

CONTAMINANTS

GUIDELINES FOR THE PREPARATION OF WORKING PAPERS FOR THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Geneva and Rome, January 2001

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PREFACE

This is the first edition of guidelines for the preparation of working papers on contaminants for the Joint FAO/WHO Expert Committee on Food Additives (JECFA). They are based on guidelines for the preparation of working papers on mycotoxins that were prepared before the fifty-sixth meeting of the Committee and they are intended primarily for WHO Temporary Advisers, FAO Consultants, and Members who prepare working papers for the Committee.

We envision that these guidelines will be modified based upon comments that we receive and experience gained in using them. These and other JECFA guidelines, including guidelines for the preparation of toxicological working papers on food additives, the working paper (monograph) format for flavouring agents, and guidelines for the preparation of working papers on intake, 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; herrmanj@who.int; fax: (+41 22)791 4848. Comments on these guidelines and suggestions for future editions are gladly accepted.

GUIDELINES FOR THE PREPARATION OF WORKING PAPERS ON CONTAMINANTS FOR THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. Introduction

These notes are designed to guide authors in the preparation of working papers for consideration at meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). These working papers summarize the data that are used to assess the safety of contaminants that may be present in food. They are designed to cover situations in which either a tolerable intake or a quantitative risk assessment is performed. The main content of the working paper will be broadly similar in each case, except for the way the evaluation is conducted. In the former case, the derivation of the tolerable intake will include an explanation of the reasons for selecting the pivotal study/basis for the evaluation and the magnitude of the safety factor applied to the toxicological data. In the conduct of a quantitative risk assessment, greater emphasis will be placed on the derivation of estimated toxic/carcinogenic potency in humans (including sensitive population groups where appropriate) and on the mathematical models used in the process. In this case, an additional section will be required explaining the basis for the estimated potency in humans and the quantitative risk assessment.

Working papers are published as monographs after meetings of the Expert Committee. To facilitate their editing and to avoid delays in their publication, the Secretariat would appreciate close adherence by authors to the style described in these guidelines.

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

Working papers should be submitted in **single spacing**. They should be provided in electronic format at the time of the meeting. If a Macintosh computer is used, the file should be converted to PC format. To facilitate editing, tables and figures should be submitted as separate electronic files, rather than being included with the text of the working paper.

The primary objective of a working paper is to summarize the relevant toxicological, epidemiological, and intake data used by JECFA to quantitatively assess the risk or evaluate the safety of the contaminant. Studies that are used for establishing a tolerable daily or weekly intake or for deriving a quantitative assessment of risk should be summarized in more detail than those that are peripheral to the safety evaluation/risk assessment, i.e. those of limited design or minor importance.

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

Even though data will be submitted by governments and/or trade associations, authors should search the literature for relevant data. The types of literature searches that were performed should be indicated.

2. Monograph structure and content

This section summarizes the types of information that are usually included in working papers and the way this information should be organized. The studies that are listed do not comprise a check-list 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 contaminant.

2.2 Explanation – If the substance has not been evaluated previously by the Expert Committee, it should be so stated, along with a brief description of the contaminant, its origins and occurrence. If it has been evaluated before, previous evaluations should be referenced by number using the standardized reference list of JECFA publications, which is included as Annex 1 in recent reports (*WHO Technical Report Series*) and evaluations (*WHO Food Additives Series*). Thus, the report of the forty-ninth meeting would be referenced as (Annex 1, reference 131) and a toxicological monograph prepared after the forty-ninth meeting would be referenced as (Annex 1, reference 132). Reference to previous Committees should be made by number (such as the forty-ninth 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 many years two meetings have been held, which would create confusion if meetings were referenced by year. Reasons for the present re-evaluation should be given (e.g. at the request of the Codex Committee on Food Additives and Contaminants) and, if a full monograph on a contaminant 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 reproduced in its entirety below.

2.3 Biological data – This section contains summarized descriptions of studies that are of importance in the safety evaluation or risk assessment of the contaminant. Studies that elucidate the mode of action, provide the basis for the safety evaluation, or are used to estimate toxic/carcinogenic potency in humans 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 conclusions of the author of the study should be summarized in this section. When the author of the working paper disagrees with the conclusions of the author of the study, he should discuss the controversial issues and present his own conclusions. If the conclusions are not straightforward, it is important that the person or group that arrived at the conclusion is identified. In this situation, the usual language would be “the authors concluded that...”. If the author of the working paper came to a different conclusion, then language such as “the present reviewer concluded that...” should be used. If the Committee agrees with the conclusion of the author of the study, the language will be changed after the meeting to “the Committee concluded that...”.

