.

When more than one article by the same author(s) in any one year is cited, the year should be followed by the lower-case letters "a", "b", "c", etc.

The names of authors are not always provided. In this case, the name of the organization associated with the generation of the data, followed by the year, should be cited, for example, (IARC, 1983) or (BIBRA, 1976).

Personal communications should be cited only in the text; they should not be included in the reference list. The name of the author, the recipient, and the date should be given. If the original recipient was not the World Health Organization, the submitter of the communication to WHO should be included.

Examples:

(Personal communication from Prof. R. Truhaut, Director, Toxicological Research Centre, Department of Pharmaceutical and Biological Sciences, René Descartes University, Paris, France, to WHO, 1975).

(Personal communication with attachments from R. Patterson, Northwestern University, Evanston, IL, USA, to S.A. Anderson, Federation of American Societies for Experimental Biology (FASEB), Bethesda, MD, USA; submitted to WHO by FASEB).

2.8.2 Reference list at the end of the working paper - The layout indicated below should be used. The order in which the information is presented is particularly important.

References should be listed in alphabetical order. All authors= names and initials should be listed, the name of the first author establishing the placement in the list of references. When more than one article by the same author(s) is cited, they should be placed in chronological order and, as indicated above, the lower-case letters "a", "b", "c", etc., should be used when more than one article by the same author(s) in any one year is cited. Only initial letters are capitalized.

When the name of an author is not available, the organization associated with the generation of the data should be given first in the citation (do not use the word "anonymous").

Translated titles appear in square brackets and the original language in parentheses. Titles of articles originally in French should remain in French.

2.8.2.1 Published studies - References should include authors (if provided), the year of publication, the title of the article, the journal and volume number, and inclusive page numbers. Names of journals should be abbreviated according to the ISDS (International Serials Data System) List of Serial Title Word Abbreviations or otherwise given in full. The initial letter of each abbreviation is capitalized. The volume number is indicated in bold print and is followed by the issue number (if any) in parentheses. First and last page numbers must be given.

Examples:

Bartlet AL, Harvey S & Klandort H (1990) Contrasting effects of nitrofurans on plasma corticosterone in chickens following administration as a bolus or diet additive. *J Vet Pharmacol Therap*, **13**:261-269.

Laubstein VH & Niedegesass G (1970) [Examination of human sensitivities to nitrofurans]. *Derm Mschr*, **156**:1-8 (in German).

2.8.2.2 Unpublished studies - The essential elements of unpublished studies that should be included are:

- @ The name of the author(s) who performed the research work, if provided.
- @ The year in which the experimental work was completed.
- @ The title of the experimental study; if the title is in a language other than English, translation of the title into English is preferred (except titles in French).
- @ Study number, if provided.
- @ An indication that the study is unpublished.
- @ The name of the institution at which the experimental study was performed.
- @ The name of the institution that submitted the report to the World Health Organization.

Examples - These examples provide guidance on the appropriate format to use under varying conditions, including when the names of authors are not provided, when the institution submitting the study to WHO did not perform the study, and when the institution submitting the study to WHO did perform the study.

Baker RC, Mastri CW, Kinoshita FK & Keplinger ML (1976) Acute irritation tests with a sample coded N252-C10406, Lot No. BL7668, in albino rabbits. Unpublished report No. 8530-08861 from Industrial Bio-Test Laboratories, Inc., Northbrook, IL, USA. Submitted to WHO by Uniroyal Inc., Bethany, CT, USA (validated by the Canadian Health Protection Branch).

Bailman JJ & Barber ED (1985) Evaluation of mono-t-butylhydroquinone in the CHO/HGPRT forward mutation assay. Unpublished report No. 85-0061 from Health and Environment Laboratories, Eastman Kodak Co., Rochester, NY, USA. Submitted to WHO by Eastman Kodak Co., Kingsport, TN, USA.

BIBRA (1976) A study of the haematological effects of caramel in human volunteers. Unpublished report No. 1/172/76 from the British Industrial Biological Research Association, Carshalton, Surrey, England. Submitted to WHO by International Technical Caramel Association.

2.8.2.3 Conference proceedings - The following elements are necessary: Name(s) and initial(s) of author(s), the year of publication, title of paper, the word "In:" the editors of the proceedings; the full title of the conference (not abbreviated); the place and date of the conference; the place of publication; the publisher; the volume number (if any) and the page numbers.

