



Food and Agriculture Organization
of the United Nations



World Health
Organization

**JOINT FAO/WHO EXPERT
COMMITTEE ON FOOD ADDITIVES
(JECFA)**

**PROCEDURES FOR RECOMMENDING
MAXIMUM RESIDUE LIMITS-
RESIDUES OF VETERINARY DRUGS IN FOOD
(1987-1999)**

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

and

WORLD HEALTH ORGANIZATION

Rome 2000

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1. INTRODUCTION

In response to a recommendation of the fifteenth session of the Codex Alimentarius Commission, a Joint FAO/WHO Expert Consultation was held in Rome in November 1984, to consider issues pertaining to the presence of veterinary drug residues in food resulting from their use in animal husbandry and veterinary medicine. Among the recommendations of that consultation was that the Codex Alimentarius Commission establish a Codex Committee on Residues of Veterinary Drugs in Foods and that the Directors-General of FAO and WHO give consideration to convening an appropriate scientific body, from time to time as necessary, to advise Member governments and the Codex Committee on issues of public health hazards and barriers to international trade as a consequence of residues of veterinary drugs in foods of animal origin. The Directors-General have subsequently convened regular meetings (32nd, 34th, 36th, 38th, 40th, 42nd, 43rd, 45th, 47th, 48th, 50th and 52nd) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifically to address residues of veterinary drugs in food of animal origin. JECFA had evaluated some veterinary drugs at its 12th, 25th, 26th and 27th meetings as part of their assessments of other food additives.

The FAO/WHO/GATT Conference on Food Standards, Chemicals in Food and Food Trade (Rome, 1991) and the 21st Session of the Codex Alimentarius Commission (Rome, 1995) emphasized the importance of transparency in the food safety evaluations performed by JECFA, as an independent, scientific, advisory body to the parent organizations and their member governments. The evaluation procedures developed by JECFA have evolved during the independent meetings referenced above, in part, from the need to have guiding principles so that its evaluations may withstand critical review by scientific peers, and to address specific scientific issues raised by compounds on the agendas. The WHO Technical Report Series publications document the procedures from these Meetings under the heading "General Considerations", however, some of the evaluation procedures have been noted within the evaluation reports of specific compounds.

This manuscript is to document the procedures developed by JECFA for the evaluation of residues of veterinary drugs in food. It includes in many instances, the historical development leading to the current procedures. In consolidating the pertinent evaluation procedures, it is intended to provide guidance to present and future members of JECFA and to provide transparency on how the food safety assessments performed by JECFA for residues of veterinary drugs in food are conducted. While informative for establishing ADIs and recommending MRLs, it is not intended to be a proscriptive document on how Member Governments might develop their national regulations for residues of veterinary drugs in food.

2. TERMS OF REFERENCE

The JECFA meetings responsible for the safety assessment of veterinary drugs in foods have been charged consistently with advising and providing guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on four broad tasks:

- (a) To establish and further elaborate principles for evaluating the safety of residues of veterinary drugs in foods and for determining acceptable and safe levels of such residues when the drugs are administered to food producing animals in accordance with good practice in the use of veterinary drugs;
- (b) To determine criteria for appropriate methods of analysis for detecting or quantitating residues of veterinary drugs in foods;
- (c) To evaluate or re-evaluate the safety of residues of certain veterinary drugs;
- (d) To discuss and provide advice on matters of interest arising from the reports of the Sessions of the Codex Committee on Residues of Veterinary Drugs in Foods.

3. RISK ASSESSMENT PRINCIPLES

Principles of risk assessment have long been a component of public health organization activities. The application of risk assessment principles has experienced a substantial growth and refinement particularly with the expert bodies that have advised the Codex Alimentarius Commission during the last ten years. The expert bodies that advise the Commission, such as JECFA, provide an explicit and critical link between those that conduct the necessary scientific research and those responsible for risk management (including the Codex Committees). The twentieth session of the Commission endorsed the position that expert bodies, such as JECFA in its assessment of residues of veterinary drugs in food, need to develop their risk assessment criteria and explicitly characterize the uncertainties inherent in their safety evaluations such that an interactive framework can be developed for all policy (and risk management) decision making activities on residues of veterinary drugs in food.

The Committee affirms that the description and application of these principles facilitates the risk management process, makes the process more transparent, and provides a critical framework for peer review by the broader scientific community and national governments.

The FAO/WHO Joint Expert Committee on Food Additives meetings for veterinary drug residues has been developing and narrating its risk assessment principles since the first JECFA meeting in 1987 devoted explicitly to veterinary drugs and has further elaborated its principles, as necessary, based on the compounds on its agenda and the specific scientific issues raised by individual compounds. Further, the Committee has applied all the principles noted below in determining ADI and MRL for compounds on its agenda at different times and summarized the principles of its assessments in its individual reports. The significant risk assessment procedures developed by the Committee include safety factors applied to no-observed-effect-levels (NOEL) in allocating an ADI and conservative procedures for recommending MRLs. Examples include utilizing food factors and intake data to determine theoretical maximum residue exposures, a decision-tree approach to determine the basis of specific MRLs considering the toxicology and availability of practical analytical methods. In addition, procedures to evaluate the contribution and significance of bound residues and their bioavailability for recommending MRLs, requirements for toxicology and residue data for compounds with a long history of use, the suitability and application of microbiological safety, evaluating issues of pharmacologically active residues at injection sites have been developed. Most recently, issues related to aquaculture and to the assessment of compounds used either as veterinary drugs or pesticides have been considered. It is important to emphasize that while having specific documented scientific principles to guide assessments, each compound has been assessed on its own merits considering the availability of data provided or that which is available in the public scientific literature.

4. TOXICOLOGY EVALUATION

A. Data Requirements

A significant portion of general toxicological data requirements have been established for food additives and contaminants (1), and many are equally applicable to veterinary drug residues. Initial descriptions of toxicological data evaluation requirements on veterinary drug products were elaborated at the 26th and 27th meetings of the Committee (3,4) although these were not exclusively devoted to residues of veterinary drugs in food. A generalized statement on toxicological data described the need for adequate, relevant and comprehensive data about the kinds and levels of residues. The 26th report (3) affirmed that the toxicological data for xenobiotic anabolic agents should be relevant to the potential tumorigenic activity of these compounds and as well as the endocrinology or toxicology consequences in humans. The 32nd meeting of the Committee noted that the extent of the toxicological data needed depended in part, for example, on the likely dietary exposure of humans to the compound of interest and whether the compound and/or its metabolites are normally present in human tissues (5). This is particularly applicable, for example, with the

endogenous hormones and the recombinant bovine growth hormones. The 32nd meeting also noted that when drugs used for veterinary purposes are also used therapeutically in humans, information from human case reports and epidemiological studies may be applicable, particularly when no animal model exists to measure endpoints using *in vitro* or *in vivo* model systems. The Committee made particular note that data from human case reports may provide critical evidence of possible adverse reactions in humans that are not detectable in model systems. The 32nd Committee also reaffirmed that the availability of toxicological data in humans may influence the amount of toxicological data required from animal studies.

Regarding toxicological data needs, the 42nd report (10) explicitly indicated the need for detailed reports, including individual animal data, on short-term and long-term carcinogenicity, reproduction and developmental studies in experimental animals and genotoxicity studies. The Committee requested, as necessary, special studies to investigate specific effects such as those on mechanisms of toxicity (e.g., the neurotoxicity phenomena of the CF-1 mouse with avermectins), no-hormonal-effect levels, immune responses or macromolecular binding (e.g., bound residue considerations with the benzimidazoles and trenbolone acetate). For compounds with antimicrobial activity, the Committee has often requested studies designed to evaluate the potential for adverse effects on the microbiological ecology of the human intestinal tract (i.e., microbial risk). In some instances, the Committee has identified the need for relevant data on the use of, and exposure to the drug in humans, including studies on effects after occupational exposure and epidemiological data following clinical use in humans (e.g., isometamidium and ocular treatment with chloramphenicol).

The Committee uses toxicological assessments from other expert committees as appropriate. For example, the 50th meeting of the Committee reviewed the neurotoxicity of avermectins and milbemyicins (18) because of prior evaluations of two anthelmintics of the same structural class. Because of wide spread use of these substances, the Committee was concerned about a sub-population of CF-1 mice being highly sensitive to the neurotoxic effects of avermectins due to a deficiency of P-glycoprotein, a component of the plasma membrane that controls the rate of passage of xenobiotics across membranes. New data on abamectin were reviewed by the 1997 Joint FAO/WHO Joint Meeting on Pesticide residues (19). That meeting concluded the increased sensitivity to homozygous CF-1 mice and postnatal rats was due to reduced expression of the protein in these species and the CF-1 mouse was not appropriate in establishing an ADI for avermectins. On this basis, the 50th Committee concluded that the additional safety factor used at the 45th meeting was no longer necessary for avermectins and milbemyicins not tested in CF-1 mice (18).

The 48th meeting of the Committee commented on data generation issues (14). The report notes that the 48th and subsequent meetings will specify whether studies submitted in dossiers have been performed

according to present-day standards as exemplified by adherence to Good Laboratory Practices (GLP). As a matter of interest, the guidelines or protocols used to generate data are cited in the monographs that are published in the *WHO Food Additives Series* and the *FAO Food and Nutrition Paper* series. The report makes clear that GLP codes were not always used in the design and conduct of older studies, however, many studies followed protocols that are adequate for assessing the safety of substances under consideration. As a cautionary note, the 48th Committee report makes it clear that while standards for study protocol and conduct are valuable for ensuring the proper conduct of studies, they do not necessarily ensure scientific quality or the relevance of the design to resolving the scientific issue being addressed.

B. Microbiological Risk

Microbiological risk issues have been addressed in several Committee reports (5, 7, 8, 10, 12, 13, 18 and 20) and the reports of the Codex Committee on Residues of Veterinary Drugs in Food. The Committee does not consider risk that pertains to the potential health effects associated with ingestion of food of animal origin containing resistant bacteria selected under the pressure of antimicrobial therapy, because this is outside the terms of reference.

The Committee has consistently expressed awareness to those veterinary drugs where the antimicrobial properties become the determining factor in the safety evaluation because the toxicity of some antimicrobial drugs is so low (e.g., spiramycin) that their residues in food, from a toxicological perspective, could be tolerated at therapeutically effective tissue concentrations (7). Of particular concern has been the issue of whether or not residues of these substances ingested as food residues pose a danger to human health by exerting a selective pressure on the intestinal flora, resulting in a microbial environment that favors the growth of microorganisms with natural or acquired resistance. In evaluating the effect of residues of antimicrobial drugs on the human gut flora, the Committee noted that the characteristics of the human gut flora should be taken into account. The 36th Committee report (7) expressed its view, based on the available data, that plasmid-mediated resistance was unlikely to develop. It further noted that studies estimate that more than 90 percent of the 10^{11} microbes per gram of feces are anaerobic bacteria and that the flora is stable indicating that the bacterial ecology generates important "barrier effects" which tend to prevent intrusion by foreign microbes.

Given these factors, the 36th Committee noted that for evaluating the effects of residues of antimicrobial drugs on the human gut flora, preferred data included (1) information on the identification of bacteria that constitute

human gut flora; (2) data pertaining to bacteria that are representative of the whole flora; and (3) data obtained from *in vivo* experiments that take barrier effects into account. The Committee recognized that, at that time, human epidemiological studies were not readily able to provide adequate information in this area, given the variation in resistant bacterial flora due to human drug therapy and other relevant factors. Nonetheless, published data have demonstrated that experimentation in human volunteers is an appropriate methodology. The 36th Committee indicated that when human data are not available, data from animal model experiments may be considered. As a third tier, in the absence of *in vivo* data from human or animal model studies, *in vitro* data including information on minimum inhibitory concentration established under standard conditions and incorporating factors such as the impact on gut pH and anaerobiosis (the resorption and degradation of antimicrobial drugs in the gut), and volume of the ingested bolus of food containing the antimicrobial drug residues may be used. The Committee concluded that when only *in vitro* data on the Minimum Inhibitory Concentration are available, these MICs should be used only on a temporary basis for safety evaluations.