If studies on more than one animal species are summarized under one heading, then the studies should be grouped in such a way that studies in smaller rodent species are listed first, with larger species and species more closely related to humans last.

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

Biological data should be grouped under four headings, *Biochemical aspects*, *Toxicological studies*, *Observations in domestic animals/veterinary toxicology*, 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 sub-headings under **Toxicological studies** except for **Special studies**, the heading should be included in the working paper with the statement “No information available”.

2.3.1 Biochemical aspects – These studies include those designed to identify the pathways of metabolism and to measure the toxicokinetics of the contaminant and its metabolites, i.e. the concentration/time profiles of the substance and its metabolites in the various tissues and organs of the body. They also include studies of effects on enzymes and other biochemical parameters designed to elucidate the mode of action and toxicodynamics of the contaminant. The Committee uses the results of these studies in interpreting the toxicological studies and they facilitate the determination of the provisional tolerable weekly intake (PTWI) or provisional maximum tolerable daily intake (PMTDI) or the execution of a quantitative risk assessment. Comparison of the data between different experimental species and humans helps to determine the relevance of toxicity observed in animals, which is likely to result in a more rational assessment of the risk to humans; such comparisons should be summarized at the end of this section.

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

2.3.1.1 Absorption, distribution and excretion

- Hydrolysis/metabolism of the parent compound and its products in the mammalian gastrointestinal tract (including products of metabolism by the gut microflora) (distinguish between hydrolysis/metabolism before absorption and of biliary excretion products).
- The bioavailability of the unchanged compound and hydrolysis products/intestinal metabolites.
- 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

2.3.1.2 Biotransformation

- Metabolism of the parent compound, if absorbed as such, and of its products. The toxicological importance of the identified metabolites, whether accumulated or excreted, should be discussed if known or indicated. If the biochemical transformation pathway is known, a figure depicting the pathway should be included along with an identification of the species to which it applies.

2.3.1.3 Effects on enzymes and other biochemical parameters

- The effects of the absorbed contaminant and/or metabolite(s) on cellular and tissue enzyme expression, regulation (induction/repression), and post-transcription processing.
- Effects on hormonal regulation, interactions with cellular receptors.
- Effects on membrane/cytosolic biochemical composition or physicochemical state.

2.3.2 Toxicological studies – Summaries of toxicological studies normally comprise a large proportion of the working paper. These refer to studies conducted under controlled experimental conditions on well-characterized substances and specified doses/mode of administration. They should be distinguished from observations made in veterinary case studies or clinical practice (see section 2.3.3) where the circumstances of exposure are less well defined and may involve exposure to multiple contaminants. Five categories of experimental toxicological studies are normally considered routine: **acute toxicity, short-term toxicity, long-term toxicity/carcinogenicity, genotoxicity, and reproductive toxicity.** Sometimes these routine studies point toward the need for investigations of particular target organs or tissues; these 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 test should be given in terms of “mg of test substance/kg bw per day”. If the author of a study presents administration levels in terms of “mg/animal per day”, these values should be converted to mg/kg bw per day using animal weights if they are included in the report. With substances mixed in the diet, authors frequently express dosage levels in terms of ppm (parts per million) or percentage of the substance in the diet. When feeding levels are presented in this way, they should be expressed as “mg/kg of diet” in the toxicological summaries. However, for determining a NOEL or making a calculation of dose/response relationships, these values should be converted to “equivalent mg/kg bw per day” figures using the conversion factors provided in Appendix C (see section 2.7). Numbers should be reduced to two significant figures, i.e. 273 ? 270 or 0.273 ? 0.27.

2.3.2.1 Acute toxicity – Such studies, when properly designed and performed, provide useful information relating to target tissues and species or sex differences. When data are available on several species of both sexes, these may be useful for predicting whether toxicity is mediated via a hormonal mechanism or whether one species should be investigated more extensively than others. For highly, acutely toxic contaminants, such data may form the basis of an acute reference dose.

The results of acute toxicity studies that are expressed in terms of the LD₅₀ (lethal dose, median; oral, intramuscular, intraperitoneal, or dermal administration) and/or LC₅₀ (lethal concentration, median; inhalation) should be presented in tabular form as shown in Appendix D. Scatter or deviation should not be recorded. When 3 or more LD₅₀ or LC₅₀ determinations by the same route in the same species 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.

