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**SPECIFICATIONS FOR THE IDENTITY
AND PURITY OF FOOD ADDITIVES AND
THEIR TOXICOLOGICAL EVALUATION :
SOME ANTIBIOTICS**

**Twelfth Report
of the Joint FAO/WHO Expert Committee
on Food Additives**

Geneva, 1-8 July 1968



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JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Geneva, 1-8 July 1968

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Specifications for the antibiotics considered in this report, and descriptions of methods for analysing their residues in foods, will be issued by FAO and WHO in a separate publication entitled :

Specifications for the identity and purity of some antibiotics and methods of analysis of their residues in food.

FAO Nutrition Meetings Report Series, 1968, No. 45a ;
WHO/Food Add./69. 34.

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A Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 1 to 8 July 1968. The meeting was opened by Dr P. Dorolle, Deputy Director-General of WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization. Dr J. W. Howie was elected Chairman, Dr W. W. Wright Vice-Chairman, and Professor H. S. Goldberg Rapporteur.

INTRODUCTION

As a result of the recommendations of the Joint FAO/WHO Conference on Food Additives held in September 1955,¹ eleven Joint FAO/WHO Expert Committees on Food Additives have met (see Annex).

The present meeting was convened on the recommendations made in the report of the 1962 WHO Expert Committee on the Public Health Aspects of the Use of Antibiotics in Food and Feedstuffs² and in the eleventh report of the Joint FAO/WHO Expert Committee on Food Additives.³ Its tasks were : (1) to draw up specifications for and to make a toxicological evaluation of certain antibiotics used as food additives, and (2) to assess the efficacy and direct and indirect potential health hazards of a number of other antibiotics and chemotherapeutic agents that, under certain conditions, might leave residues in foods of animal origin. The substances considered had been suggested by the Codex Committee on Food Additives, to which the Expert Committee acts as an advisory body on questions of

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1956, No. 107 (*FAO Nutrition Meetings Report Series*, No. 11).

² *Wld Hlth Org. techn. Rep. Ser.*, 1963, No. 260.

³ *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 383 (*FAO Nutrition Meetings Report Series*, No. 44).

specifications for identity and purity, methods of analysis, and evaluation of safety.

The Committee gave major attention to specifications for these compounds, to methods of analysing them, to an evaluation of their biological effects and toxicity, and to an assessment of the levels at which they may be used with safety. Their efficacy has been dealt with elsewhere and therefore was not considered.

Antibiotic residues in food might also arise from the use of these substances to treat ornamental and food plants and (in honey) from their use to treat bee diseases. However, this report deals only with foods of animal origin. The Committee considered that the hazard that may arise from such other uses of antibiotics should be examined by an appropriate group on a future occasion.

OCCURRENCE OF ANTIBIOTIC RESIDUES IN FOOD

The ways in which antibiotic residues may occur in foods, and the problems presented by the presence of such residues, are reviewed in the following sections.

Antibiotic residues may occur in foods in any of the following ways: (1) They may occur naturally in some foods. (2) Certain antibiotics may be added to food for technological reasons. (3) For similar reasons, certain antibiotics may be introduced into the immediate environment of food, and may contaminate the food itself. For example, antibiotics may be added to the ice or water used for chilling fish and poultry, or they may be applied to cheese rind or banana skins to control the growth of mould. (4) Antibiotics may be added to animal feeds to permit more rapid growth and more economic feed-conversion (see below). (5) Antibiotics¹ and certain other antimicrobial agents may be added to animal feeds in larger amounts for special purposes—e.g., they may be added to artificial milk substitutes used for the economical rearing of young animals—without the prescription of a veterinarian. (6) Antibiotics may be used for prophylactic purposes in the hope of controlling infection in groups of animals. (7) Antibiotics may be used by veterinarians to treat sick animals. (8) Antibiotics may be unintentionally included in food materials (e.g., by-products of fermentation processes) given to animals, unless the use of such food materials is properly controlled. (9) Antibiotics may occur naturally in animal feeds. Any of the last six sources might result in the presence of antibiotic residues in the tissues of animals at the time of slaughter, and such residues might subsequently reach the consumer.

¹ See also *Wld Hlth Org. techn. Rep. Ser.*, 1963, No. 260.