Example:

Wassermann M (1984) L'étude de la toxicologie des pesticides en climat tropical In: Smith JH ed. Proceedings of the 14th international Congress of Occupational Health, Madrid, 16-21 May 1983. Amsterdam, Excerpta Medica, vol 3, 1728-1733.

2.8.2.4 Books

Examples:

Windholz M ed. (1983) The Merck Index: an encyclopedia of chemicals, drugs, and biologicals, 10th ed. Rahway, New Jersey, Merck and Co., Inc.

Reference to a chapter in a book should be given as follows:

Rall TW (1990) Oxytocin, prostaglandins, ergot alkaloids, and other drugs; tocolytic agents. In: Gilman AG, Rall TW, Nies AS & Taylor P eds. the pharmacological basis of therapeutics, eighth edition. New York, Pergamon Press, pp 933-953.

2.8.2.5 Agency reports

Example:

US EPA (1984) Mercury health effects update: health issue assessment. Washington, DC, US Environmental Protection Agency (EPA-60018-84-019F).

2.8.3 Order of entries in the list

The following rules are applied:

- a) Several papers by different authors with the same surname are listed alphabetically according to their initials.
- b) Several papers by one author are listed chronologically.
- c) Several papers by the author plus a co-author are listed alphabetically.
- d) Several papers by the author plus two or more coauthors are listed chronologically.

Examples:

Smith DE (1985)
Smith JH (1983)
Smith JH (1984)
Smith JH & Barns MP (1986)
Smith JH & Jones TD (1979)
Smith JH, Jones TD, & Barnes MP (1981)
Smith JH, Barnes MP, & Jones TD (1983)

Appendix A

Working paper pattern

This appendix provides the format that should be used in preparing working papers for the Joint FAO/WHO Expert Committee on Food Additives. Close adherence to this pattern will facilitate the editing of those working papers that are subsequently published as toxicological monographs.

Please adhere to the heading hierarchy shown in this appendix. The headings are indented here to clearly show the hierarchy, but they should not be indented in the text. The headings should be indented as shown in this appendix in the table of contents at the beginning of the working paper. Boldfacing should be used with all titles. The listed animal species and special studies are meant to serve as examples, not as exhaustive lists.

An extra space should be left between paragraphs when they summarize different studies; when adjacent paragraphs summarize the same study, an extra space should not be left between them.

TITLE Table of contents

1. EXPLANATION
2. BIOLOGICAL DATA
 - 2.1 Biochemical aspects
 - 2.1.1 Absorption, distribution, and excretion
 - 2.1.2 Biotransformation
 - 2.1.3 Effects on enzymes and other biochemical parameters
 - 2.2 Toxicological studies
 - 2.2.1 Acute toxicity [In tabular form; see Appendix C]
 - 2.2.2 Short-term toxicity
 - 2.2.2.1 Mice }
 - 2.2.2.2 Rats } Smaller rodent species first, with larger
 - 2.2.2.3 Hamsters } species last; this order holds for all
 - 2.2.2.4 Rabbits } categories of animal studies, not just
 - 2.2.2.5 Dogs } short-term toxicity studies.
 - 2.2.2.6 Pigs }
 - 2.2.2.7 Monkeys }
 - 2.2.3 Long-term toxicity/carcinogenicity
 - 2.2.4 Genotoxicity [In tabular form; see Appendix D]
 - 2.2.5 Reproductive toxicity
 - 2.2.5.1 Multigeneration reproductive toxicity
 - 2.2.5.2 Developmental toxicity
 - 2.2.6 Special studies on cardiovascular effects
 - 2.2.7 Special studies on immune responses
 - 2.2.8 Special studies on macromolecular binding
 - 2.2.9 Special studies on metabolites
 - 2.2.10 Special studies on microbiological effects
 - 2.2.11 Special studies on no-hormonal effect levels
 - 2.2.12 Special studies on ocular toxicity
 - 2.2.13 Special studies on photoisomerization products
 - 2.2.14 Special studies on relay toxicity
 - 2.2.15 Special studies on thyroid function
 - 2.3 Observations in humans
3. COMMENTS
4. EVALUATION
5. REFERENCES

Appendix B

Toxicological summaries

The summaries of experimental data form the bulk of a working paper. They are aimed at giving an overview of the essential elements of an experimental study that sometimes is several hundred pages long. Studies that are used for setting the ADI or are otherwise used for making a determination of safety should be summarized in more detail than those that are peripheral to the safety evaluation.