2.3.2.2. Short-term toxicity – The Committee defines short-term studies as those animal studies in which effects produced by the test material, when administered in repeated doses (or continuously in food or drinking-water) over a period of about 90 days, are investigated. However, in preparing working papers, toxicological studies in which substances are administered in regularly repeated doses over periods up to one year (two years for 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 studies are often performed to ensure proper dose selection in long-term studies, and they can point toward target tissues and organs. In some cases, short-term studies can help clarify lowest-effect levels for effects observed in long-term studies and they can provide retrospective information that is useful for the interpretation of long-term/carcinogenicity studies e.g. early signs of toxicity in the liver or kidney when tumours appear in these organs after long-term administration of the test substance.

In some cases, toxicological studies are reported on, for example, fungal biomass or naturally contaminated feed with determined levels of specified contaminant(s). Such studies should be reported separately as special studies (e.g. special studies on fungal biomass or special studies on contaminated corn) since they may be associated with intake of other contaminants than those specified, or other factors, and may not reliably be used for dose-response or relative potency evaluations.

The same general format and type of information as outlined under **Long-term/carcinogenicity studies** (section 2.3.2.3) should be followed when summarizing short-term studies.

2.3.2.3 Long-term toxicity/carcinogenicity – Toxicological studies in which substances are administered in regularly-repeated doses or continuously in food or drinking-water 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 short-term studies or which are progressive on longer administration. Long-term studies that are designed to investigate specific chronic effects, such as carcinogenicity, should be included in this section.

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

- Species, strain and number of animals 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 features of the study design considered to be noteworthy. When histopathological examinations were performed, the number of tissues that were examined should be indicated along with identification of tissues that were of particular significance to the evaluation.

- Toxicological findings and dose levels at which effects were observed. Results of relevant statistical analyses may be included here.
- No-observed-effect levels (NOELs) for specific toxic effects. Negative findings should be limited to general statements on survival, growth, organ weights, tumour incidence, organ function tests and gross and microscopic appearance of tissues. Statistical significance of a result should be given when the effect is also biologically significant.

2.3.2.4 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 substances. Results of these studies may also be considered when evaluating the results of rodent carcinogenicity bioassays. To present data in a more understandable form and to conserve space, the results of short-term genotoxicity tests should be tabulated. While tables provide information in a format that facilitates the review, it is recognised that further details of the protocols and results are sometimes necessary. Such information should be provided in footnotes or in separate paragraphs in the text. Appendix E provides an example of tabular representation of genotoxicity data.

2.3.2.5 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 behaviour, conception, parturition, lactation, weaning, and the growth and development of the offspring until the age of weaning. **Developmental toxicity studies** are used for assessing effects on the developing organism, which can include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) a functional deficiency.

2.3.2.6 Special studies – Special studies are those designed to test for specific effects such as neurotoxicity and effects on organ function (e.g. thyroid function) or particular systems (e.g. the immune system). Appendix A lists, in alphabetical order, most of the special studies that will be encountered. Placing such studies in alphabetical order facilitates locating them when searching through the monograph. Special studies on naturally contaminated feed components should also be included in this section. Special studies designed to elucidate qualitative interspecies differences in the manifestations of toxicity (e.g. different target organs in different species) should also be included in this section.

2.3.3 Observations in domestic animals/veterinary toxicology – Observations made in veterinary practice, such as veterinary case studies of mycotoxicoses or retrospective epidemiological studies of disease of domestic animals attributed to intoxication, may provide useful ancillary information in relation to qualitative interspecies differences in toxic effects and potency. However, because of the uncontrolled nature of exposure in such circumstances they would not usually provide quantitative data suitable for identifying a NOEL or for a quantitative risk assessment. Such studies should therefore be summarized separately. The summary should indicate the species, sex, and number of animals involved, the circumstances and duration of consumption of the contaminated feed, and the estimated dose or dietary concentration of the contaminant, where available. Brief details of the observed effects and other details important to the evaluation should also be provided. Similar cases may be summarized as a group.

2.3.4 Observations in humans – Results of observations in humans are useful for assessing the validity of animal studies and for confirming tolerable intakes or evaluation of risk. All studies dealing with humans except for those summarized under **Biochemical aspects** should be included in this section, including epidemiological surveys, anecdotal observations, health effects related to occupational or accidental exposure, and (in few cases) volunteer studies. Details such as sex, age, numbers of subjects, incidence and severity of observed effects etc. should be included as indicated in Appendix B.