The amounts of approved antibiotics that may be added to feeds without a veterinary prescription vary from one country to another. The 1962 WHO Expert Committee on the Public Health Aspects of the Use of Antibiotics in Food and Feedstuffs¹ judged an antibiotic concentration of 20 ppm in feeds to be sufficient for growth and feed-conversion purposes, but noted that under certain circumstances it might be necessary to use higher levels (up to 100 ppm). National regulations in most countries adhere to the limit of 20 ppm for growth purposes. However, some regulations appear to permit levels up to 100 ppm, but do not clearly specify the antibiotics, the diets, or the uses for which such levels are permissible. It is difficult to distinguish between the use of feed additives for growth and feed-conversion purposes, and their use for prophylactic objectives, since growth and feed-conversion effects may be at least partly prophylactic in nature. Nevertheless, although it is widely accepted that the addition of small amounts of antibiotics (levels of about 20 ppm) is unlikely to give rise to serious problems, the addition of 100–200 ppm may well result in problems of residues in food and in the development of antibiotic-resistant organisms. In view of the complications that might arise from the indiscriminate use of antibiotics (including interference with their proper use), it cannot be too strongly emphasized that effective control of their use is essential.

The Committee believe that a distinction should be made between (a) the addition of antibiotics to animal feeds for growth and feed-conversion purposes (and for other special purposes that are not thought to require direct veterinary control) and (b) the use of antibiotics for all forms of prophylactic treatment for which prescription by a veterinarian is considered advisable. The Committee also believe that national regulations deserve critical review in the light of such a distinction.

Control measures

Since antibiotics are potent substances that are known to have certain toxic effects, attempts have been made in many countries to control the residues of such substances that might be found in human food as a result of their use for different purposes. It is a general principle that only as much of a given additive as is necessary to achieve the desired effect should be used in food.² It is also generally agreed that the amount of extraneous substances that may be inadvertently introduced into food should be kept as low as possible. In dealing with the intentional addition of antibiotics to food for human consumption, to its immediate environment, or to animal feeds, it has been the general practice to control residues in food by appro-

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1963, No. 260, p. 18.

² *Wld Hlth Org. techn. Rep. Ser.*, 1957, No. 129 (*FAO Nutrition Meetings Report Series*, No. 15), p. 14.

priate legislation. However, scientific information is continually changing, and one of the objectives of the Committee was to review the available evidence and to indicate the residue levels that might be regarded as acceptable on scientific grounds.

The veterinary use of antibiotics in prophylaxis and therapy raises rather different problems.¹ The choice of approved antibiotics, the dosage administered, and the time that elapses between treatment and slaughter are matters that should be decided by the veterinarian concerned, in accordance with the applicable regulations. In this connexion the Committee considered it useful to define as clearly as possible the limits that are regarded as permissible for each antibiotic residue and to indicate the most suitable methods for assessing such levels. This information, based on evaluation of the available toxicological and microbiological evidence, should make it easier for authorities concerned with animal husbandry, veterinary and medical practice, and public health to devise the most appropriate and effective control measures.

The problem of mixtures containing antibiotics being fed to animals was also given general consideration by the Committee.

Residue levels likely to occur in foods

When antibiotic residues occur in foods they are usually present only in trace quantities. Legislation may prescribe that "no residues" of antibiotics should be present in food. However, a residue may be present but undetected, depending on the sensitivity of the method used. Consequently, either the amount of residue detected should be reported, or it should be stated that no residue was detected by means of a given method, whose sensitivity should be reported. It is also important to report the results of analyses in terms of whether or not a given substance was detected. The use of words such as "contamination" and "pollution" should be avoided in reports of analyses, since they carry some degree of toxicological significance.

It is important to have information on the residue level that is likely in any edible material of animal origin. The residue level in muscle may differ from that in liver, kidney, adipose tissue, or milk, yet all these substances may be constituents of the human diet. Measurement of the concentration of an antibiotic or its breakdown product in an animal's urine may also be useful as a general guide to the residue levels in the carcass.

The residue levels in foods of animal origin may be affected by the way in which the foods are treated subsequent to the slaughter of the animal—e.g., the duration and conditions of storage, cleaning, and cooking.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1963, No. 260, p. 19.

Nature of residues

The residues found in foods may consist of unchanged antibiotic or various breakdown products that may or may not retain antibiotic potency. Consequently, a residue may consist of (a) an antibiotically active group of substances that may cause either toxic or microbiological effects or both, and (b) an antibiotically inactive group of breakdown products that may cause toxic but not microbiological effects. Ideally, analysis should yield adequate information about both groups of substances.