The 38th meeting of the Committee (8, see Annex 5) commented extensively on microbiological risk and further considered procedures to use for evaluating the potential effects on the human intestinal flora. Three procedures were described that could be used to establish an ADI:

1. Doses of the antimicrobial substance that are without effect on the intestinal flora of human volunteers, together with appropriate safety factors, may be directly used to calculate an ADI.
2. In the absence of human data, no-effect doses obtained from specifically designed studies using human intestinal flora, such as studies with holoxenic rodents, combined with higher safety factors may serve as a basis for the calculation of an ADI. The validity of the particular animal model used must be evaluated in each case,
3. In the absence of data from *in vivo* studies, and where justified, results from *in vitro* experiments using relevant human gut microflora to identify MICs may also be used to establish a temporary ADI and/or MRL. Data from *in vivo* studies are required for the establishment of a full ADI.

The 38th Committee assumed that for given combinations of microorganisms and antimicrobial substances, pressure to select resistant mutants would only be appreciable when concentrations of the antimicrobial substance in the medium were greater than or equal to the minimum inhibitory dose for a sufficiently long time, and that the pressure would likely be highest in the distal portion of the digestive tract. On this basis the Committee suggested two items of information as necessary in any MIC-based assessment

of microbiological risk.

1. An estimate of the concentration without microbiological effect on the relevant microorganisms colonizing the distal part of the human intestine.
2. An estimate of the fraction of the ingested amount of the antimicrobial substance available to the bacteria in that part of the intestine.

The Committee agreed that the selection of the standard MIC concentration of residues without microbiological effect would not necessarily be an appropriate estimate because it may not take into consideration the pH, bacterial counts or other relevant factors determining the conditions of growth. MIC values also need to be corrected to cover the complete range of MICs from individual microbial species. To transform a MIC in the intestine into a no-effect dose, three factors need to be considered: a) the fraction of the orally administered antimicrobial active substance that retains its microbiological activity after passage through the digestive tract (considered bioavailable to the microorganisms in the distal part of the intestine, b) the estimated mass of the daily fecal bolus, and c) the safety factor to be applied to cover the variability of the above conditions within the human population. The upper limit of the temporary ADI may be calculated using formula 1 with this data.

$$\text{Temporary ADI} = \frac{\text{Concentration without effect on human gut flora } (\mu\text{g/ml}) \times \text{Daily fecal bolus (g)}}{\text{Fraction of oral dose bioavailable} \times \text{Safety factor} \times \text{Weight of human (60kg)}} \quad (1)$$

The Committee considered that a conservative safety factor should be applied with the above model, and that it should only be used to establish a temporary ADI because the procedure employs estimates of the intestinal flora no-effect value containing too many uncertainties. A cautionary note indicated that a change in status to a full ADI would require a change in safety factor, and use of a different model based on a directly determined no-effect level for microbiological effects. This reaffirmed the basic principle noted by the 36th Committee. The 38th meeting of the Committee used this approach extensively in its food safety assessments.

Notwithstanding the evolution of the models for assessing human food safety noted above, the 42nd meeting of the Committee acknowledged that antimicrobial activity may be an appropriate end-point for

establishing the ADI (10). They noted that evidence of microbiological risk associated with exposure to very low concentrations of antimicrobial drugs present as residues in food is minimal and that other methods for examining microbiological end-points were being developed and may be of assistance to JECFA. The Committee therefore, modified its earlier conservative decision and stated that they should remain flexible in their approach to establishing an ADI for residues of antimicrobial drugs in food. The Committee concluded that until there is more general agreement on the appropriateness of the new and different models, the Committee may accept extensive *in vitro* studies on antimicrobial activity on human intestinal bacteria as an alternative to more direct evidence that ingestion of residues will not have an adverse effect on human intestinal ecology. The Committee stated that the resulting ADI may be either temporary or final, depending on the quantity and quality of the available data, a qualified shift in posture from the previous Committees.

The 45th meeting of the Committee reviewed further the appropriateness of the calculation (formula 1, above) then being used by JECFA. They acknowledged the high potential of increasing the development of acquired resistance in pathogenic enteric bacteria, while noting that there is an insufficient understanding about whether or how the intestinal microflora is adversely effected by exposure to low concentrations of veterinary antibiotic residues in food. A variety of *in vitro* and *in vivo* methods for studying the effects of antibiotic residues on the intestinal microflora were summarized in the Committee report (12). The Committee did not draw conclusion to the appropriate determination and, perhaps, application of an upper limit of antimicrobial exposure for determining an ADI. The Committee did affirm that model systems to assess these residues should be validated based on studies intended to evaluate their relevance to human exposure.

A cautionary note was made by the 45th Committee on the difficulty it had encountered in evaluating data submitted using formula 1. For this reason, the 45th Committee agreed to develop a modification of the above described procedure for comment and further consideration at the 47th meeting for determining an upper limit of the ADI based on a microbiological endpoint.

The 47th Committee provided extensive comments on the assessment of the effects of antimicrobial drug residues in food on the human intestinal microflora (13, see also Annex 3). The comments were based on a draft document initially considered at the 45th meeting. The Committee reaffirmed its view that the possible adverse effects of residues of antimicrobial agents on human gastrointestinal microflora should always be investigated. They noted that more research is needed to evaluate the potential health risks associated with the consumption of foods containing low levels of residues of antimicrobial agents since such

substances occur naturally in food of animal and plant origin.

In addition, the 47th Committee stated that it would normally require all available data on the microbiological activity of a substance and an evaluation report relating such data to possible effects on the human intestinal microflora. These investigations may be based on the results of studies using *in vivo* or *in vitro* models and/or other relevant data. The Committee recognized that the nature of the data may differ, depending on the class of drug and the extent it may have been tested or used previously in humans and animals. The Committee reaffirmed that useful types of information include, but are not be limited to, (1) the stability and bioavailability of the drug in the gastrointestinal tract; (2) its spectrum of activity against different species of the gastrointestinal microflora; (3) its effect on population changes in the intestinal microflora; (4) its influence on the barrier to colonization *in vitro* and *in vivo*; and (5) its potential to cause gastrointestinal disturbances in animals and humans.

The 47th Committee recognized, as had earlier Committees, that there were shortcomings to the current methods used to evaluate the antimicrobial drug residue effects on the human intestinal microflora as well as insufficient validation of the test models. Therefore, the Committee recommended that in the absence of data from studies in humans, sponsors should submit data on the safety evaluation of antimicrobial drugs for use in animals including:

1. Results of studies on *in vivo* models such as germ-free rodents colonized with human intestinal microflora (human flora-associated [HFA] rodents), or *in vitro* models such as MIC data or continuous culture systems. The Committee noted that HFA rodent studies have been used in several laboratories as an *in vivo* model for determining the effects of therapeutic doses of antimicrobials and may have moderate to high relevance in determining the effects of low dose (e.g., residue) levels, the model needs to be validated.
2. When MIC data from *in vitro* studies are used for assessing the safety of antimicrobial agents it is important to recognize the limitations of the microbiological assay. The Committee reaffirmed the importance of ensuring that the microorganisms chosen for evaluation are representative of the human intestinal microflora and that the MIC determinations are made under conditions that mimic those in the gastrointestinal tract.
3. The Committee encouraged the development of better *in vitro* and *in vivo* methods that are relevant to determine the effects of low concentrations of antimicrobial agents on the human gastrointestinal microflora.

The 47th Committee commented that it would consider developing an alternative procedure, if the data are sufficient, based on the provision that residues of antimicrobial substances do not exceed a threshold such as an average amount of 1.5 mg per day, corresponding to 1 mg/kg in the diet, equivalent to 25 µg/kg body weight per day.

The 47th Committee further commented that while the model developed by the 38th meeting of the Committee was attractive in its simplicity, it had not been validated. In addition, when using the equation, issues had arisen regarding the values that should be applied. Considering these factors, the Committee reviewed MIC factors (e.g., *in vitro* activity of antimicrobials against bacterial pathogens, inoculum density, etc.), daily fecal bolus, fraction of oral dose bioavailability, human body weight and safety factors. Accordingly, the 47th Committee modified the equation as noted in formula 2:

$$\text{ADI} = \frac{\text{Mean MIC}_{50} (\mu\text{g/g}) \times \text{Mass of Colonic Content (g)}}{\text{Fraction of bioavailable dose} \times \text{Safety Factor} \times \text{BW(kg)}} \quad (2)$$

(µg/kg body weight)

The significant changes are use of mean values for the MIC and use of 220 g versus 150 g for the daily mass of colonic content. The fraction of bioavailable dose remains the same (0-1) as does the safety factor (1-10) and the adult body weight (60 kg). Though not reflected directly in the formula 2, to determine an upper limit of antimicrobial exposure (µg/kg of body weight) the modified formula considers the daily food consumption in grams per day. Referenced papers (13, Annex 3) in the 47th Committee report were used for selecting a 220 g colonic mass in place of the original estimated fecal bolus (which was considered too low for the colonic volume of a 60 kg person).

The 47th Committee reaffirmed its view that the possible adverse effects of antimicrobial drug residues on gastrointestinal microflora should always be investigated and that research is needed to evaluate potential health risks associated with consumption of food with concentrations of antimicrobial residues. The 47th Committee cautioned that microbiological safety should not normally be limited to one simple test system or the use of MIC data (only) for deriving an ADI.

The 52nd Committee proposed a more systematic and comprehensive tiered decision-tree system that permits use of all relevant data from *in vitro* and *in vivo* model test systems in addition to minimum inhibitory concentration (MIC) assays (20). The procedure is described at the end of this report (see Item 15). The

Committee concluded that using the new procedure would not require additional microbiological data if there is evidence for at least one of the following:

1. Residues in milk and edible tissue do not have antimicrobial properties.
2. Ingested residues do not enter the colon.
3. Ingested residues are transformed to inactive metabolites before entering the lower bowel or bound quantitatively to colon contents soon after entry to the lower bowel.
4. A literature survey on residues of the veterinary drug provides a basis to establish an ADI sufficient to protect the intestinal microflora.
5. Human clinical data from therapeutic use indicates the incidence of toxicological effects are substantially higher than gastrointestinal side-effects on disruption of the microflora.

If necessary, additional types of microbiological studies recommended for establishing an ADI, are:

1. Consideration of the drug class to discern whether the main concern is emergence of resistance or disruption of the flora.
2. For colonization barrier effect concerns, MIC data against 100 bacterial strains comprising 10 isolates of suitable organisms to represent the most sensitive, relevant genera.
3. For colonization barrier disruption concerns, drug residue concentration studies to indicate no disruption of the colonization barrier. Studies in a monogastric animal model are recommended
4. For antimicrobial emergence concerns, in vitro or in vivo data to show no change of antibiotic resistance of resident populations.
5. Lack of evidence of enzymatic activity concerns specifically linked to an adverse consequence in humans.

C. Allergenic Potential

Allergenic potential occurring from residues of veterinary drugs in foods was addressed by the 36th Committee (7). The Committee recognized that several drugs used in human and animal medicines have produced allergenic reactions when used in humans but that there were very little data published concerning the possible role of residues of veterinary drugs in food with allergenic reactions in humans. The Committee recognized that generally, initial sensitization in an individual occurred following administration of a relatively large dose of a drug with allergenic potential after which a much smaller dose could elicit a response. These true allergenic responses are immunologically mediated and should be distinguished from

drug intolerance, due to pharmacological or toxicological properties of the drug, and drug idiosyncrasy caused by factors such as enzyme deficiency in an individual that leads to an unusual adverse response. Exposures to the very small amounts of any drug residue with allergenic potential likely to occur after exposure to those residues in foods of animal origin were believed to be unlikely to lead to sensitization in humans. The Committee also concluded that hypersensitivity reactions due to the ingestion of drug residues with allergenic potential in food of animal origin were unlikely to be of major health significance. The Committee noted, however, that anaphylactic reactions have been infrequently reported from penicillin residues in foods of animal origin. Thus, the Committee recognized that reactions could occur in highly sensitized individuals and therefore recommended that residues of drug with known or suspected allergenic potential (e.g., β -lactams) be kept as low as possible. This conservative approach has influenced decisions on penicillins as well as ceftiofur, for example. For determining a causal relationship between residues of veterinary drugs and allergenic reactions in humans, four criteria were established. They are:

1. Residues of the veterinary drug are present in the food causing the allergic reaction;
2. An individual was not allergic to the same food without the veterinary drug residues;
3. An immunological mechanism could be demonstrated for the response; and
4. The origin of the drug residue in food was likely to have been from its use in animals.