2.4 Analytical methods – Analytical methods for reliable screening tests and quantitative determinations in the appropriate matrix or matrices, with appropriate performance characteristics, should be described in this section. Conclusions should be provided concerning the limit of detection and limit of quantification in relation to any specific requests that may have been made by the Codex Committee on Food Additives and Contaminants.

2.5 Sampling protocols – Assessments of various sampling protocols and their influence on the analytical results should be provided in this section.

2.6 Effects of food processing – Typical ways in which contaminated commodities are processed and information relating to the effects of processing on the level of the contaminant in the product that is consumed (reduction or concentration) should be provided. If the contaminant is degraded during processing, the products of degradation that may be present in food should be described.

2.7 Levels and patterns of contamination of food commodities – Surveillance data should be provided in tabular form as shown in Appendix F, which include the commodity, year and/or season during which the commodity was studied, the method of sampling, the number of samples, the limit of quantification (LOQ) and the number of samples that exceeded the LOQ, the mean, maximum, and 90th percentile levels, the number of samples that exceeded any hypothetical standards (maximum levels) that have been proposed by the Codex Committee on Food Additives and Contaminants, and the reference. Distribution curves and data on annual variation and contamination levels should be provided in the working paper.

2.8 Food consumption/dietary intake assessments – Dietary intake estimates for contaminants are an integral part of the risk assessment/safety evaluation process. Such estimates are based on surveillance data (which are assessed in view of analytical methods used in collecting the data and their performance characteristics, sampling protocols, and effects of processing) and on available information on food consumption. Information on biomarkers of exposure, e.g. levels in body fluids, may also be useful for assessing intake. When the contaminant causes acute toxicological effects, short-term intake should be estimated. Information on significant non-dietary exposure to the contaminant should be summarized and quantitated to the extent possible. Some of the data will have been submitted electronically using the GEMS/Food format, the instructions of which are available at www.who.int/fsf/.

These guidelines provide an abbreviated overview of the procedures used for estimating the intake of contaminants. The report of a Joint FAO/WHO Workshop on *methodology for exposure assessment of contaminants and toxins in food* that was held on 7-8 June 2000 (available at www.who.int/fsf/) provides more details relating to the major elements of an intake assessment of contaminants and toxins in food.

2.8.1 National assessments – These assessments are based on residue levels of the contaminant in food and estimates of food consumption in the corresponding country, so they are evaluations of *national assessments of intake*. Such data should permit estimation of both mean and ‘high consumer’ intakes. The residue data are evaluated as described in section 2.7. The assessments of intake should be tabulated, which include the country or region, population groups studied with their estimated levels of intake of the contaminant, and assumptions (such as the definition of ‘high consumer’) used. The type of food consumption survey used should be specified either in the text or the table.

2.8.2 Regional estimates – In order to obtain a global perspective and to permit regional comparisons of the potential intake of the contaminant, analyses should be performed using GEMS/Food regional diets and various assumptions about the concentrations of the contaminant in relevant foods as described in the next section.

2.8.3 Impact of alternative maximum levels on intake – Using the above data, the effects of alternative scenarios on intake can be evaluated by, for example, using the available data on occurrence and distribution of contamination, assuming application of alternative upper limits. Assumed concentrations in food may, for example, be maximum levels proposed by the Codex Committee on Food Additives and Contaminants, ‘typical’ concentrations of the contaminant in selected foods, or realistic ‘maximum’ concentrations in selected foods. Such estimates were performed for lead at the fifty-third meeting of JECFA, and the monograph may be consulted to see examples of the presentation of such data (WHO Food Additives Series No. 44). They can then be applied to assessing intake relative to determined tolerable daily or weekly intakes or for risk assessment purposes (see below).

2.9 Prevention and control – Pre- and post-harvest procedures for preventing or minimizing contamination of food, including good agricultural practices and good manufacturing practices, should be presented in this section. If effective procedures are available for decontaminating food and/or feed are available, they should be described and evaluated.

2.10 Dose-response analysis and estimation of carcinogenic/toxic risk – This section, in which the risk is characterized, replaces the usual **Comments** section. It includes narrative relating to the relevance and significance of the data that were summarized and the experimental/epidemiological basis for the conclusions reached by the author of the working paper. When it is concluded that the effect is a threshold phenomenon and a tolerable daily or weekly intake is proposed, relevant NOELs and the basis for them should be included. When it is concluded that a non-threshold mechanism of carcinogenicity/toxicity is operative, the relevant potency estimates should be included in this section. Under these conditions, the conclusions of the experimental and epidemiological studies should be outlined and the most significant information provided relating to the identification and characterization of the carcinogenic hazard, the most relevant experimental species and target tissues, the mode of action, evidence of human carcinogenicity and relative potency, and estimates of human exposure.