A good scientific summary contains the following elements:

- (a) Purpose or objective of the experiment.
- (b) Identification, specifications, and purity of the substance administered.
- 8 (c) Designation of animal species and strain employed in the study.
- (d) Number of animals in test and control groups.
- (e) Sex.
- (f) Dose levels of treatment (preferred units are mg test substance/kg of body weight per day).
- (g) Route(s) of administration.
- (h) Duration of treatment and/or the experiment, if they differ in length.
- (i) Biological parameters examined.
- (j) Effects observed (percent survival should be included in long-term toxicity/carcinogenicity studies).
- (k) Author's conclusions and conclusions of the Temporary Adviser, if different from those of the author (if the conclusions are not straightforward, identify who made them).
- (l) Reference(s).

The following paragraph is an example of a typical summary of a toxicity study prepared by a Temporary Adviser in preparation for a meeting of the Committee.

2.2.2 Short-term toxicity

2.2.2.2 Rats

Groups of 20 male and 20 female Crl:CD(SD)BR rats were given diets containing 0, 2000, 10 000, or 50 000 mg spiramycin embonate (purity not specified)/kg feed for 13 weeks. At the end of this time, 10 rats per sex per group were killed and the remainder maintained on the treatment diets for a further week and then allowed a 4-week recovery period (i.e. up to week 18). The doses on a body-weight basis varied over the test period, being equal to 220, 1200, or 5900 mg/kg of body weight per day in males and 210, 1000, or 5200 mg/kg of body weight per day in females at 2000, 10 000, or 50 000 mg/kg feed, respectively, at week 1 of the study, while at week 13 the respective values were 89, 470, or 2500 mg/kg of body weight per day in males and 120, 580, or 2800 mg/kg of body weight per day in females. The mean calculated doses in females (the sex that gave the lower values) were 140, 720, and 3900 mg/kg of body weight per day.

Clinical observations were made daily, while body weights and food consumption were measured weekly. Ophthalmological examinations were conducted at weeks 6 and 13 in animals in the control and highest-dose groups. Haematological and biochemical analyses and urinalyses were carried out at weeks 6 and 13, and at weeks 16 and 18 in recovery groups. At necropsy, all major organs were weighed and the animals were subjected to full histopathological examination.

No compound-related mortality occurred during the study and no major clinical signs attributable to spiramycin intake were observed. Significant reductions in body-weight gain were noted in males in the highest-dose group.

Haematological examinations revealed reductions in neutrophils in males and females in the mid- and highest-dose groups at week 6, although the effect in the latter group was not statistically significant. At week 16 of the recovery period, reductions in both neutrophil and lymphocyte counts were noted in males in the mid- and highest-dose groups. Significant biochemical effects were not

observed, but urinalysis revealed reductions in urinary protein at weeks 6 and 13 in high-dose males, although the effect was not statistically significant at the latter time point.

At termination, reduction in mean relative liver weights was observed. Bone marrow examination showed reductions in lymphocytes in high-dose males. The authors considered the effects on circulating lymphocytes to be unrelated to treatment because they were not observed during the treatment period. The only other major effect noted on histopathological examination was dilatation of the caecum. Testicular degeneration was not observed. The NOEL was 2000 mg/kg feed, equal to 140 mg/kg of body weight per day (Powell et al., 1990).

Appendix C

Tabular representation of acute toxicity data

Table 1. Acute toxicity of nitrofurural

Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference
Mouse	?	oral	380	Krantz & Evans, 1945
	M&F	oral	590	Miyaji, 1971
	?	i.p.	300	Smith <i>et al.</i> , 1963
Rat	M	oral	590	Krantz & Evans, 1945
	M&F	oral	590	Miyaji, 1971
	M	oral	800	Anderson, 1983