Considering the allergic potential as an end point in its safety assessment, the Committee did not apply its normal ADI procedures in evaluating benzylpenicillin (7). The 36th meeting of the Committee concluded that allergy was the determining factor in the safety evaluation of benzylpenicillin. Having insufficient adequate data available to establish a no effect level, the Committee recommended that the daily intake from food should be kept as low as possible (below 30 μg per person of parent drug). The Committee concluded that the quantity was the important factor and that the risk associated with the occurrence of mild hypersensitivity reaction at this level was considered to be insignificant. The Committee may have to consider other veterinary drugs with allergenic potential. As noted previously, the Committee would conduct its food safety assessment based on the best available scientific data.

D. Pharmacological Effects

The importance of pharmacologic activity in the Committee's safety assessment of residues of veterinary drugs in food was first addressed briefly by the 32nd Committee (5). It noted that the Committee sought information on the pharmacological activity of the drug and that it would be desirable to have

information on its mechanism of action. The 38th Committee (8) recognized that a desired pharmacological effect in animals might well be an undesirable effect for consumers, particularly if they were very sensitive to such an effect (e.g., tranquilizers, β -adrenoceptor agonists, vasodialators, etc). Consequently, the Committee noted that these major pharmacological properties should be regarded as adverse effects and assessed accordingly with other toxicological effects of the veterinary drug. The Committee further noted that if there are no toxicological data of overriding importance or if the pharmacological effects are the most relevant and sensitive, the ADI should be determined on the basis of the pharmacology of the substance. The No Effect Level (NOEL) derived from the results of the most relevant means of administration (e.g., the oral route) and the most sensitive pharmacological test should be chosen in establishing the ADI. Data from human use would be the most preferable if available. In this situation, a lower safety factor could then be considered in establishing an ADI (the Committee suggested a safety factor of 10 as a possibility). The Committee, however, acknowledged that a case-by-case approach was the most prudent and appropriate for evaluation of pharmacologically active compounds because of the nature and variety of the effects and their relevance to the overall toxicological assessment.

Of particular concern and relevance are residues of drugs used as animal medication or as aids to animal production and the frequency (those used widely and for potentially lengthy time frames) with which consumers might be exposed to them. A related concern to the Committee was the situation where a drug's presence in food could result in a pharmacological effect in the consumer in the absence of conventional toxicological effects. This was envisioned as an intrusion into the body's homeostasis, against which consumers should reasonably be expected to be protected. Several compounds evaluated at the 38th Committee meeting were in this category of veterinary drugs. Of particular concern were those tranquilizers and β -adrenoceptor-blocking agents used in pigs during transportation and immediately prior to presentation for inspection (carazolol and azaperone, for example, were of particular concern). The Committee concluded that the continued use in animals of any such substance capable of leaving residues likely to be ingested by humans in food is permissible only when that use can be shown to be acceptably safe. Acceptable safety for the consumer would require the demonstration not only that the residues of such substances are toxicologically acceptable, but also that no pharmacological effect is produced from potential consumption of residues from animal food injection sites. The Committee noted that for those substances allocated a zero withdrawal period by some national authorities, it is not likely that these criteria would be met. The Committee strongly supported that immediate pre-slaughter use should not be allowed unless specific studies show that the substance is without unacceptable toxicological or pharmacological risk for consumers. Concern was expressed that consideration should be noted both for the concentrations in animal tissues and

more particularly for the unabsorbed residue of the administered dose (e.g., an injection site). For this reason the 38th Committee requested specific studies on carazolol and azaperone to define a pharmacological no-effect level in humans.

The 40th meeting of the Committee reaffirmed the seriousness of concerns regarding pharmacological effects. They noted that the risk from residues of drugs with a pharmacologic effect that might still be present in injection sites at slaughter will be an important risk consideration in JECFA evaluations (9). Significantly, this Committee recognized that such residues could arise as the result from either the recommended or the improper use of a veterinary drug. In its evaluations, the Committee stressed that they will only consider the correct use of the product in accordance with the manufacturers' recommendations (e.g., recommended dose, route of administration, species use and duration of treatment) in their safety assessments.

E. Endogenous Substances

Although the 32nd report of the Committee was the first devoted exclusively to residues of veterinary drugs in food, and the three endogenous hormones were evaluated at that meeting, portions of the 26th and 27th meetings briefly addressed the xenobiotic anabolic hormones (3,4). However, those Committee reports do not contain any guiding principles for toxicological assessment and establishment of ADI.

The 32nd Committee report acknowledged that residues from veterinary use may occur in tissues from treated animals resulting from their application as therapeutic (e.g., reproduction control) or prophylactic (e.g., growth promotion) agents in veterinary practice. The Committee concluded that residues resulting from their use as growth promoting agents should be considered separately from residues after their use for other purposes because the administration of the drug and recommended withdrawal periods may be different (5). The 32nd Committee assessments, therefore, were limited to use as growth promoting agents in bovine animals. The Committee noted that there were insufficient data on use of these substances as implants in veal calves to determine whether or not residues were within the normal ranges for untreated animals.

These endogenous substances occur naturally in humans and as such, exert hormonal effects in humans. The Committee evaluated the potential for toxicological and pharmacological effects in humans, if any, resulting from consumption of residues from treated bovine animals. The Committee did note that several of the hormonally active substances under consideration were used in combination (one with another) and where substances having similar physiological activities were combined, evidence that their hormonal effects were additive, rather than synergistic, should be provided. The Committee agreed that data on the

residues of each of the substances that are used in a combination product should be available for evaluation, whether or not their physiological activities were similar. For toxicological evaluation, studies regarding carcinogenicity, embryotoxicity and mutagenicity are necessary as well as the routes of administration (e.g., oral and parenteral).

The 52nd Committee reevaluated the three natural growth promoting hormones – estradiol, progesterone and testosterone (20). The Committee reviewed the available toxicology and, in particular for estradiol, the extensive literature on estrogen replacement therapy on post-menopausal women in its risk assessments. The Committee concluded that there were sufficient data to enable the Committee to establish ADIs for the three substances. Extensive statistical analysis of all available residue data was used to estimate the theoretical maximum additional exposure that might result from treatment of food animals in accord with the good practices in the use of veterinary drugs. Considering the ADIs and the wide margin of safety for the theoretical consumption of the residues in food, the Committee concluded that MRLs for estradiol, progesterone and testosterone were “not specified” in cattle tissues. The Committee recommended, however, that the total intake of estrogenic residues resulting from the use of any approved hormonal product be kept below the theoretical calculated excess intakes.

5. RESIDUE EVALUATION

A. Data Requirements

The 26th and 27th reports of the Committee contained only generalized statements regarding data required for residue evaluation (3,4). They suggested that comprehensive data about the kinds and levels of residues should be provided when the substances are used according to good animal husbandry practice. Evidence is required on the efficacy of the substances, the amounts used to produce the effect, the residue amounts in animal tissues based on field trials and information about methods of analysis of residues that could be used for control purposes. The 32nd meeting of the Committee (5) concurred and stated that the criteria listed below will usually provide adequate information on residues to allow the setting of acceptable withdrawal periods for the veterinary drug (the Committee was silent with regard to 1) residue data for the purposes of recommending MRL, and 2) use of dose studies greater than the recommended dose):

1. The mode of administration, dose, and formulation of the drug should be the same as proposed for its intended use(s) in food producing animals;
2. The animal groups should be large enough to allow meaningful statistical assessment of the data;

3. After animals have been treated with the drug, tissues and biological fluids should be collected at appropriate times for residue analysis so that a recommended withdrawal period can be set.

With regard to metabolism and pharmacokinetics, the 32nd Committee report noted that before the safety of residues can be assessed, appropriate metabolic studies in the food producing animal are generally required to identify and quantify the residues (5). The studies should simulate the conditions of use of the veterinary drug in animal husbandry as closely as practical. The pharmacokinetics of the veterinary drug should be examined between the time of administration of the drug and the time the animals enter the human food supply. Metabolic studies may also be required in the animal species used for toxicological investigations, in order to ensure that these laboratory animals are exposed to the same array of compounds as are those who consume residues from treated animals.

It was not until the 42nd meeting of the Committee that explicit requirements for residue data were presented as well as some data requirements for toxicological evaluation (10). The Committee requested detailed reports, including individual animal data, on the following types of studies:

1. The chemical identity and properties of the drug;
2. The use and (recommended) doses;
3. Pharmacokinetic, metabolic and pharmacodynamic studies in experimental and food producing animals, and in humans, where available;
4. Residue depletion studies with radiolabelled drug in target animals from zero withdrawal time to periods extending beyond the recommended withdrawal time. These studies should provide information on total residues, including free and bound residues, and major residue components to permit selection of a marker residue and target tissue;
5. Residue depletion studies with unlabeled drug for the analysis of marker residue in target animals and in eggs, milk and honey. These should include studies with appropriate formulations, route of application, and species, at up to the maximum recommended dose;
6. A review of routine analytical methods that may be used by regulatory authorities for the detection of residues in target tissue;
7. A description of the analytical procedures used by the sponsor for the detection and determination of parent drug residues. The sponsor is also required to describe a method that may be used by regulatory authorities for the specific determination of the marker residue with a sensitivity equal to or less than the MRL (ideally, ≤ 0.5 MRL); and

8. In addition, studies designed to assess the impact of residues of antimicrobial agents on food processing may be required.

The 42nd Committee concluded that the list may not be exhaustive and that other studies may, in some instances, be of assistance in the evaluation. Possible instances would include MIC residue data for those substances where an ADI may be established and an MRL recommended based on an antimicrobial end point, bound residue and bioavailability studies.

B. Bound Residues

Bound residues were addressed by the 34th and 36th meeting of the Committee (6,7). It was recognized by the Committee that the use of veterinary drugs in food producing animals can result in residues that are neither extractable from tissue nor readily characterized. The generation of bound residues may occur through a variety of means, including conjugation of the parent drug or a metabolic product with endogenous proteins, enzymes, etc. In addition, extensive hydrogen bonding to similar endogenous material, or extensive metabolism of the parent drug to reactive, low molecular weight components that either bind to endogenous materials or are incorporated synthetically into endogenous materials may occur. The 34th meeting defined the terms used and outlined its approach to the evaluation of such compounds (6), while details were more explicitly described in the 36th report (7).

The 34th JECFA recommended that in the absence of other data, a bound residue should be considered of no greater toxicological concern than the compound for which the ADI was set. When the total residue in an animal-derived food does not exceed the recommended MRL, the bound residue should not be examined further. If the total residues exceeded the recommended MRL, the Committee recommended that the best information available should be used to evaluate the contribution of the bound residue to the toxicity of the veterinary drug. The information requested includes, but is not limited to data on the chemical structure, bioavailability, metabolism and toxicological activity of the bound residues. At the 34th meeting, the Committee developed a procedure to estimate the maximum daily intake of residues of a drug that has a bound residue component. It takes into account the toxicological potency and bioavailability of the residues.

For purposes of describing the approach noted below, the 34th Committee defined “total residues” of a drug in animal-derived food as consisting of parent drug, all metabolites and drug-based products that

remain in the food after the administration of the drug to food producing animals. The amount of total residues is generally determined by using radiolabelled drug studies and is expressed as the parent drug equivalents in mg/kg of the food. Residues may be further characterized as either extractable or non-extractable, based on accepted residue extraction procedures that ensure that the compounds of interest are not destroyed (6). Typically, the non-extractable residues are calculated by subtracting the amount of extractable residues from the total residues. The 34th Committee explicitly noted that all non-extractable residues might be of toxicological concern. Specifically, (1) drug residues that are incorporated through normal metabolic pathways into endogenous compounds (e.g., amino acids, proteins, nucleic acid) are of no toxicological concern, whereas (2) chemically bound residues derived by the interaction of parent drug residues or its metabolites with macromolecules, may be of toxicological concern. The information listed below is required to estimate the maximum daily intake of residues:

1. The concentration of total residues, extractable residues and bioavailable residues in muscle, liver, kidney, fat, milk and eggs at a known treatment or withdrawal time;
2. The chemical identity of the residues;
3. The biological/toxicological potency of the residues of major metabolites;
4. The ADI for the compound.