A quantitative risk assessment of a contaminant involves consideration of (a) choice of data, (b) measure of exposure, (c) measure of response, and (d) choice of mathematical model. These considerations are addressed in this section and the basis for the selection of data (e.g. only positive epidemiological data vs all epidemiological data; whether intake estimates used in risk assessment are based on dietary intake or biomarkers of exposure) should be provided.

When estimating potency, interspecies differences and the experimental bases for the estimates should be indicated. Estimates of potency in humans derived from epidemiological data should be included separately. When addressing published estimates of potency and risk assessments, the mathematical models used by the authors of the studies should be made explicit.

The general types of data required to resolve any outstanding safety issues or to render risk estimates more precise can be indicated, but there is no need to list the specific studies that are listed under **Further Work or Information** in this section.

2.11 Evaluation – The form of this section will depend on whether a tolerable daily or weekly intake is derived, or whether a quantitative risk assessment is made.

In the former case, this section consists of three headings, Level causing no toxicological effect, Estimate of tolerable intake for humans, and Further work or information.

1. Under the first heading NOELs should be listed by species in ascending order of size. NOELs should be given in terms of mg/kg bw per day or week. There are generally three ways of deriving NOELs in these terms:
 - (a) When the author(s) of the study has presented the data in these units (gavage studies) the NOEL simply should be given in terms of “x mg/kg bw per day or week.
 - (b) When the author(s) present the data in terms of mg/kg of diet (ppm) and food consumption data are available, then intake in terms of mg/kg bw per day or week can be calculated directly. In such situations, the NOEL should be presented as “x mg/kg of diet, equal to y mg/kg bw per day or week.
 - (c) When the author(s) present the data in terms of mg/kg of diet (ppm) and food consumption data are not available, the table in Appendix C should be used for calculating intake. In these situations the NOEL should be presented as “x mg/kg of diet, equivalent to y mg/kg bw per day or week.
2. An estimate of the tolerable daily or weekly intake is presented in terms of mg/kg bw. The author should consult *Principles for the safety assessment of food additives and contaminants in food* (WHO Environmental Health Criteria, No. 70; full text available online at <http://www.who.int/pcs/jecfa/jecfa.htm>) or *Principles for the assessment of risks to human health from exposure to chemicals* (WHO Environmental Health Criteria, No.210) for guidance on selection of safety factors in establishing PTWIs or PMTDIs.
3. Under the heading **Further work or information** should be indicated information that is required in order to complete an evaluation of a contaminant when only a tentative evaluation is possible because of limitations in the database. In such circumstances the PTWI or PMTDI would normally have been derived using larger safety factors to reflect the shortcomings of the database and the work stated as being required should complete the database required for a full evaluation. In some cases, better mechanistic or human data may indicate that a safety factor smaller than the conventional one may be appropriate and work to this end might be listed as “Desirable”

When the evaluation takes the form of a quantitative risk assessment, there will be two sub-headings: **Potencies** and **Population risks**.

The section on potencies should list the estimated toxic/carcinogenic potencies in experimental species and, where possible, in humans. The range of estimates with estimates of central tendency and upper and lower bounds for each species should be indicated graphically if possible with accompanying narrative as necessary (see the monograph on aflatoxins, *Safety Evaluation of certain food additives and contaminants*, WHO Food Additives Series No.40, pp 446-452 for an example (document is available from the Secretariat)). The section on population risks should contain an estimate of the intake and associated risk (derived where possible from human potency) in various scenarios covering the levels of contamination and dietary patterns in different regions. The effects of applying (reasonable) hypothetical maximum contamination standards to major contributors to dietary intake of the contaminant on the percentage rejection rate and residual risk should be estimated to provide guidance to risk managers on the consequences of alternative strategies.

An overall summary of conclusions may be useful at the end of this section.

2.12 References

2.12.1 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).

When 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).

2.12.2 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.

2.12.2.1 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:

Dean, I., Jackson, F. & Greenough, R.J. (1996) Chronic (1-year) oral toxicity of erythritol in dogs. *Regul. Toxicol. Pharmacol.*, **24**, S254-S260.