Appendix D

Tabular representation of genotoxicity data

Table 2. Results of genotoxicity assays on trenbolone

Test system	Test object	Concentration	Results	Reference
<i>in vitro</i>				
Ames test ¹	<u>S.typhimurium</u> TA98, TA100	0.06-2 µg/ plate	Negative	Schiffman <u>et al.</u> , 1985
Cell transformation assay	Syrian hamster embryo fibroblasts	5,10,15 µg/ml	Equivocal ²	Schiffman <u>et al.</u> , 1985
Cell transformation assay	Mouse C3H10T1/2	2-25 µg/ml (-S9 mix) ³ 5-20 µg/ml (+S9 mix) ⁴	Equivocal (-S9 mix) Positive (+S9 mix)	Henderson <u>et al.</u> , 1987a
Forward mutation assay ¹	Chinese hamster ovary cells (HGPRT locus)	25-500 µg/ml ³	Negative	Henderson <u>et al.</u> , 1986a
DNA repair assay	Cultured human epithelioid cells	1-512 µg/ml ⁵	Negative ⁶	Allen & Proudlock, 1983
<i>in vivo</i>				
cytogenetics assay	Rat bone marrow	100 mg/kg bw once; 25 or 50 mg/kg bw 4 times ⁷	Negative	Richold & Richardson, 1982

¹ Both with and without rat liver S9 fraction.

² There was an inverse dose relationship, with the largest number of transformations occurring at the lowest dose.

³ 20-Methylcholanthrene was used as a positive control.

⁴ 2-Acetamidofluorene was used as a positive control.

⁵ 4-Nitroquinoline-1-oxide (-S9 mix) and 2-aminoanthracene (+S9 mix) were used as positive controls.

⁶ Increases in nuclear grain count were observed in a limited number of cultures in one experiment only.

⁷ Mytomycin C was used as a positive control.

mals in the highest-dose group survived. Survival rates were decreased in the progeny of animals given lower doses, but there were no findings that could be attributed to effects on fertility. The NOEL in this study was 0.4 mg per kg of body weight per day. In a three-generation reproductive toxicity study in rats given doses of up to 0.83 mg per kg of body weight per day for a 70-day period before mating, there were no effects on mortality or on fertility except at the highest dose, where slight reductions in male body weights and significant reductions in pup survival were seen. The NOEL was 0.4 mg per kg of body weight per day.

In a teratogenicity study in rats dosed at 10 or 12 mg moxidectin per kg of body weight per day, there was evidence of both maternal toxicity and fetotoxicity, as shown by increased incidences of cleft palate and wavy or incompletely ossified ribs. There was no evidence of teratogenic effects, and the NOEL in this study was 5 mg per kg of body weight per day. In a teratogenicity study in rabbits, there was evidence of maternal toxicity at 5 and 10 mg per kg of body weight per day, but no evidence of fetotoxicity or teratogenicity. The NOEL in this study, based on maternal effects, was 1 mg per kg of body weight per day.

In a long-term toxicity/carcinogenicity study in CD-1 mice, moxidectin was administered in the diet at concentrations equal to 2.5, 5.1, or 12 mg per kg of body weight per day for 2 years. After 9 weeks, the highest dose was reduced to 7.9 mg per kg of body weight per day because of toxic effects that included deaths, hunched posture, decreased activity, tremors, and laboured breathing. There were no effects on haematological parameters and no increases in the incidence of any types of tumour were observed. The NOEL was 5.1 mg per kg of body weight per day.

A 2-year toxicity/carcinogenicity study was conducted in Sprague-Dawley rats, which were given moxidectin in the diet at concentrations equal to 0.8, 3.2, or 9.8 mg per kg of body weight per day. After 8 weeks, the highest dose was reduced to 5.1 mg per kg of body weight per day because of signs of toxicity that included hunched posture, tremors, hyperactivity, urine-stained fur, and hypersensitivity to external stimuli. At the end of the 2-year period there was no evidence of toxicity and no increased incidence of any type of tumour. The NOEL was 5.1 mg per kg of body weight per day.

The Committee concluded that moxidectin has no carcinogenic potential.

Moxidectin has been tested in bacterial mutation assays, in a forward mutation assay in Chinese hamster ovary cells, in a test for unscheduled DNA synthesis in primary rat hepatocytes, and in an *in vivo* cytogenetic assay in rat bone marrow. All gave negative results, and the Committee concluded that moxidectin had no genotoxic potential.