HAZARDS CAUSED BY ANTIBIOTIC RESIDUES IN FOOD

The hazards that may arise from the presence of antibiotic residues in food are similar to those that might be brought about by any chemical substance. However, as with other antimicrobial agents, antibiotics cause the added hazard of unwanted microbiological effects. Some of the factors involved in the possible hazards are reviewed in the following sections.

Direct toxic effects

Direct toxic effects are effects of the residue on the consumer, modifying either structure or function. Biochemical and *in vitro* studies of effects on cells or on enzyme action may be helpful in evaluating such effects. The toxic effects are often demonstrable in appropriate experimental animals by feeding them with the substance being tested and comparing the results with those observed in similar control groups. Long-term studies, including life-span observations of tumour incidence, are of particular importance in the investigation of food additives. Although animal studies are of great value, it is possible that the toxic effect of a given substance may occur only in man. If so, individuals showing the effect must be sought among those who may be accidentally, occupationally, or therapeutically exposed to the substance, and such persons should then be studied.

Indirect toxic effects

The following three types of indirect toxic effect are commonly studied.

(a) Effects of the additive or residue on the food itself, perhaps causing the formation of a toxic agent.

(b) Effects of the additive or residue on the consumer, leading to hypersensitivity or allergy. A similar effect may also be observed in a consumer who has been sensitized by some other means. This is relevant to the problem of antibiotic residues in food, since the consumer may have been

sensitized by having previously received antibiotics for therapeutic purposes. Such effects are not readily revealed by animal studies unless the substance concerned is an exceptionally potent sensitizing agent. It is possible that allergic effects caused by foods or substances in foods are more common than would appear from the medical and scientific literature. Careful observation of the incidence of such effects in human subjects is called for, since their true extent and importance is at present unknown. Since reactions of this type tend to be based on individual idiosyncrasy, it is usually not feasible to control them directly by regulation. However, if a certain form of sensitization is known to be relatively common in a given community, it would seem desirable to avoid contamination of important foods (e.g., milk) with the allergen concerned. Special consideration should always be given to the possible presence of antibiotic residues, particularly penicillin, in staple foods that are extensively consumed by children.

(c) Remote effects, such as the modification of micro-organisms. Since this is a matter of particular importance with antibiotic residues, it is discussed in greater detail in the following section.¹

Microbiological effects

Antimicrobial agents, including antibiotics, are used to modify or control the growth of certain micro-organisms, but they may affect a wider range of organisms than intended. The ingestion of low levels of antibiotic residues may lead to minor changes in the natural body flora in humans. Of much greater importance is a possible alteration in the sensitivity of microbial populations to antibiotics that are ingested, or even to other antibiotics. There are a number of mechanisms by which resistance to antibiotics may be brought about, and several ways in which the number of resistant organisms in a bacterial population may be increased.

Chromosomal resistance (genetic resistance)

Resistance to antibiotics may be genetically produced as a result of selection of spontaneous mutations. Provided a sufficient number of resistant mutants are present, a level of antibiotic that is sufficient to inhibit the growth of parent strains will favour the development of the drug-resistant mutants. If the mutants are highly resistant to the antibiotic, a predominantly resistant bacterial population may develop in one step, as is commonly observed with streptomycin. However, the development of resistance may depend upon repeated exposure of the micro-organisms to increasing concentrations of antibiotics, as is commonly observed with

¹ Microbiological problems may also arise in connexion with emergency slaughter; see *Wld Hlth Org. techn. Rep. Ser.*, 1963, No. 260, p. 20.

penicillins. Chromosomal resistance develops only when organisms are exposed to an antibiotic concentration within a certain critical range.

Extrachromosomal resistance (infectious resistance)

In the past few years considerable attention has been given to the transfer of resistance from one micro-organism to another by direct contact. Upon contact, which need be only brief, an intracellular element (R-factor) may be transferred from the donor to the recipient cell, with the result that both cells possess the antibiotic-resistant properties of the donor cell. One R-factor can confer resistance to several antibiotics and antimicrobial agents. R-factors can multiply faster than the cells from which they are derived, and under favourable conditions transfer between cells can be extremely rapid. In this way, a bacterial population that is resistant to many antibiotics may be built up. The presence of antibiotics in the environment tends to favour the growth of such resistant strains.