IARC (1983) IARC (International Agency for Research on Cancer) monographs on the evaluation of the carcinogenic risk of chemicals to humans: Miscellaneous pesticides, **30**, 329-344.

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

2.12.2.2 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.

Herken, H. (1961) [Pharmacological expertise on tolerance to natural and synthetic menthol.] Unpublished report from Pharmakologisches Institut der Freien Universität, Berlin. Submitted to WHO by Schering AG, Berlin, Germany (in German).

2.12.2.3 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.

2.12.2.4 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.

2.12.2.5 Agency reports

Example:

US EPA (1984) Mercury health effects update: health issue assessment. Washington, DC, US Environmental Protection Agency (EPA-600/18-84-019F).

2.12.3 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 assist the integration of the sections of the monographs prepared by different individuals, will facilitate the editing of those working papers that are subsequently published as toxicological monographs, and consequently their timely publication.

It is recognised that a different pattern may need to be adopted for contaminants for which a tolerable daily (weekly) intake can be established using a threshold model from those contaminants for which no threshold can be assumed (e.g. carcinogens with a genotoxic mode of action). In the former case, the format should be similar to that adopted at the fifty-third meeting of the Committee for zearalenone (WHO Food Additives Series No. 44); in the latter case, it should generally follow the pattern adopted for aflatoxins at the forty-ninth meeting (WHO Food Additives Series No. 40).

Please adhere to the hierarchy shown in this appendix. The headings are indented here to show clearly the hierarchy, but they should not be indented in the text. A table of contents of the working paper should be prepared with the headings indented as below. Bold facing should be used with all titles (not included here for easier reading). The listed animal species and special studies are meant to serve as examples and are not 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 D]
 - 2.2.2. Short-term toxicity
 - 2.2.3.
 - 2.2.3.1. Mice }
 - 2.2.3.2. Rats }
 - 2.2.3.3. Hamsters }
 - 2.2.3.4. Rabbits }
 - 2.2.3.5. Dogs }
 - 2.2.3.6. Pigs }
 - 2.2.3.7. Equidae }
 - 2.2.3.8. Primates }
 - 2.2.4. Long-term toxicity/carcinogenicity
 - 2.2.5. Genotoxicity [In tabular form with annotations; see Appendix E]

- 2.2.6. Reproductive toxicity
 - 2.2.6.1. Multigeneration reproductive toxicity
 - 2.2.6.2. Developmental toxicity
- 2.2.7. Special studies
 - 2.2.7.1. Covalent binding to nucleic acids and/or proteins
 - 2.2.7.2. Immunotoxicity
 - 2.2.7.3. Neurotoxicity
 - 2.2.7.4. Hormonal activity/effects
 - 2.2.7.5. Thyroid function
 - 2.2.7.6. Pancreatic function/glucose tolerance
 - 2.2.7.7. Metabolites
 - 2.2.7.8. Breakdown products [e.g. of “detoxification” processes]
 - 2.2.7.9. Related contaminants [where relevant]
- 2.3. Observations in domestic animals/veterinary toxicology [distinguish from experimental toxicological studies]
- 2.4. Observations in humans
 - 2.4.1. Biomarkers of exposure
 - 2.4.2. Biomarkers of effects
 - 2.4.3. Clinical observations
 - 2.4.4. Epidemiological studies
- 3. ANALYTICAL METHODS
 - 3.1. Chemistry
 - 3.2. Description of analytical methods
 - 3.2.1. Introduction
 - 3.2.2. Screening tests
 - 3.2.3. Quantitative methods
- 4. SAMPLING PROTOCOLS
- 5. EFFECTS OF PROCESSING
- 6. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES
 - 6.1. Surveillance data
 - 6.2. Distribution curves
 - 6.3. Data on annual variation in contaminant levels
- 7. FOOD CONSUMPTION/DIETARY INTAKE ESTIMATES
 - 7.1. Introduction and background [nature and occurrence in food]
 - 7.2. Methods [distinguish between short-term and chronic intake if both are of relevance, e.g. if it is considered necessary to derive an acute reference dose]
 - 7.3. Estimates of dietary intake
 - 7.3.1. National estimates
 - 7.3.1.1. Country A
 - 7.3.1.2. Country B, etc.
 - 7.3.2. Regional estimates
 - 7.3.3. Impact of alternative maximum levels on intake