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 [Comments section of working paper, to be completed by the Temporary Adviser]

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 [Evaluation section of working paper, to be completed by the Member]

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The Committee concluded that the most relevant effects for the toxicological evaluation of moxidectin were those observed in the 90-day study in dogs, where the NOEL was 0.3 mg per kg of body weight per day. Based on this NOEL and using a safety factor of 200 to account for the uncertain sensitivity of the test systems used to assess the neurotoxicity of moxidectin (see section 2.2), the Committee established an ADI of 0-2 µg per kg of body weight for moxidectin.

The ADI was rounded to one significant figure, consistent with accepted rounding procedures (Annex 1, reference 91, section 2.7). This ADI provides an adequate margin of safety for the effects noted in the reproductive toxicity studies in rats.

Appendix F

Approximate relation of parts per million in the diet to mg/kg of body weight per day¹

Animal	Weight in kilo-grams	Grams food consumed per day (liquids omitted)	Type of diet	1 ppm in food is equivalent to, in mg/kg bw per day ²	1 mg/kg bw per day is equivalent to, in ppm of the diet ²
Mouse	0.02	3		0.150	7
Chick	0.40	50	Dry laboratory chow diets	0.125	8
Rat, young	0.10	10		0.100	10
Rat, older	0.40	20		0.050	20
Guinea pig	0.75	30		0.040	25
Rabbit	2.0	60		0.030	33
Dog	10.0	250		0.025	40
Cat	2	100	Moist, semi-solid diets	0.050	20
Monkey	5	250		0.050	20
Dog	10	750		0.075	13
Man	60	1500		0.025	40
Pig or sheep	60	2400	Relatively dry grain forage mixtures	0.040	25
Cow, maintenance	500	7500		0.015	65
Cow, fattening	500	15 000		0.030	33
Horse	500	10 000		0.020	50

¹ Lehman, A.J. (1954). **Association of Food and Drug Officials Quarterly Bulletin** 18: 66. The values in this table are average figures, derived from numerous sources.

² 1 ppm / 1 mg/kg feed.

Example: What is the value in mg/kg feed and mg/kg of body weight per day of 0.5% substance x mixed in the diet of a rat?

Solution

- I. 0.5% corresponds to 5000 mg/kg feed (see below)
- II. From the table, 1 mg/kg in the feed of a rat is equivalent to 0.050 mg/kg of body weight per day. Consequently, 5000 mg/kg diet is equivalent to 250 mg/kg of body weight per day (5000 X 0.050).

Unit relationships

$$\begin{aligned}
 1.1 \text{ g} &= 1000 \text{ mg or } 10^6 \text{ ug} \\
 0.1 \text{ g} &= 100 \text{ mg or } 10^5 \text{ ug} \\
 0.01 \text{ g} &= 10 \text{ mg or } 10^4 \text{ ug} \\
 0.001 \text{ g} &= 1 \text{ mg or } 10^3 \text{ ug}
 \end{aligned}$$

$$\begin{aligned}
 2. \quad 1 \text{ mg} &= 1000 \text{ } \mu\text{g or } 10^{-3} \text{ g} \\
 0.1 \text{ mg} &= 100 \text{ } \mu\text{g or } 10^{-4} \text{ g} \\
 0.01 \text{ mg} &= 10 \text{ } \mu\text{g or } 10^{-5} \text{ g} \\
 0.001 \text{ mg} &= 1 \text{ } \mu\text{g or } 10^{-6} \text{ g}
 \end{aligned}$$

$$\begin{aligned}
 3. \quad &1 \text{ mg/kg diet} = 0.0001\% \\
 &10 \text{ mg/kg diet} = 0.001\% \\
 &100 \text{ mg/kg diet} = 0.01\% \\
 &1000 \text{ mg/kg diet} = 0.1\% \\
 &10\,000 \text{ mg/kg diet} = 1\% \\
 &100\,000 \text{ mg/kg diet} = 10\% \\
 &1\,000\,000 \text{ mg/kg diet} = 100\%
 \end{aligned}$$

4. Units of weight (decimal system):

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kilogram (kg)	-----+	+-----	picogram (pg)
gram (g)	-----++	++-----	nanogram (ng)
milligram (mg)	-----++	++-----	microgram (ug)