R-factors have been demonstrated for a wide range of antibiotics in common use. Some R-factors are inborn, and it has been shown that they were already present in micro-organisms before discovery of the therapeutic properties of the antibiotics to which they confer resistance. The resistance brought about by R-factors may result partly from changes in cell permeability and partly from the induction of antibiotic-inactivating enzymes. Furthermore, the transfer of R-factors may affect the fertility of the cells. There are many interesting features of this phenomenon that are not yet clearly understood. This type of transfer of resistance to antibiotics appears to be peculiar to gram-negative members of the Enterobacteriaceae.

Significance of antibiotic resistance

There is no significance *per se* in the lack of sensitivity of a group of micro-organisms to one or more antibiotics. It assumes importance only if it interferes with the need for controlling a given micro-organism in the interests of the animal or human community. Micro-organisms that are pathogenic to man or animals and those that may bring about food spoilage are of particular interest in connexion with the possible appearance of antibiotic residues in foods, and it was deemed important to consider whether there is any danger of causing the development of resistance in such organisms in this way. However, attention should not be restricted to the possibility of direct contact between an antibiotic residue and pathogenic or food-spoilage organisms, since it is possible that resistance may also be transferred by means of intermediate organisms that are neither pathogens nor involved in food spoilage. This transfer of resistance is such that it seems most likely to occur naturally in the intestinal lumen. Even within the intestinal lumen, a high density of both the donor and the recipient cells seems to be important.

In evaluating the possible microbiological complications that might arise from the presence of an antibiotic residue in food, the following points should be considered :

- (1) the nature and the amount of the residue present ;
- (2) the likelihood that ingestion of the residue will be regular or intermittent ;
- (3) the possible effect of the residue on the flora of the food, and the influence of the conditions of storage on this effect ;
- (4) the possible effect of the residue on the natural flora of the consumer ;
- (5) the significance of ingestion of the residue in relation to subsequent invasion of the gastrointestinal tract with pathogens ;
- (6) the effect of the development of antibiotic-resistant pathogens on the therapeutic control of disease ; and
- (7) the possibility that resistant pathogens produced as a result of exposure to antibiotic residues might become a public health problem .

These points are discussed in the monographs, but a few are briefly considered below for illustrative purposes.

If chlortetracycline is added to the ice or water used to chill fish or poultry, respectively, there may be little risk of the development of resistant organisms if the conditions are such as to restrict the growth of microorganisms. However, if the conditions become modified (e.g., by a temperature rise or an alteration in the frequency of water changes), or if the hygiene of the processing equipment is not properly maintained, rapid growth of chlortetracycline-resistant organisms may occur, causing spoilage of the food or affecting the health of the consumer.

The incidence of antibiotic-resistant salmonellas has increased considerably in some countries in recent years, although in some cases this may be the result of faulty agricultural or medical practices rather than the presence of antibiotic residues in feeds or foods. The significance of antibiotic resistance in salmonellas is a problem of some complexity in human and veterinary medicine, and much further work is necessary in order to clarify it. Studies of the problems involved are being undertaken in the United Kingdom, the USA, the USSR, and other countries. If the intestinal flora of the consumer were to develop resistance to chloramphenicol, and to transfer this resistance to *S. typhi* or *S. paratyphi* during infection with these organisms, it would be a serious matter, since chloramphenicol is the most effective agent available against such infection. (The mortality in typhoid epidemics prior to the discovery of chloramphenicol was often considerable.) Consequently, there seems to be an *a priori* case for insisting that chloramphenicol should not be used for any purpose that might result

in its presence as a residue in food. A case could also be made on toxicological grounds against the indiscriminate use of chloramphenicol.

The above examples illustrate the complexity of the microbiological problems that may arise from the presence of antibiotics in foods, and the importance of giving careful consideration to the possibility that any given antibiotic might produce such effects.

COMMENTS ON MONOGRAPHS

Specifications and assays *

Analytical methods

Analytical methods with improved sensitivity, specificity, accuracy, and reproducibility are continually being developed. Any method cited in the monographs is acceptable according to present knowledge. Alternative methods or refinements of those described may be available now or in the near future.

In the assay of antibiotics and their breakdown products in tissues, it is necessary to know the type of residue that is present. This information can be obtained from qualitative tests, which may include direct simple zone inhibition methods with tissue or urine specimens and separation by electrophoresis or chromatography.