For data analysis, the withdrawal times should be chosen so as to include both the time when the residue concentration is at a maximum and the time when the estimated maximum daily intake of residues falls and remains below the ADI for a 60 kg person. The amount of residues of toxicological concern is calculated in the extractable and the bound fraction using the diet consumption factors described in Part 7.c. The concentrations are expressed as parent drug equivalents.

$$\text{Residues} = \text{Free residues} + \text{Bioavailable bound residues}$$

$$\text{Bound residue} = \text{Total residue} - (\text{extractable fraction} + \text{endogenous fraction})$$

$$\text{Residues} = P_0 + \sum_{n=1}^{n_x} (M_n \times A_n) + (\text{Bound residue} \times \text{fraction bioavailable} \times A_b) \quad (3)$$

where

P_0 = amount of parent drug per kg of tissue

$n_1..n_x$ = different metabolites of the parent drug

M_n = amount of (unbound) parent drug metabolite n per kg of tissue

A_n = toxicological potency of n relative to that of parent drug

A_b = estimated relative toxicological potency of the metabolites in the bound

residue (when no information is available, use $A_b = 1$)

Where the endogenous fraction (residues incorporated through normal metabolic pathways into endogenous compounds) is not known, it should be considered equal to zero. The bioavailable fraction is estimated from bioavailability data. If no data are available, the fraction should be considered equal to 1.

The 36th Committee described an approach for determining the amount of releasable (recoverable) residues from animal tissue (7). In considering the safety evaluation of bound residues, if they make up an insignificant portion of the total residue, the Committee agreed that a suitable extractable residue may usually be selected as a marker compound and used for recommending an MRL. Where bound residues become a significant portion of the total residues of toxicological significance, then the procedure prescribed by the Committee ought to be used to assess their safety.

The procedure proposed by the Committee involves, as a first step, a mild extraction procedure commonly used for residue analysis and determining the amounts (or percentage) of extractable and non-extractable residues. As noted above, if the amount of non-extractable residues in tissues is insignificant, then a marker compound needs to be identified for recommending tissue MRL from the extractable residues. For situations where there are significant non-extractable residues, the tissue samples should be independently subjected to *in vivo* and *in vitro* digestion and extraction procedures. The residues that are releasable (recovered) from *in vitro* procedures should be identified and quantitated (for identifying a marker compound) prior to assessing their toxicological potential. Similarly, those residues determined to be bioavailable through *in vivo* techniques should be identified prior to subjecting them to a toxicological assessment. The *in vitro* procedures used for quantitating and characterizing the amounts of residues is a more vigorous procedure than the mild extractions noted above. Examples include incubation of the bound residue with acid or appropriate enzyme preparations that mimic the human digestive system. However, the Committee noted that the procedures used should not destroy the compounds of interest. The Committee suggested the Gallo-Torres procedure (15) as one acceptable to evaluate the extent to which the bound residues are absorbed when the food containing the bound residue is ingested by test animals (bioavailability). With the exception of this *in vivo* procedure, the Committee has not attempted to define or describe specific studies that might be used to assist in the safety assessment of bound residues. The Committee noted that any procedure used by drug sponsors needs to be clearly justified. The use of bound residue data for the purpose of safety assessment will be evaluated on a case-by-case basis by the Committee.

C. Injection Site Residues

Drug residues in edible tissues resulting from their parenteral administration or as implants have been reviewed at the 38th, 42nd and 45th meeting of the Committee (8,10,12). Consistently, the Committee has affirmed that it does not include residues that persist at or near the injection site in assessing their contribution to drug residues in edible tissues to the total daily intake. It has, however, expressed serious concern about the possible large amounts of residues at injection sites that may be in edible tissues and exhibit a physiological response in humans. The 38th Committee considered four circumstances of use by parenteral administration or implantation, noting that the first one must always be considered in Committee food safety assessment (8). The Committee declared that if it is to assess the safety implications of residues at the injection site, it requires information regarding drug dose, formulation and time elapsed since injection. The four considerations are:

1. Use of an allowed drug in accordance with approved specifications.
2. Use of an allowed drug in a manner not in accordance with approved specifications resulting from:
 - a. administration at an unapproved site
 - b. administration to an unapproved species
 - c. administration for an unapproved indication
3. Improper use of an allowed drug by failure to observe the prescribed withdrawal time.
4. Use of a prohibited substance.

The second and third situations noted above are of concern only to recognize that the Committee wishes to draw attention to the additional residue burden for consumers that might result from these uses as an allowed drug. The fourth circumstance is regarded as the responsibility of member countries through their national residue control programmes. The Committee did not address the situation or conditions where a greater than recommended dose may be warranted. Examples noted for uses that may cause high amounts of residues at injection sites include:

1. Administration of a drug immediately before slaughter (e.g., pigs given a tranquilizing drug immediately before transport, where by the full pharmacological effect may be exerted right up to the moment of slaughter, and having no possible withdrawal period).
2. Treatment of animals intended for human consumption before the appropriate withdrawal times are observed.

3. Implantation or injection of long-acting drugs with slow rates of absorption (e.g., sustained release anabolic agents).

The 38th Committee also noted that in order to prevent improper practices in the use of veterinary drugs, the Committee may recommend specific restrictions on the use of a drug in particular situations. Thus, the Committee drew attention that it considered some of the issues noted above to be a general principle that the Committee may act upon while certain matters (e.g., the use of a prohibited substance) may be outside the scope of the Expert Committee. Providing advice to the Codex Committee on Residues of Veterinary Drugs in Food based on the information available to the Expert Committee that is relevant to public health is considered to be within the scope of the Expert Committee.

The Committee acknowledged that there is difficulty in ensuring that the injection site is excised and discarded at slaughter because the site may not be readily recognizable. The site, its dimensions, and the efficiency of its removal are subject to considerable variation, and thus, the Committee noted that a relatively large amount of tissue would have to be discarded. No additional recommendations or further action was taken by the 38th Committee.

The 42nd meeting of the Committee reaffirmed comments provided by the 38th JECFA and commented briefly on the particular concern of veterinary drugs formulated as long-acting injectables (dexamethasone esters were a specific concern at that meeting). These formulated veterinary drugs, on hydrolysis, can result in persistent and relatively high concentrations of residues at the injection site. For this reason the Committee advised that it would be helpful to future Committees to have additional pharmacokinetic (and residue) information on those veterinary drugs that may be slowly hydrolyzed to parent drug.

The 45th meeting further addressed residues at the injection site, recalling that previous meetings of the Committee had addressed specific concerns about pharmacologically active residues at the injection site (e.g., carazolol). The Committee also noted that several of the drugs evaluated by the 45th Committee had injection site residues at concentrations that were comparable to, and may even exceed the recommended MRL, at practical withholding times. For this reason, the Committee recommended a sampling procedure currently utilized by both the European Community's Committee on Veterinary Medical Products (CVMP) and the U.S. Food and Drug Administration (FDA). The intention of the Committee was to standardize sampling of injection sites for results provided for review in sponsor generated dossiers. The

recommendation calls for the permanent marking of the injection site while estimating that the injection site is at the center. At sampling, the recommendation is to collect 500 g of tissue including the injection site and that this should take the general shape of a cylinder with approximate dimensions as follows:

10 cm in diameter and 6 cm in depth for intramuscular injections

15 cm in diameter and 2.5 cm in depth for subcutaneous injections

When collecting tissue, care should be taken to ensure that the needle track is included in the sample where possible. If the site of injection includes edible skin, the skin should be included in the sample in normal proportions that may be consumed. These data may be used by future Committees to assess consumption of these residues in comparison to the ADI.

The 48th Committee reviewed a recent report on residues of veterinary drugs at injection sites (14). Several recommendations were deemed applicable to the Committee's safety assessment of injection site residues. The recommendations commented on:

1. The need for a standard-setting process to be established for residues of veterinary drugs that considers the public health significance of injection site residues;
2. The need for harmonization of standard-setting procedures for such residues;
3. The recognition that the likelihood of human exposure to residues that exceed the MRL at injection sites is extremely small; and
4. The standard-setting process for veterinary drugs administered by intramuscular injection should be based on the safety evaluation of the possibility of acute adverse effects arising from ingestion of injection-site tissue from animals treated with a single dose of a given drug.

The most important recommendation in that report for the Committee was that the injection-site tissue should be excluded from normal muscle when MRLs are recommended when the particular residues have not been shown to cause adverse health effects in animals treated with a single dose of the drug. This latter point reflects the approach the different Committees have used in consideration of injection site residues. The Committee noted that these recommendations were addressed also in the 38th and 42nd meetings of the Committee. The 45th meeting the Committee standardized sampling procedures at the injection site to minimize the variability of information obtained for review by the Committee (see above).

The 52nd Expert Committee was asked for additional advice by the Codex Committee on Residues of Veterinary Drugs in Food regarding the safety of residues that may be present at the injection site. Specifically the Committee was asked to consider establishing an acute reference dose (acute RfD, see glossary of terms, Annex 1) in such cases. The 52nd Committee reaffirmed that the primary objective in performing a safety assessment is to assure that the average daily of consumption of residues over a lifetime does not exceed the ADI and that consumption on a single day does not exceed the acute RfD (20). The Committee acknowledged, however, that while safety is usually assessed on long-term exposure, drug residues at the injection site may pose an acute hazard and require an acute RfD. In addition, the Committee expressed the view that under some circumstances identification and removal of injection sites after slaughter may not be a practical means of ensuring that consumers are not exposed to residues that exceed the acute RfD. The Committee concluded that in these circumstances a withdrawal period should be specified sufficient for residues at the injection site to deplete below the acute RfD.

6. ACCEPTABLE DAILY INTAKE (ADI)

A. End Point Assessment

A significant contribution to the principles applied in evaluating a substance for the purposes of establishing an ADI was published in the *Principles for the Safety Assessment of Food Additives and Contaminants in Food* (1). The 32nd JECFA meeting elaborated many of these principles as a framework for the specific assessment of residues of veterinary drugs in food (5). Most importantly, where possible and appropriate, an ADI based on determination of a No Observed Effect Level (NOEL) from animal or human toxicological data will be used as the end point of the safety evaluation with use of an appropriate safety factor. The 32nd Committee recognized that in some instances it might be inappropriate to establish an ADI (e.g. the naturally occurring growth promoting hormones). When it has been determined that establishing an ADI is unnecessary because of a large margin of safety, the recommendation of an MRL is also unnecessary.

As noted above, the 32nd Committee determined that an ADI was unnecessary for the naturally occurring growth promoting hormones. At the 40th meeting, an ADI “not specified” was established for the bovine somatotropins (9). The Committee noted the lack of oral activity of the recombinant somatotropins and the Insulin-like Growth Factor-1 (IGF-1) as well as the low amounts and non-toxic nature of the residues of these compounds even at exaggerated doses. The Committee concluded that these results provide an extremely large margin of safety for humans consuming dairy products from animals treated with the recombinant somatotropins and, therefore, warranted the establishment of “ADI not specified”. While

explaining its decision, the Committee did not comment on whether this terminology superseded “ADI unnecessary”.

The Committee has noted that an ADI for a drug is usually based on the toxicity of the parent drug rather than on its metabolite(s). However, it may sometimes be necessary to calculate an ADI for individual metabolites. Although most compounds have been evaluated as individual substances, there are instances where an ADI has been established as a group ADI (e.g., streptomycin/dihydrostreptomycin, and enrofloxacin/ciprofloxacin) and where an ADI has been established on a microbiological endpoint rather than a toxicological endpoint (spiramycin and spectinomycin are examples). The 38th meeting of the Committee (8) noted that if there are not toxicological data of overriding importance or if the pharmacological effects are the most relevant and sensitive, the ADI should be established on the basis of pharmacology. The acute toxicity of some veterinary tranquilizers are examples of this principle.