Appendix G

Tabular summary of pivotal oral toxicity studies

Summary of pivotal oral toxicity studies with triclabendazole

Species (strain) No. of animals	Exposure duration (purity)	Dose levels	NOEL (mg/kg bw per day)	LOEL (mg/kg bw per day)	Critical effects	Refer-ence
Short-term toxicity						
Rat (Charles River CD) 20/sex/group	13 weeks (purity 97%)	0, 10, 100, or 1000 ppm in the diet, equal to 0.7, 6.6 or 69 mg/kg bw per day in males and 0.8, 7.9, or 87 mg/kg bw per day in females.	0.7	6.6	1000 ppm: decreased food and water intake, decreased body-weight gain; decreased red blood cell counts, haemoglobin, haematocrit, lymphocytes; increased serum alkaline phosphatase, cholesterol, albumin and total protein; reduced urine volume. 100 ppm: decreased body-weight gain.	Hunter et al., 1982
Dog (beagle) 6/sex/group	13 weeks (purity 97.6%)	0, 10, 100, or 1000 ppm in the diet, equal to 0.35, 3.4, or 37 mg/kg bw per day in males and 0.35, 3.5, or 39 mg/kg bw per day in females.	0.35	3.4	1000 ppm: body-weight depression, prolongation of QT interval and ATc value; decreased red blood cell counts, haemoglobin, and haematocrit values; increased serum alkaline phosphatase, GPT and cholesterol; increased liver weight and centrilobular hepatocellular pigment granules with basophilia, glycogen depletion and foci of pigmented macrophages; immature ovaries and testes; incomplete spermatogenesis and failure to reach oestrus. 100 ppm: increase in serum alkaline phosphatase.	Taupin, 1981
Long-term toxicity/carcinogenicity						
Mouse (Tif:MAGf) 80/sex/group	2 years (purity 99.5%)	0, 3, 15, 60 or 300 ppm in the diet, equal to 0.39, 1.4, 5.7, or 30 mg/kg bw per day in males and 0.27, 1.4, 5.4, or 29 mg/kg bw per day in females.	0.27	1.4	300 ppm: increased serum alkaline phosphatase, GPT, and GOT. Increases in absolute and relative liver weight. 15 and 60 ppm: increases in absolute and relative liver weight. Incidence of hepatomas increased in all treated females, but significance not reached at 99% level.	Basler et al., 1988a
Rat (Charles River CD) 60/sex/group	2 years (purity 99.5%)	0, 3, 15, 30, or 100 ppm in the diet, equal to 0.1, 0.6, 1.2 or 4.0 mg/kg bw per day in males and 0.2, 0.7, 1.5, or 5.2 mg/kg bw per day in females.	1.2	4.0	100 ppm: decreased body weight gain, decreased kidney weights in males at interim (1 year) but not at terminal sacrifice.	Charlley et al., 1986

Species (strain) No. of animals	Exposure duration (purity)	Dose levels	NOEL (mg/kg bw per day)	LOEL (mg/kg bw per day)	Critical effects	Refer-ence
Reproductive toxicity						
Rat (Tif:RAIF) 20/sex/group	62 days prior to mating until post partum day 35 of the 3rd generation pups. (purity 97.6%)	0, 3, 15, or 75 ppm, equal to 0.2, 1.1, or 5.5 mg/kg bw per day in males or 0.3, 1.4, or 7.4 mg/kg bw per day in females.	5.5	---	No treatment-related effects were noted.	Fritz et al., 1984
Teratogenicity						
Rat (Tif:RAIF) 25/group	Gestation days 6-15, killed on gestation day 21. (purity 97.6%)	0, 10, 30, or 100 mg/kg bw per day	30	100	100 mg/kg bw per day: decreased food consumption and body-weight gains, decreased live fetus weights. No external, visceral or skeletal malformations were noted.	Giese et al., 1981a; Giese, 1987
Rat (Sprague Dawley) 20/group	Gestation days 8-15, killed on gestation day 21. (purity 100%)	0, 10, 25, 50, 100, or 200 mg/kg bw per day	50	100	100 and 200 mg/kg bw per day: decreased maternal weight gain, reduced fetal body weights.	Yoshimura, 1987
Rabbit (Chinchilla) 20/group	Gestation days 6-18, killed on gestation day 28. (purity unknown)	0, 3, 10, or 20 mg/kg bw per day	3	10	20 mg/kg bw per day: unossified phalanges of fore and hind limbs, rare omphalocele in one fetus. 10 mg/kg bw per day: unossified phalanges of fore and hind limbs.	Giese et al., 1981b