An alternative approach would be to submit the sample to each of the quantitative assays for different groups of antibiotics. However, quantitative measurement of any specific antibiotic requires a knowledge of the antibiotic involved, as different response curves may exist for different antibiotics in the same group.

In practice more than one antibiotic may be present in a given sample. The methods recommended by the Committee (see p. 4) are for single substances. In the analysis of samples containing more than one antibiotic, where interference may occur, appropriate procedures should be used.

Feed-grade antibiotic material

Specifications are given in terms of the pure antibiotic. Similarly, safety and methods for determining tissue residues were studied in terms of the active antibiotic involved, rather than on the basis of the form in which it was administered. However, feed-grade antibiotic materials often contain complexes and partially refined sources of the antibiotic. Therefore, it is recommended that in evaluating the acceptability of a given feed-grade

* As previously noted (see p. 4), specifications and descriptions of analytical methods are not included in this report.

antibiotic, the actual form being administered be studied, with attention to its purity and to the possibility of the presence of decomposition products, inactive ingredients, etc. All safety evaluation tests and tissue residue determinations should be made with reference to the actual feed-grade material. When appropriate, the possibility of the presence of complexing chemicals and other non-antibiotic materials should also be considered. Only where specifications are available can feed-grade antibiotic materials be adequately evaluated.

Tentative specifications

A tentative specification was drawn up for kanamycin, which could not be fully evaluated toxicologically on the basis of available data.

Breakdown products of antibiotics

Antibiotics may break down to a certain extent during the processing and storage of animal feeds and tissues that contain them. Furthermore, like other biologically active substances, antibiotics may undergo metabolic modification in the animal body.

Ideally, analytical procedures would identify the breakdown products; however, most of the methods of assaying the products considered by the Committee are not capable of such discrimination and differential quantification, and measure only total antimicrobial activity. The Committee recommend that, in future, information on all products should cover this aspect of the problem, so that a more complete analytical and toxicological review can be made of the substances and their breakdown products.

Material balance studies for the antibiotic administered will in general be a measure of the magnitude of analytically undetected components. Direct toxicity due to breakdown and metabolic products is assessed in 2-year chronic toxicity studies, since such products are normally formed within the body during the long-term feeding process. However, it may also be necessary to carry out toxicity studies of breakdown products in concentrations higher than those that occur normally.

Evaluations

Acceptable daily intake

As at previous meetings the Committee decided to express an acceptable daily intake (ADI) where adequate information is available. It was further decided to use the terms "unconditional ADI", "conditional ADI", and "temporary ADI", where appropriate, in the evaluation of the antibiotics.

An unconditional ADI was allocated only to those substances for which the biological data available included the results of adequate short-term and long-term toxicological investigation and information on the biochemistry and metabolic fate of the compounds. A conditional ADI was

allocated either for specified uses or when the data fell short of the requirements for an unconditional ADI. A temporary ADI was allocated if the available data, while indicating that the use of a given substance is likely to be safe, were considered inadequate to justify a final conclusion. In such cases, the additional evidence required should be submitted within the stated time limit, or the Committee's conditional acceptance of the substance may be withdrawn.

Acceptable residues in human food

If adequate biological information about a given antibiotic residue is available, it may be possible to establish a definitive tolerance level. If this is done, further improvement in analytical methods should not necessitate any modification of the acceptable tolerance level. However, if the biological evidence for a given antibiotic residue is incomplete, it is not possible to establish a tolerance level. In such cases, the Committee will require that there be no detectable level in food (they will also state the smallest amount detectable by the recommended method). Since it is stated that the amount of the residue should be as low as possible in such cases, it necessarily follows that the smallest amount detectable will change if a more sensitive method of analysis is introduced, and more stringent control may be needed. Consequently, the user of antibiotics may suffer the inconvenience of having to modify his practice as analytical methods of increased sensitivity become available. This situation results from the lack of adequate long-term studies, which makes it impossible to establish definitive tolerance levels, and it is the position with many of the antibiotics considered in this report.

ANTIBIOTICS EVALUATED BY THE COMMITTEE

Evaluations of the following antibiotics will be found on the pages indicated.¹

	Page		Page
Aminoglycosides		Polypeptides	
Streptomycin, Dihydrostreptomycin, Kanamycin, Neomycin . . .	16	Bacitracin	30
Macrolides		Nisin	33
Erythromycin, Leucomycin, Oleandomycin, Spiramycin . . .	18	Polymyxin B	35
Tylosin	20	Tetracyclines	
Penicillins	23	Tetracycline, Chlortetracycline, Oxytetracycline	38
Polyenes		Other antibiotics	
Nystatin	25	Chloramphenicol	41
Pimaricin	27	Novobiocin	43

¹ Monographs have not been included on hygromycin or peptolides, since the Committee could not obtain the detailed information necessary for the preparation of specifications and the assessment of the sensitivity of available methods of analysis.