8. PREVENTION AND CONTROL

- 8.1. Preharvest control
- 8.2. Postharvest control
- 8.3. Decontamination

9. DOSE RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/TOXIC RISK

- 9.1. Contribution of above data to assessment of risk
 - 9.1.1. Pivotal data from biochemical and toxicological studies
 - 9.1.2. Pivotal data from human clinical/epidemiological studies
 - 9.1.3. Biomarker studies
- 9.2. General modelling considerations
 - 9.2.1. Selection of data
 - 9.2.2. Measure of exposure
 - 9.2.3. Measure of response
 - 9.2.4. Selection of mathematical model
- 9.3. Potency estimates
 - 9.3.1. Potency estimates in humans based on epidemiological data
 - 9.3.2. Potency estimates in humans based on biomarkers
 - 9.3.3. Potency estimates in test species [and basis of extrapolation to humans where relevant e.g. comparative biochemical indices]

10. EVALUATION

- 10.1. Carcinogenic/toxic potency
- 10.2. Population risks [or level at which no appreciable risk is expected i.e. tolerable daily or weekly intake with the basis for it]
- 10.3. Conclusions; requirements for further information

11. REFERENCES

Appendix B

Toxicological summaries

The summaries of the experimental data are aimed at giving an overview of the essential elements of studies that are sometimes several hundred pages long. Studies that are used for establishing a tolerable daily or weekly intake or for a quantitative risk assessment should be summarized in more detail than those that are peripheral to the safety evaluation/risk assessment.

Examples of toxicological and epidemiological summaries may be found in previous toxicological monographs. Please contact the Secretariat to obtain copies of selected monographs if copies are required.

A good scientific summary of a toxicological study contains the following elements:

- (a) purpose or objective of the study
- (b) Identity, specification and purity of the substance administered (and, where naturally contaminated material was administered, co-contaminants)
- (c) Animal species and strain employed in the test
- (d) Number of animals in test and control groups
- (e) Sex
- (f) Dose levels (preferred units are mg contaminant/kg bw per day)
- (g) Route(s) of administration
- (h) Duration of treatment and/or experiment if they differ in length
- (i) Biological parameters examined
- (j) Effects observed (percent survival should be included in long-term studies).
- (k) Study author's conclusions and conclusions of the author of the working paper, if different (if the conclusions are not straightforward, identify who made the conclusions).
- (l) Reference

Summaries of epidemiological studies are intended to provide an overview of studies relevant to establishing the nature of the effect(s) that may be associated with exposure to the contaminant and the toxic/carcinogenic potency in humans. Studies that are able to provide quantitative relationships between exposure and incidence/severity of effects should be described in greatest detail. The summary should contain the following elements:

- (a) The nature, purpose or objective of the study
- (b) The location, number, sex, and age of the population(s) studied
- (c) The circumstances of human exposure (occupational, dietary, acute, sustained or episodic, etc.)
- (d) The magnitude of estimated intake (expressed on a body-weight basis where possible)
- (e) Whether the estimated exposure (intake) is supported by biomarkers of exposure
- (f) Duration of exposure and of observation/health records
- (g) Parameters examined
- (h) Possible confounders
- (i) Effects observed (incidence and severity)
- (j) Study authors' conclusions and conclusions of the reviewer if different from those of the author(s).
- (k) Reference

Appendix C

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

Animal	Weight in kilograms	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		0.15	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 bw 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 bw per day. Consequently, 5000 mg/kg diet is equivalent to 250 mg/kg bw per day (5000 X 0.050).

Unit relationships

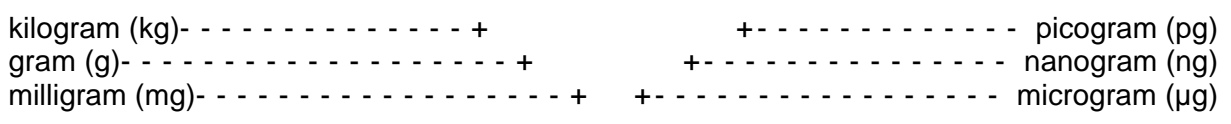
- 1. 1 g = 1000 mg or $10^6 \mu\text{g}$
- 0.1 g = 100 mg or $10^5 \mu\text{g}$
- 0.01 g = 10 mg or $10^4 \mu\text{g}$
- 0.001 g = 1 mg or $10^3 \mu\text{g}$