Where ADIs for veterinary drugs have been established, the numerical value is almost always expressed as a range extending from zero to an upper limit. This indicates that where MRLs are recommended, efforts should be made to reduce consumer exposure to residues as far as possible below the upper limit of the ADI. There have been a limited number of situations where an ADI numerical value or range was not identified. For example, with carbadox at the 36th meeting of the Committee (7), no ADI was established for toxicological reasons due of the genotoxic and carcinogenic nature of carbadox. However, on the basis of other scientific data (e.g., metabolism) the Committee concluded that residues of carbadox in pigs were acceptable provided the recommended MRLs were not exceeded (e.g., limited acceptance of residues). For allergenic considerations, the Committee did not establish an ADI for benzylpenicillin as there was insufficient data to establish a NOEL (7). The Committee recommended that the daily intake from food should be kept as low as possible (below 0.03 mg per person per day). When establishing the numerical expression of an ADI, the 36th Committee also agreed to express the ADI to only one significant figure. If the ADI is calculated from a NOEL that has more than one significant figure, the number is rounded to one significant figure, consistent with acceptable rounding procedures (closantel, for example).

The 38th meeting of the Committee (8) also addressed the issue of establishing an ADI for pro-drugs -- those synthetic substances that are rapidly converted to the biologically active drug when administered to the target animal or host. The Committee recognized that there may be occasions when these drug metabolites are devoid of the specific biological activity possessed by the parent drug that may be of toxicological concern, yet have unique toxicological factors associated with these rapidly generated

substances. In these situations, the parent drug activity would be discounted in establishing the ADI (upon which the MRL is recommended); the ADI would be established on a toxicological property of the metabolites with an appropriate safety factor applied (e.g., febantel).

The 40th Committee noted that certain conditions apply regarding the identity and quality of veterinary products subject to Committee review (9). The Committee evaluations depend on studies performed with a chemical substance or product of defined identity, purity, and physical form. In particular, the ADI is valid only for products that do not differ significantly in identity and quality from the material used to generate the data used for the Committee's evaluation.

B. Safety Factors

The 38th meeting of the Committee affirmed that in calculating the ADI, the Committee has usually followed the procedures described in *Principles for the Safety Assessment of Food Additives and Contaminants in Food* (1), applying a safety factor to the NOEL derived from the most relevant and appropriate toxicological, microbiological or pharmacological end-point study.

As there has been some inquiry about the safety factors used in establishing some previous ADIs, the 38th meeting of the Committee (8) explained its approach more comprehensively. The safety factor usually chosen is 100 in the situation where a NOEL is derived from a long-term animal study on the assumption that humans are ten-times as sensitive as the test animal(s) used in such studies and that a ten-fold range of sensitivity within the human population may exist. When no adverse health effects are seen in long-term studies, a safety factor of 100 may be applied to the NOEL derived from short-term studies where higher dose levels have been used and an effect has been noted. Typically, acceptable short-term studies need to be at least 3-month studies. The Committee noted, however, that depending on the quantity, quality and nature of the available data, a safety factor of 100 might be insufficient. This may occur when the required data are incomplete, when the end-point study upon which the NOEL is established is inadequate (e.g., insufficient numbers of animals per test group or when no individual animal data are reported), or when irreversible effects such as teratogenicity or carcinogenicity are noted. The Committee may, and on limited occasions has employed higher safety factors (e.g., 200, 500, 1000) depending on the quality and quantity of relevant data. The Committee noted that safety factors are usually not appropriate for genotoxic carcinogens. When the only noteworthy toxicological effects are observed in human studies, a lower safety factor (e.g., ten) may be applied. The Committee stressed that the safety factor applied with each drug would be assessed on its own

merits considering all the above factors.

Different factors are taken into account when considering the establishment of an ADI based on a microbiological basis (e.g., microbial inhibition concentration (MIC) or adverse effects on the human gut microflora). In these cases the safety factor is used in an entirely different way than when applied to an ADI based on toxicological data. When establishing a microbiological based ADI (see above, formulas 1 and 2) the safety factor is used to account for uncertainty about the amount and relevance of the MIC data available for review. For example, where inhibitory effects are reported on only a limited number of microorganisms, a safety factor of greater than 1 may be used. Safety factors considered appropriate for microbiological endpoints are 1-10 considering the quantity and quality of the data.

C. Temporary Acceptable Daily Intake (ADI)

Several meetings of the Committee on residues of veterinary drugs in food have had substances with limited toxicological data available upon which to establish an ADI. The 36th Committee (7) noted that when the Committee, in its scientific judgement, is confident that the consumption of residues of the veterinary drug is without toxicological hazard over a limited amount of time (e.g., the amount of time required to generate and evaluate further data for toxicological assessment), but not sufficiently confident that consumption of these residues over a lifetime may pose a public health concern, it may establish a temporary ADI. In applying this approach, the Committee considers whether that data might be made available to the Committee within a relatively short period of time. As will be noted later, temporary MRLs may be recommended for similar or additional reasons, such as the availability of reliable residue methods or additional information on the nature of the quantification of residues.

Where the Committee has established temporary ADIs, it specifies what information is required to resolve the data needs and sets a date when the data are requested for re-evaluation by the Committee. The same approach is applied with MRLs. At the reassessment, if one is done, the Committee has the option to (a) establish a full ADI; (b) extend the temporary ADI; or (c) not extend the temporary ADI (the ADI is withdrawn). The same options are available with temporary MRLs. The 36th Committee established a temporary ADI for levamisole and temporary MRLs, and requested additional toxicological and residue data for re-evaluation by the Committee. Based on the additional data provided, the 42nd Committee established an ADI, however, it withdrew the temporary ADI on levamisole in milk as no additional data were made available. Similarly, the Committee withdrew the MRL in eggs, because of high amounts of residues (10).

7. MAXIMUM RESIDUE LIMITS (MRLs)

A. End Point Assessment

General principles on recommendations for maximum residue limits were addressed at the 32nd meeting of the Committee (5). Where the amount of residues determined from administration of the veterinary drug according to good animal (or veterinary) practice are below (less than) those permitted within the ADI, then the amount of residues determined under the above conditions will be used as the upper limit to recommend the Maximum Residue Limit (Acceptable Residue Level was used in the 32nd report). The Committee recommendation is on the proviso that practical analytical methods are available for routine residue analysis. However, the Committee noted that if the residues found under existing conditions of use exceed (are greater than) those determined to be acceptable based upon the ADI, then the drug formulation, route of administration, extension of withdrawal times, or other comparable approaches may be necessary to reduce concentrations of residues in edible tissues. When the Committee has determined that an ADI is unnecessary because the compound of interest is produced endogenously in humans and animals or for other valid toxicological considerations, then the Committee recommendation of an MRL is also deemed unnecessary. When an ADI is not allocated because the safety of the compound on toxicological considerations cannot be assured, then an MRL should not be recommended. Application of the concept of limited acceptance of residues in the absence of an ADI has occurred only rarely (e.g., carbadox and benzylpenicillin).

To promote clarity in identifying all appropriate species and tissues to which a MRL applies, the 38th meeting of the Committee (8) decided that it would be reasonable to apply the following guidelines when recommending an MRL for residues of a veterinary drug:

1. All animal species would be named individually;
2. Target tissues (muscle, liver, kidney or fat) or food products obtained from treated animals (milk and eggs) would be identified (honey and fish were not specified by the 38th Committee);
3. The general term "edible tissues" would be used when the MRL referred to all edible tissues of the named animal species; and
4. Marker residues would be identified where appropriate. Where no marker residue is named, it is assumed that the MRL is recommended on the basis of the parent drug.

The 38th Committee also decided that at least two target tissues (i.e., edible tissues) would be identified whenever possible, one being muscle or fat and the other, liver or kidney. The rationale was that selection of an appropriate target tissue permits regulation of the MRL in international trade of animal foods (e.g., muscle or fat tissue) as well as in national residue control programs (e.g., liver or kidney). Identification of the target tissue is ultimately determined by the properties of the residues of concern.

In recommending a MRL in a specific species, the Committee did not wish to preclude the occasional and limited prescribed therapeutic use of drugs in unnamed species that should not give rise to residues in food of animal origin. For any widespread or recommended new uses in food animals, submissions containing sufficient information about metabolism and residues would be required to establish additional MRLs.

B. Decision Rule Principles

Arising from comments from the Codex Committee on Residues of Veterinary Drugs in Food on how JECFA recommended some MRLs (e.g., zeranol), the 36th meeting elaborated the principles upon which it made its decisions (7). Several factors are taken into account by the Committee when recommending MRLs. Among them are the results of toxicological and radiolabel residue studies, available residue data based upon its use according to good practice in the use of veterinary drugs and the bioavailability of bound residues, for example. Other factors include the identification of target tissue(s), the existence of a residue marker for determining compliance with safe residue limits, withdrawal periods for adequate residue depletion, and practical analytical methods for residue analysis. Other factors may be appropriate depending on the specific nature of the animal drug.

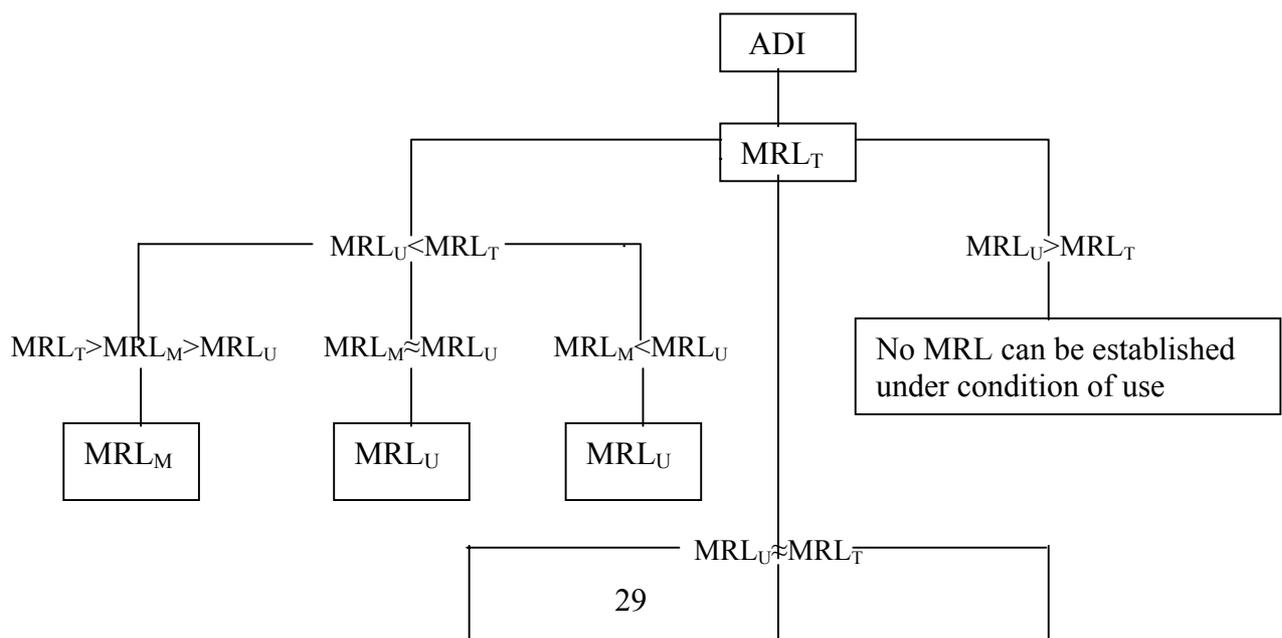
The primary consideration in recommending MRLs is the establishment of an ADI (based upon either available toxicological data or microbiological data). The Committee will not recommend MRLs so that the theoretical maximum daily intake of residues significantly exceeds the ADI. It is important to recognize that the consumption of residues, based upon the ADI and the theoretical food factors, is the upper limit consideration in recommending MRLs by the Committee. The recommendation of MRLs is not derived directly from the ADI. They are recommended in accord with the ADI.

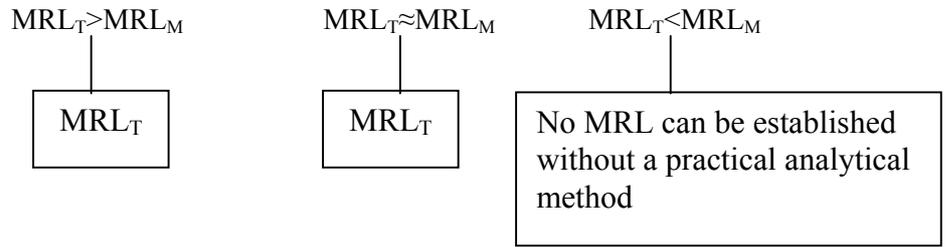
The Committee does take into consideration three principal factors. If the residues in food animal tissues based upon good practice in the use of veterinary drugs yields concentrations of residues lower than

those necessary to comply with the ADI, the MRLs will be reduced accordingly based upon approved use. However, if the residues cannot be reliably measured using a practical analytical method under the conditions of use, the recommended MRL will be modified so that compliance with the MRL may be verified analytically. This takes into consideration that the theoretical maximum consumption of residues does not exceed the theoretical consumption of residues based upon the ADI. If the residues arising from its use, according to good practices in the use of the veterinary drug, exceed the MRLs that could be recommended based on toxicological considerations, no MRL can be recommended under these conditions of use. Changes in either formulation, extension of withdrawal times or route of administration would be required, for example. Similarly, if considered MRLs based on the limits of the practical analytical method would exceed the theoretical maximum consumption of residues, no MRL can be recommended without an improved method (e.g., having a lower limit of quantification). These principles for recommending MRLs have been adhered to regularly by the Committee and for review are described in Figure 1 (7).

A very limited number of exceptions have occurred regarding the principle of recommending MRLs for a substance or substances that exceed the ADI (for example, see the group MRLs for the tetracyclines, 45th report of the Committee). Another important consideration in recommending MRL for a veterinary drug is a conservative set of dietary intake values for animal food products including milk and eggs. The quantities of muscle, liver, kidney, fat, milk and eggs are described in Section 7.c.

Figure 1. Decision Tree for Establishing Recommended MRLs





Definitions

- MRL_T** Maximum Residue Limit considered to be without hazard to human health as determined from the Acceptable Daily Intake (ADI)
- MRL_U** Maximum Residue Limit based on residues of the drug when used according to good practice in the use of veterinary drugs
- MRL_M** Maximum Residue Limit based on the availability of a validated, practical analytical method for measuring residues of the compound of interest

C. Food Consumption Factors

One of the most important considerations in determining MRLs in edible tissues and other products of animal origin is the amount of food used for these calculations. The 34th meeting recognized the complexity

of the issue and acknowledged that accurate food intake data are difficult to obtain, particularly at the international level, where dietary habits are influenced by ethnic origin, religion, climate and many other factors (8). In addition, there are limited publications on the fate of veterinary drug residues during processing of foods (e.g., curing, fermentation, pasteurization and sterilization, to name a few) and the domestic preparation of food of animal origin (e.g., cooking procedures, smoking, etc.). To protect all segments of the population, the Committee deemed it reasonable to use available intake data at the upper limit of the range for individual consumption of edible tissues and animal products. The Committee, therefore, agreed to use a conservative approach by selecting daily intakes of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of tissue fat, 100 g of eggs and 1.5 liter of milk. It should be noted that these values are now used by the U.S. Food and Drug Administration and the EU Committee on Veterinary Medical Products, however, the EU Committee amended values for poultry offal (10 g) and fat tissues (40g) but the total theoretical food intake, in grams, remains the same. The 34th meeting of the Committee noted that other assumptions and variables are involved in determining MRLs, including safety factors used in establishing ADIs, withdrawal times, the contribution of bound residues and the bioavailability of residues. The Committee believed that the potential errors in estimating food intake are not likely to be of great significance, and for this reason did not support great effort be devoted to further refining of food intake estimates. The Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) has surveyed dietary intake of veterinary drug residues in Member Countries to provide information to the Committee and other parties on these specific dietary intakes.

The 40th meeting of the Committee (9) reviewed the dietary consumption factors from data provided by Member Governments to the Sixth Session of the CCRVDF. The 40th JECFA believed that, in general, while the above food consumption estimates were statistically supported submissions, the data were not necessarily compatible since the data had been provided using different statistical approaches, including market basket surveys, dietary recall, and national food consumption data. Nevertheless, the data indicated that the values established at the 34th meeting, were realistic yet conservative values for consumption of edible animal products and, therefore, protective of human health. On this rationale, the 40th meeting of the Committee stated that no great effort should be made to further refine food intake estimates (9). It is worth

noting that the Committee evaluated a substance (abamectin) that is approved for use as a pesticide and a veterinary drug. The MRLs recommended for abamectin by JMPR posed a specific issue for the 45th JECFA, in part, because of the different food consumption data bases used by the two Expert Groups to recommend MRLs. Resolution on the MRLs was achieved at a subsequent meeting in accord with harmonization efforts with JMPR by including an allotment for MRLs from both uses, for example.

The 42nd meeting of the Committee (10) noted that in a few instances, consumption of food containing drug residues at the recommended MRL could result in a residue intake that was marginally higher than the ADI. However, the Committee was of the opinion, that overall, the approach used by JECFA provides a very adequate margin of safety for the consumption of animal food products. The 42nd meeting of the Committee did note that the consumption value for milk was particularly high, but the Committee reaffirmed this value to be appropriate as it would ensure that young children do not consume residues of veterinary drugs at amounts that exceed the ADI on a per kilogram body weight basis. The 42nd Committee acknowledged the need for better dietary intake data for use in assessing risks of substances in foods in general, but did not believe it was necessarily the case for veterinary drugs in foods. It should be noted, however, that the 45th meeting when commenting on an appropriate food consumption factor for determining an ADI on a microbiological end point, suggested a 500 g consumption factor for young children (12).

D. Target Tissue and Marker Residue

In recommending MRLs, the 38th meeting of the Committee (8) commented on its decision that at least two edible tissues would be identified whenever possible, one being muscle or fat and the other being liver or kidney. The selection of an appropriate target tissue in this manner, they reasoned, would permit regulation of the MRL in international trade as well as in national residue control programs. The 40th Committee reaffirmed this reasoning and provided the working definitions noted in the annex of its report (9). The 40th Committee noted that the identification of a marker residue is extremely important because it is the substance to be used to determine, for control purposes, enforcement of MRLs by national governments and industry. The Codex requires recommended methods of analysis to be identified for determining compliance with the MRLs (i.e., the marker residue and target tissue) adopted by the Commission as food safety standards.

E. Temporary MRLs

As noted previously, the 36th meeting of the Committee (7) addressed temporary ADIs. Likewise, this Committee also commented that temporary MRLs are sometimes recommended to provide sponsors time to generate additional data on the nature and quantification of residues. In this regard, Committees have specified what information is required to resolve outstanding issues and indicated a date by which these data should be submitted. At the time the requested data are to be provided to the Committee, the Committees have the option of establishing a full MRL, extending the temporary MRL, or not extending (withdrawing) the temporary MRL. Types of additional data requested may include, but are not limited to, radiolabel pharmacokinetic and metabolism data, further characterization of residues, identification of a marker residue or an appropriate analytical method. The specific requests by the Committees are summarized in their Committee report.

F. Transparency of the MRL Setting Process

One of the main reasons for this document on JECFA food safety assessment procedures for residues of veterinary drugs in food is to provide clarity on the principles and procedures developed by the various Committees. The 48th Committee acknowledged that there were some tasks relating to recommendations on MRLs that require deliberations at future meetings of the Committee (14). Because of the relatively high cost of developing the necessary data for JECFA evaluations the additional need for guidance on policies and procedures for substances was addressed. Among the items considered at the 48th meeting were MRLs for therapeutic substances for use in minor animal species and the concerns on why some tissues do not have MRLs. Other issues include substances used as a pesticide and a veterinary drug (addressed elsewhere in this document), considerations on how MRLs will be recommended for highly lipophilic substances in muscle and milk (in particular), guidance on analytical methods validation, the relationship of the analytical method limit of quantification and the MRL, and the statistical determination of an MRL. These transparency items were not completed at the 48th meeting, however, they were addressed at the 50th and 52nd meetings (see Section 11).

At the 52nd meeting the Committee developed recommended guidelines on the establishment of MRLs for minor animal species, MRLs in honey and other bee products, and on tissues for which MRLs are to be established for animal food products in international trade (19).

The document addressing MRLs for minor animal species outlines proposed guidelines to be used by JECFA when MRLs for veterinary drug residues are to be considered for minor species such as deer (e.g., moxidectin) and rabbits (e.g., diclazuril). Regarding MRLs in honey, the Committee recognizes that honey

and other bee products are not a significant part of the usual diet, however, they are within the terms of reference. Since the 38th meeting, whenever possible, the Committee has identified a minimum of two target tissues for recommending MRLs. However, some Member Governments have expressed the need for MRLs in all tissues when examining imported animal food products. The papers on these issues propose guidance on and will seek comment from the Codex Committee on Residues of Veterinary Drugs in Food. Additional guidance papers are being developed for consideration at the 54th Committee meeting. They will address residue considerations for MRLs in fish, principles for expressing numerical values for MRLs, and guidance for recommending MRLs for minor species when no MRL exists for the drug residue in a major animal species (e.g., goat and salmon).

G. Statistical approaches for determining MRLs

The 52nd meeting of the Committee noted that developing a well defined, scientifically acceptable approach for recommending MRLs based on the variability of individual data points from well controlled residue studies were addressed at the 50th meeting of the Committee (21). In addition, the 52nd Committee, in order to improve the transparency and scientific basis for recommending MRLs continued to develop two approaches that are complementary, yet individually applicable that are dependent on the quality and quantity of the residue data provided. In cases when the residue database is limited (e.g., three animals per group) on a substance with a long history of use, the Committee has agreed in principle to use mean values and consider incorporating three standard deviations for determining upper limits for residues at an individual time point. This approach was applied to two compounds evaluated at the 52nd meeting (Nicarbazin and Imidocarb). In situations where there is a large amount of highly reliable data available that permits a comprehensive statistical analysis, a more refined statistical approach may be applied. An example of this approach was used at the 50th meeting in recommending MRLs for eprinomectin (18). The Committee agreed to continue to evaluate other approaches for recommending the most suitable MRLs for a substance. Summaries of these two approaches will be developed further and provided to the Codex Committee, sponsors and other interested parties for information and comment.

8. DRUGS WITH A LONG HISTORY OF USE

The earlier meetings of the Committee indicated that for several veterinary drugs submitted for evaluation, particularly those with a long history of use, toxicological and residue data were often insufficient to meet contemporary criteria for a comprehensive safety assessment. However, it was recognized that these

data might be helpful in the safety assessment, particularly when the data can be complemented with the published scientific literature and case history experience with human use of these substances. The main concern of the 40th meeting of the Committee in considering alternative sources of information was to ensure that the safety of these veterinary products can be, to the extent possible, equivalent to that achieved for newer veterinary products (9). The 40th Committee agreed that the basic data necessary to assess the relevant food safety issues must adequately address pharmacological effects, general toxicity, reproductive toxicity, embryotoxicity/teratotoxicity, genotoxicity, carcinogenicity, other effects identified as being of importance, metabolism, tissue residue, and analytical methodology. Although pharmacokinetic data was not specifically identified, it was requested that each submission should contain information on all available and relevant animal studies on the pharmacology and residue chemistry data. The Committee also suggested that if human food safety can be adequately assessed from a combination of animal studies and alternative sources of information, then an ADI and MRL can usually be established for these veterinary drugs.

An important consideration by the 40th Committee was that the sponsor should provide an evaluation report that includes a comprehensive expert review of the scientific literature, relevant human and/or relevant data for the target species when all areas of concern listed above are not addressed by animal (and other relevant) studies. The Committee also stated that information on the scale and use pattern of the drug should be provided to enable assessment of the potential human exposure. This report should provide additional information on the specific veterinary drug and the general class of compound to which it belongs if it is appropriate. In the latter case, this would include a structure-activity analysis indicating whether extrapolation from the general class to the specific compound is warranted. In addition, any toxicologically significant effect(s) identified in the evaluation report need to be investigated. The report should also include any other necessary toxicological or residue information not provided by animal studies, or give the reasons why this information is not required. Importantly, the Committee considered that this evaluation report should present a logical and comprehensive analysis of the available data rather than a summary of the data and that a complete bibliography be provided, including full texts of all pertinent references that form the basis of the evaluation report (9). On this latter point, the Committee requests full texts of all references forming the basis of the evaluation report to be provided.