- 2. 1 mg = 1000 μg or 10^{-3} g
- 0.1 mg = 100 μg or 10^{-4} g
- 0.01 mg = 10 μg or 10^{-5} g
- 0.001 mg = 1 μg or 10^{-6} g

- 3. 1 mg/kg diet = 0.0001%
- 10 mg/kg diet = 0.001%
- 100 mg/kg diet = 0.01%
- 1000 mg/kg diet = 0.1%
- 10 000 mg/kg diet = 1%
- 100 000 mg/kg diet = 10%
- 1 000 000 mg/kg diet = 100%

4. Units of weight (decimal system):

1.001.001.001.001.001



Appendix D

Tabular representation of acute toxicity data

Table 1. Results of studies of the acute toxicity of zearalenone

Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference
Mouse	M/F	Oral	> 2 000	National Toxicology Program (1982)
Mouse	F	Oral	> 20 000	Hidy et al. (1977)
Mouse	F	Intraperitoneal	> 500	Hidy et al. (1977)
Rat	M/F	Oral	> 4 000	National Toxicology Program (1982)
Rat	M/F	Oral	> 10 000	Hidy et al. (1977)
Rat	M	Intraperitoneal	5 500	Hidy et al. (1977)
Guinea-pig	F	Oral	> 5 000	Hidy et al. (1977)
Guinea-pig	F	Intraperitoneal	2 500	Hidy et al. (1977)

M, male; F, female

Appendix E

Tabular representation of genotoxicity data

Table 2. Results of assays for genotoxicity with some low-molecular-mass isoparaffins

End-point	Test object	Chain length	Concentration	Results	Reference
In vitro					
Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	C11-13	7.7-77 000 µg/ plate	Negative	Xerox Corp. (1981)
Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	C10-11	7.5-75 000 µg/ plate	Negative	Xerox Corp. (1983)
Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	C10-13	≤10 000 µg/ plate	Negative	Phillips Petroleum Co. (1990)
Reverse mutation ^a	<i>E. coli</i> , WP2	C10-11	7.5-75 000 µg/ plate	Negative	Xerox Corp. (1981)
DNA damage	<i>E. coli</i> , Pol A ⁺ /A	C10-11	7.5-75 000 µg/ plate	Negative	Xerox Corp. (1981)
DNA damage	<i>E. coli</i> , Pol A ⁺ /A ⁻	C11-13	7.7-77 000 µg/ plate	Negative	Xerox Corp. (1981)
Cell mutation ^a	Mouse lymphoma L5178Y cells, Tk ^{+/-} locus	C10-13	≤1000 µg/ml	Negative	Phillips Petroleum Co. (1990)
Sister chromatid exchange ^a	Chinese hamster ovary cells	C10-13	≤50 µg/ml	Negative	Phillips Petroleum Co. (1990)
In vivo					
Micronucleus formation ^b	Mouse bone marrow	C10-11	19 g/kg bw, intaperitoneally	Negative	Xerox Corp. (1983)
Dominant lethal mutation	Sprague-Dawley rat	C10-11	0, 300, 900 mg/kg feed, 6h/day, 5 d, by inhalation	Negative	Exxon Corp. (1978)

^a In the presence and absence of Arochlor-induced rat liver microsomal fraction

^b Killed at 48 and 72

Appendix F

Tabular representation of levels and distribution of contamination of food commodities

Summary of ochratoxin A monitoring data in cereals

Commodity	Year/season	Ref. to sampling method	n	LOQ (µg/kg)	n<LOQ	Mean ^a /max (µg/kg)	90 th (µg/kg)	n>5 µg/kg ^b	n>20 µg/kg ^b	Ref.
Wheat	1998/spring	unknown	345	1.0	256	0.25/34	unknown	20	2	Ason (1999)
	1998/summer	unknown	125	1.0	50	0.90/38	unknown	18	5	Ason (1999)
Wheat flour	1995/unknown	Bson (1997)	45	3.0	44	<LOQ/7.5	6	1	0	Bson (1997)
Wheat bran	1995/unknown	Bson (1997)	30	5.0	28	1.0/15	3	2	0	Bson (1997)
	2000/spring	Eson (2000)	80	0.1	7	2.0/15	5	1	0	Eson (2000)

Ref., reference; n, number of samples; LOQ, limit of quantification; max, maximum level; 90th, 90th percentile

^aTrue mean: for n analytical values, the true mean is the sum Xi/n, where Xi is the value of each analytical result.

^b5 and 20 µg/kg are maximum levels that have been proposed by the Codex Committee on Food Additives and Contaminants.