The Committee noted that the toxicological data required depends on the specific characteristics of a compound. Specific studies listed by the Committee include genotoxicity data that provide a range of endpoints sufficient to enable the genotoxic potential of the veterinary product to be adequately determined. A teratogenicity study was requested for compounds that are suspect teratogens or where a high or significant

potential exists for human exposure to residues in foods. The Committee noted that some toxicological studies may not meet currently established criteria, and where warranted, the Committee may compensate for these inadequacies by increasing the safety factor applied when establishing the ADI. The Committee also noted that a lack of information about a potential toxicological hazard may not be compensated for this way. Commenting on microbiological risk, the 40th Committee noted that information is normally required for the assessment of antimicrobial compounds, however, the Committee did not recommend any specific studies given the current level of development of studies designed to assess this risk.

The 40th meeting of the Committee also requested specific residue data needed to recommend an appropriate MRL(s). Pertinent data were requested for (a) an appropriate analytical marker residue for the residues of toxicological concern; (b) consideration for at least two target tissues on which to base the MRL, (one of which needs to be liver or kidney for national residue control programs and the other to be muscle or fat to facilitate testing in international trade); and (c) a suitable analytical method for the marker residue that satisfies the current performance based criteria. The 40th report did not mention data needs specifically for metabolism and pharmacokinetics. As in previous reports, the Committee agreed to remain flexible in evaluating the submitted data to determine what conclusions could be drawn based on the quality and completeness of the data provided. In recommending MRLs or temporary MRLs, the 40th Committee also agreed to consider, for practical reasons, good practice in the use of veterinary drugs.

The 43rd meeting of the Committee (11) reaffirmed that for these veterinary drugs with a long history of use, the toxicological data were likely to have been generated in their early stages of development and would, therefore, need to be evaluated by the basic guidelines in the 40th report of the Committee. However, they noted that when a new use for the veterinary drug was proposed, the Committee expected that new data would be generated according to contemporary protocols for residue and toxicological studies. As an example, more comprehensive residue data, including total residues from appropriate radiometric studies and information to assess bound residues.

The 43rd meeting of the Committee recognized that individual sponsors might be reluctant to generate the data necessary for veterinary drugs for which they may not have exclusive commercial rights. As the Committee requires sufficient data for establishing an ADI and recommending MRLs, it suggested the establishment of a consortium to provide the resources to generate the necessary data. Groups suggested include drug sponsors, government agencies, and manufacturers' organisations. Data for the reassessment of chloramphenicol at the 42nd meeting was noted as an example of the collaborative approach to provide the

necessary food safety data for an old drug. Lastly, the Committee recommended that the sponsor should stipulate at the time of submission, if the veterinary drug is to be evaluated under the protocol described above. The Committee stated firmly that it reserves the right to decide which substances qualify as drugs with a long history of use.

9. ANALYTICAL METHODS CRITERIA

A. Methods Requirements

The 32nd meeting of the Committee discussed methods requirements in general terms (5). The Committee noted that methods of analysis should be specified because they are necessary to detect, quantify and positively identify residues of veterinary drugs; support toxicology, drug metabolism, and pharmacokinetic studies; support residue studies of compounds to be evaluated. In addition, specific methods are necessary to satisfy the needs of public health agencies to determine compliance with an MRL. The Committee noted that method performance would be assessed, as appropriate, according to the following criteria: accuracy, precision, specificity, sensitivity, reproducibility, reliability and cost-effectiveness. The Committee noted that these criteria should be considered in making assessments of the residue methods. Cost-effectiveness is considered primarily in assessing the suitability of a method for residue control purposes and is not an issue for methods used for generation of pharmacokinetic and residue depletion studies for JECFA review. Equally important, for residue control programs, it was noted that ongoing quality assurance and quality control programs are essential to a laboratory quality system to ensure routine acceptable performance of analytical methods.

The 32nd Committee, however, did not provide descriptive language on analytical performance characteristics to be used in making the food safety assessments that would be useful to sponsors in preparing a dossier for the Committee. Some details have been included in the recent Codex circular letters when requesting data to be provided to JECFA for its assessments of compounds on upcoming agendas (see for example the request for information for the 47th and subsequent meetings of the Expert Committee).

The CCRVDF has developed specific guidelines for analytical method performance and a method evaluation work sheet requesting specific information for its *ad hoc* Working Group on Methods of Analysis and Sampling for consideration in recommending methods (to CCRVDF) for determining compliance with MRLs (16). The defined acceptable analytical performance for accuracy is based on residue concentration and has been published in *Codex Committee on Residues of Veterinary Drugs in Foods, Codex Alimentarius*,

Volume 3, Second Edition (16). As a result of CC/RVDF actions on analytical methods and an Expert Consultation sponsored by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (FAO/IAEA) on analytical methods for food control (17), procedures for recommending analytical methods to support compliance with MRLs are being reevaluated.

Some caution should be noted regarding suitable methods to support the recommended MRLs. While this has been a responsibility of the CCRVDF through its Tenth Session, JECFA does need to make assessments of the sufficiency of analytical or microbiological methods data in recommending MRLs, using for example, the decision tree approach described in section 7.b. It should be noted that some Committees have made recommendations for temporary MRLs (dexamethasone, for example) where the available analytical methods were not adequate to reliably quantify residues at the recommended MRL (10,11).

Other conservative approaches to recommending MRLs have been used by the Committee when considered MRLs are close to the limit of quantification of available methods. In evaluating spiramycin at the 43rd meeting, for example, the Committee chose to recommend MRLs in cattle, pigs and poultry on the basis of two times the reported limit of quantification of the methods reviewed by the Committee (11). Although the rationale for this decision was based on providing conservative assurance that analytical variability would not be a major impediment in determining compliance with the MRLs, the 43rd Committee report did not elaborate on this in the general principles section of their report. However, it should be noted that the approach was consistent with the general principle described in the decision tree approach to recommending MRLs noted previously (e.g., MRLs for zeranol). This procedure was used in the 45th meeting of the Committee in its discussion of MRLs for abamectin (12), but not elaborated on in the section of general principles.

The 45th meeting of the Committee did address the effect of analytical method recovery on assignment of MRLs (12). While this report did not address the two times the limit of quantification for recommending an MRL, an argument could be made on this issue to account for the situation where analytical recoveries presented for methods may be low (e.g., less than 50 percent). This is not to distract from the basic discussion regarding recovery factors on recommending an MRL or determining compliance with an MRL. The discussion in the 45th report of the Committee noted that the extent of analytical variation depends upon such factors as the concentration of the analyte, the analytical procedure, the recovery of the analyte and sample matrix effects, among others. In addition, loss or apparent gain of drug residue may occur during the processes of extraction from the analytical test material, purification of the extract, and determination of the

residue which may result in different analytical results on the same test material. In cases where an analytical procedure incorporates an analytical standard, the analytical result expresses the total amount of the residue because fractions of the analyte and the internal standard losses during the analytical work-up procedure are (expected to be) the same.

Some analytical methods do not necessarily contain an internal standard, therefore, different fully validated analytical procedures may yield different analytical results on the same test material. This consequence can result in an under- or overestimation of drug residues in the matrix (test sample); have a corresponding effect on the allocation of an MRL by the Committee; and influence the results of subsequent national residue control programs designed to determine compliance with the MRL. The Committee wished to recognize that, among other things, the assignment of an MRL to an analyte is based on the understanding that there is complete recovery (i.e., 100%) of the analyte. The 45th meeting of the Committee, therefore, recommended that where a method does not provide complete recovery, the analytical results should be corrected to 100 percent to determine if an analytical result is within or exceeds the recommended MRLs. The Committee requested that the sponsors provide uncorrected and recovery corrected analytical data for assessment by future Committees. (This has a potentially significant impact on recommending MRLs for new substances by the Committee and consistency with its previous MRL recommendations and needs to be reviewed further).

B. Assessment of Analytical Methods

The 47th meeting of the Committee noted that one of its major roles regarding analytical methods is to comment on the availability of suitable analytical methods capable of reliably measuring residues at the recommended MRL in a particular tissue matrix. It noted that the final responsibility for recommending suitable validated methods rests with the CCRVDF. With the great need to facilitate the availability of analytical methods for CCRVDF and for the Expert Committee to complete its evaluations, JECFA must be assured that the sponsor has provided sufficient acceptable method performance information so that the reliability of the residue data may be assessed. JECFA noted that it would also review and comment on the performance data to assess the utility of a method for residue control purposes.

The 52nd Committee reviewed a working paper on method validation requirements for generating pharmacokinetic and residue depletion data that are used for evaluating residues of veterinary drugs in food. The Committee concluded that the document reflects current guidance principles for assessing the adequacy

of methods used in studies that are reviewed by the Committee and for assessing the suitability of methods proposed for residue control programs to determine compliance with MRLs (20). The document will be published and made available to CCRVDF and other interested parties to facilitate the harmonization of method assessment for determining compliance with recommended MRLs.

C. Chemical and Microbiological Methods

Extensive discussions on use of microbiological methods for determining compliance with MRLs have been recorded in Committee reports on specific substances. For example, the 36th meeting of the Committee addressed this matter regarding benzylpenicillin (7). The Committee noted that there were established microbial bioassay methods available for measuring residues in milk that have detection limits of 1-10 µg/l. The Committee agreed that it was practical and possible to use methods with a detection limit of 4 µg/l in milk. The chemical methods reviewed at the 36th Committee were not as sensitive as bioassays and could not be used to confirm benzylpenicillin in milk at the recommended MRL. However, the Committee noted that there was poor correlation between the results obtained with different assay methods in tissues. Residue concentrations were highest in radiometric studies where total residues of benzylpenicillin are measured. This suggests that large amounts of the residues were microbiologically inactive metabolites. In addition, residues determined using high performance liquid chromatographic procedures reported higher concentrations of residues than those using the bioassay because the extraction procedures employed for the chemical method were more rigorous than the procedure used for the microbial bioassay method. Consequently, the Committee noted that it was important to consider whether the quantity of benzylpenicillin residues in tissues may be underestimated in some bioassays. The Committee noted that these bioassays are not specific for benzylpenicillin (e.g., detection of other β-lactams) and that specific chemical methods must be used for identification.

Discussions on determination of oxytetracycline residues in the same report were considerably different (7). In the case of the tetracycline class, the Committee noted that based on the data available, these substances are unlikely to be extensively metabolized in animals and, therefore, concluded that for the determination of oxytetracycline residues, either a chemical or microbiological method would provide a suitable result for residues in animal tissues and milk. Therefore, the appropriateness of a method may depend on the metabolic and pharmacokinetic characteristics of the antimicrobial.

The 43rd meeting of the Committee addressed microbiological assays for residues of spiramycin,

neomycin, gentamicin, streptomycin and dihydrostreptomycin (11). Regarding gentamicin residue methods, the Committee noted that there was a cylinder plate assay having sufficient sensitivity to measure residues at 0.01 mg/l in milk and approximately 0.1 mg/kg in tissues, respectively. The Committee noted that there was no validated chemical assay although there was a method available but that it required specific equipment that may not be available in many laboratories. The decision on an appropriate analytical method for gentamicin was addressed in a different manner than noted with benzylpenicillin and oxytetracycline. Because the gentamicin ADI was established using a microbiological end-point determination and there was no indication of significant metabolism in tissues (the MRLs were expressed as parent drug), antimicrobial methods were considered to be applicable for determining compliance with the temporary MRLs. However, a validated chemical method was requested for evaluation by the Committee.

For neomycin, the ADI was established on a toxicological end-point, using the parent drug as the marker residue because of limited metabolism of the administered dose. On this basis, the Committee considered the antimicrobial bioassay methods were suitable for determining compliance with the MRLs.

The suitability of antimicrobial bioassays and chemical methods for spiramycin were both deemed appropriate for determining compliance with the MRLs by the 43rd meeting of the Committee (11), depending on the availability of validated methods in different animal species. The sponsor provided sufficient analytical data to demonstrate that the antimicrobial bioassay gave comparable residue results when compared with high performance liquid chromatographic methods. In addition, the data indicated that other antimicrobial substances that may be used in combination with spiramycin did not interfere with the quantification of spiramycin residues because the antimicrobial method employs a chemical extraction and purification procedure prior to application of an analytical extract in the antimicrobial assay.

For streptomycin and dihydrostreptomycin, the 43rd Committee noted that there was an enzyme immunoassay, a receptor-binding assay, a microbial inhibition assay and thin layer and high performance liquid chromatographic methods for determining compliance with the MRLs. Characterization of residues of the two drugs indicated only limited metabolism in food producing animals. Therefore, the parent drug was appropriate as the residue marker, and residues could be identified and quantitated using methods noted above.

Thus, the Committees have made determinations on the suitability of antimicrobial bioassay methods based on several factors: the nature of the drug metabolism, the end-point for determining the ADI, the

performance characteristics (e.g., specificity) of available methods and factors that are unique to a particular substance. This reflects on the Committee's long standing position of assessing compounds based upon the availability of the data provided by sponsors, the scientific literature and the Committee's assessment.

10. MISCELLANEOUS

A. Aquaculture

At the 43rd meeting of the Committee, the use, consumption and residue analysis of farmed fish was reviewed (11). The Committee noted that human consumption of farmed fish varied considerably and accurate food intake data are difficult to obtain at the international level. To protect all segments of the population, the Committee noted that MRLs for food commodities, including aquaculture products (although they are not specifically listed) should be based on the food consumption factors described in the 34th meeting of the Committee. It was also noted that the European Community has decided to use a daily intake of 300 grams of muscle and skin in natural proportions for recommending MRLs. As this is the same food intake value used for muscle tissue in food producing animals, the Committee agreed that this is an appropriate value to use for recommending MRLs for veterinary drugs used in aquaculture. It was also noted that certain veterinary drugs used in aquaculture such as oxolinic acid, flumequine and oxytetracycline may have higher concentrations of residues in the skin of fish than the corresponding muscle tissue. Therefore, when analyzing farmed fish for residues of veterinary drugs, analysis should be on test samples using skin and muscle in natural proportions. Though not specifically defined, natural proportions should include muscle tissue with adhering skin only. The Committee also noted that samples should be large enough so that the contribution of residues from the skin and muscle tissue are representative of the whole fish.

B. Compounds Used as Veterinary Drugs and Pesticides

The terms of reference of the Committee limit its assessment to consumption of residual amounts of residues of veterinary drugs that occur in tissues of food producing animals. It is possible, however, to recognize instances in which residues from food may arise from a substance used both as a crop protection agent and as a veterinary drug. In this instance, it is possible the entire ADI for a compound may be fully allocated in either use. A specific issue was abamectin at the 45th meeting of the Committee (12).

Abamectin had been initially reviewed by the Joint Committee on Pesticide Residues (JMPR) in 1992 and again at the 1994 and 1997 meeting of that Committee. In their assessment of abamectin as a pesticide,

an ADI was established using a 500-fold safety factor based on a genotoxic Δ -8,9 photo-degradation product resulting from this use. The JMPR also recommended MRLs in edible tissues of food producing animals using their established procedures. Applying the JMPR food consumption factors to all uses of abamectin, the theoretical maximum daily intake of residues from such uses did not exceed the ADI. However, JMPR and JECFA use different approaches for determining theoretical maximum residue intake. Applying the JMPR-recommended MRLs to the food consumption factors for muscle, liver, kidney, fat and milk used by JECFA for determining theoretical maximum daily intake of residues yielded theoretical amounts of residues that exceeded the ADI. For this reason, the 45th meeting of the Committee (12) did not recommend MRLs for use as a veterinary drug. Having concern for this situation, the Committee recommended a joint meeting with JMPR experts to resolve this issue. As a result the 1996 JMPR meeting established an ADI for abamectin (as the parent drug) when it is used as a veterinary drug.

Recognizing further the need to harmonize MRLs, the 47th meeting of the JECFA (13) again reviewed the JMPR recommended MRLs. In considering that abamectin used as a veterinary drug is only intended for use in beef cattle, that liver and fat are the appropriate target tissues, that abamectin does not yield bound residues in fat and that bound residues are less than 15% in liver, and that validated methods are available, the Committee recommended MRLs in beef cattle in fat, liver and kidney. Only the recommended MRL for kidney was the same between the two Expert Committees. JECFA did not recommend a MRL in muscle tissue because residues depleted to non-detectable concentrations at the recommended withdrawal time. Nevertheless, JECFA recognized that JMPR had established an MRL for abamectin used as a pesticide in cattle muscle and milk. This example indicates the potential complexity of substances used as a pesticide and as a veterinary drug for which acceptable procedures are needed to harmonize food safety assessments with JMPR.

As noted above, harmonization of MRLs continue to arise with substances used as a pesticide and as a veterinary drug resulting from differing approaches employed by the Joint Meeting on Pesticide Residues and the Codex Committee on Pesticide Residues (CCPR) and JECFA and the Codex Committee on Residues of Veterinary Drugs in Food (19). At its 30th session, CCPR recommended that JMPR and JECFA work to harmonize procedures for recommending MRLs. JMPR also agreed that MRLs should accommodate both types of approved uses and that where the two recommended MRLs do not agree, the Codex MRL should be based on the higher value. Similarly, CCRVDF recommended that the joint FAO/WHO secretaries convene an informal meeting of experts from both groups to address the issues. The informal meeting was held prior to the 52nd JECFA and a report of this meeting has been published (20). In this report, 24

recommendations were agreed to by participants. The recommendations were elaborated under four sections that parallel the areas of discussion – tissue, milk, eggs and harmonisation. These recommendations will be forwarded for consideration to the two expert committees, the Codex Committee on Pesticide Residues and Codex Committee on Residues of Veterinary Drugs in Food for consideration and action as appropriate. It was also agreed at the informal meeting that additional meetings should be held as necessary to advance efforts to further harmonize MRLs.

C. Drug Characterization

At the 32nd meeting of the Committee (5) the need to characterize a veterinary drug prior to a food safety evaluation was emphasized. The Committee noted that the substance must be well characterized with regard to both the active substance and any major impurities as appropriate resulting from its manufacture. Details of chemical and physical characteristics of the drug need to be described along with the consistency and quality of the final product (the manufacturing process must be capable of yielding a reproducible product). In addition, the substance should be registered as a veterinary drug in at least one country.

D. Drug Efficacy

The 26th and 27th meetings of the Committee briefly commented on the efficacy of veterinary drugs (3,4). The 26th meeting (3) noted that in connection with good animal husbandry practice, evidence is required as to the efficacy of the substances considered by the Committee (specific reference was made to anabolic substances, however, the principle applies to all veterinary drugs), the amounts used to produce the intended effect, and the residue concentrations resulting from use in controlled field trials. Other relevant matters on data requirements for toxicology and residue evaluation have been addressed earlier. The 27th meeting (4) affirmed the considerations raised at the 26th meeting and added that data on residues from studies in which the substances under consideration are used in combination may make food safety reviews more difficult because of the need to separate factors associated with the individual components. This Committee, however, did not make specific recommendations on this issue.

E. Withdrawal Times

The 38th meeting of the Committee addressed the relationship of withdrawal time to recommended MRLs (8). Importantly, the report noted that the Committee does not attempt to derive the appropriate

withdrawal times (e.g., from the residue kinetics) to be observed in order to ensure that the concentration of residues will be below the recommended MRL. It noted also that the determination of the appropriate withdrawal time for a given veterinary drug such that its use complies with the MRL is the responsibility of national licensing authorities. The Committee, when deriving MRLs, verifies that the MRLs it recommends can be achieved through practical withdrawal times and application of good practices in the use of veterinary drugs. There are instances in Committee evaluations where it has recommended against use of a veterinary drug (e.g., levamisole in eggs from laying birds and milk from lactating cows) because residues were very high at practical withdrawal times and the time necessary for depletion of residues could not be achieved in normal agricultural practice.

A withdrawal time, as recommended by the 38th Committee (8), should be established on the basis of a statistical limit that provides an interval within which a given percentile of the population of residue data resides, with a given level of confidence. The Committee agreed to use the 99th percentile with a 95% confidence level for verifying that recommended MRLs can be achieved through realistic withdrawal times. This is because a withdrawal time based on a mean value of a set of residue data may result in instances where failure to comply with the MRL may occur in a considerable number of samples in a residue control program. There are some instances in the various Committee reports where this principle has been documented (e.g., the MRLs for ivermectin in the 40th report).

11. BIBLIOGRAPHY

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ANNEX 1.

Glossary of Terms

Definitions for terms and principles used by the Committee have been developed and subsequently employed in those meetings devoted to residues of veterinary drugs in food producing animals and birds. They are referenced in the corresponding WHO Technical Report Series. Many of the definitions relative to the food safety assessments performed by JECFA are incorporated by reference from EHC 70: Principles for the Safety Assessment of Food Additives and Contaminants in Food, published by WHO (1).

Veterinary drug. Any substance applied or administered to any food producing animal, such as meat or milk-producing animals, poultry, fish or bees, whether used for prophylactic, therapeutic or diagnostic purposes or modification of physiological functions or behavior (5).

Residues of veterinary drugs. Residues include the parent compound(s) and/or their metabolites in any edible portion of the animal product, and include residues of associated impurities of the veterinary drug concerned (5).

Acceptable Daily Intake (ADI). An estimate by JECFA of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk. It is determined using 60kg as a standard. An ADI is the end-point of JECFA evaluations for intentional food additives.

ADI not specified. (Where) available data on the toxicity and intake of the veterinary drug indicate a large margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drug. For this reason, and for reasons stated in individual evaluations, the Committee has concluded that use of the veterinary drug does not represent a dietary hazard to human health and there is no need to specify a numerical ADI (9).

Maximum Residue Level (MRL). The maximum concentration of residue resulting from the use of a veterinary drug (expressed in mg/kg or µg/kg on a fresh weight basis) that is recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in or on a food. It is based on the type and amount of residue considered to be without toxicological hazard for human health as expressed by the Acceptable Daily Intake (ADI), or on the basis of a temporary ADI that utilizes an

additional safety factor. It also takes into account other relevant public health risks as well as food technological aspects and estimated food intakes (6).

MRL not specified. (Where) available data on the identity and concentration of residues of the veterinary drug in animal tissues indicate a large margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drug. For that reason, and for the reasons stated in the individual evaluation, the Committee has concluded that the presence of drug residues in the named animal product does not present a health concern and that there is no need to specify a numerical value (9).

Total residues. The residues of a drug in animal-derived foods that consists of the parent drug, together with all the metabolites and drug-based products that remain in the food after the administration of the drug to food-producing animals. The amount of total residues is generally determined by means of a study using the radiolabelled drug and is expressed as the parent drug equivalents in mg/kg of the food (6).

Extractable residues. The residues extracted from tissues or biological fluid by means of aqueous acidic or basic media, organic solvents and/or hydrolysis of with enzymes (e.g., sulfatase or glucuronidase) to hydrolyze conjugates. The extraction conditions must be such that the compounds of interest are not destroyed (6).

Bioavailable residues. The residues that can be shown, by means of an appropriate method (e.g., Gallo-Torres method) to be absorbed when fed to laboratory animals (6).

Bound residues. Residues resulting from the use of veterinary drugs in food producing animals that are considered as non-extractable residues (6).

Non-extractable residues. Non-extractable residues are those residues obtained by subtracting the extractable residue from the total residues and comprise 1) residues of the drug incorporated through normal metabolic pathways into endogenous compounds (e.g., amino acids, proteins, nucleic acid). These residues are of no toxicological concern; 2) chemically bound residues derived by the interaction of residues of the parent drug or its metabolites with macromolecules. These may be of toxicological concern (6).

Target tissue. The edible animal tissue (muscle, fat, liver or kidney) for which the MRL is recommended and that may be analyzed for purposes of the enforcement of the MRL (9).

Marker residue. The substance that is, or is representative of, the residue of toxicological concern in the target tissue and/or milk/eggs. Identification of a marker residue is extremely important as it is the substance determined for control purposes in the enforcement of MRLs by national governments and industry (9).

Minimum Inhibitory Concentration. The minimum concentration of an antimicrobial agent that inhibits to a specified degree the growth of cultures of a particular microorganism. The MIC is usually expressed as a percent of complete growth inhibition of the cultures studied (e.g., MIC₅₀ is complete inhibition of 50% of the cultures).

Acute reference dose. The estimate of the amount of a substance in food or drinking water, that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to consumers on the basis of all the known facts at the time of the evaluation. It is usually expressed in milligrams per kilogram body weight of the chemical.

Decision tree on determining the adverse microbiological effects of residues of antimicrobial drugs in food-producing animals.