AMINOGLYCOSIDES

Streptomycin, Dihydrostreptomycin, Kanamycin, Neomycin

The aminoglycosides form a class of microbiologically, pharmacologically, and toxicologically related drugs. To this class belong streptomycin, dihydrostreptomycin, kanamycin, neomycin, and some other recently developed compounds. None of these drugs has found application in food preservation. Streptomycin, dihydrostreptomycin, kanamycin, and neomycin are used for growth purposes in animals. Kanamycin could not be fully evaluated toxicologically on the basis of the data that were available.

Uses

As food additive :	Not used
In food environment :	Not used
As feed additive for growth purposes :	Used in some countries
In veterinary medicine :	Used
In human medicine :	Used

Residues

Antibiotics of this group are poorly absorbed, and consequently their oral administration does not readily give rise to tissue residues, especially when they are used at levels of 5-20 ppm. Since they are not easily destroyed in the upper intestine, they exert their effects in the lower bowel.

Following parenteral administration, residues may remain for long periods ; for example, residues of streptomycin may remain for as long as 1 month (Durbin, C. G., unpublished report, 1968).

Biological data*Direct biological effects*

None of the aminoglycosides is readily absorbed from the gastrointestinal tract. The great majority of the clinical observations of the toxicity of these antibiotics (of which there have been a considerable number, particularly in relation to neomycin) need not further be analysed, since they were made following parenteral administration.

Other effects

Allergic reactions. Although many of the aminoglycosides were once regarded as having little tendency to cause allergy, many reports of local hypersensitivity have appeared in recent years. Streptomycin may produce

skin rashes in nurses and other persons who handle it. The extensive use of neomycin in various preparations for tropical use has led to a considerable increase in allergic reactions of the skin. In rare cases, hypersensitivity to the aminoglycosides may be severe.

Microbial resistance. Streptomycin resistance is a common finding during the clinical use of the drug. Feeding very small amounts (as low as 2 ppm) to animals may cause selective multiplication of resistant *E. coli*.¹ It has been shown that *E. coli*, *Salmonella*, and *Shigella* may carry resistance to all aminoglycosides, including streptomycin, in one R-factor.²

Evaluation and comments

Acceptable levels of residues in food

If members of this group are used they should not be allowed to give rise to detectable residue levels in human food. If the methods of analysis recommended by the Committee (see p. 4) are used, it will be possible to ensure that the residue levels in food for human consumption will be within the following limits (ppm):

<i>Antibiotic</i>	<i>Milk</i>	<i>Meat</i>	<i>Eggs</i>
Streptomycin and dihydrostreptomycin (calculated as base)	0-0.2	0-1.0	0-0.5
Neomycin (calculated as base)	0-0.15	0-0.5	0-0.2

Effects of residues

Microbiological effects. At the levels noted above, aminoglycoside residues are not likely to cause microbiological effects in the consumer. However, there is a possibility of the transfer of resistance among gram-negative organisms in animals receiving these antibiotics in feeds.²

Toxic effects. No acute toxic effects are likely. There is insufficient information with regard to long-term effects.

Allergic effects. At the levels noted above, aminoglycoside residues are not likely to cause allergic effects.

Recommendations

- (1) The use of aminoglycosides should be restricted to present practice.
- (2) The long-term effects of small amounts of those aminoglycosides that are used in animal feeds or for veterinary purposes should be further studied and the results reported to WHO within 5 years.

¹ Gurnée, P. A. M. (1963) Thesis, Utrecht.

² Anderson, E. S. & Datta, N. (1965) *Lancet*, 1, 407; Lebek, G. (1963) *Zbl. Bakt., I Abt. Orig.*, 188, 494.

(3) The importance of transfer of resistance resulting from the use of aminoglycosides in agricultural practice, especially in feeds, should be further investigated.

MACROLIDES

Erythromycin, Leucomycin, Oleandomycin, Spiramycin

Members of this class of antibiotics are mainly effective against gram-positive bacteria ; at least one, erythromycin, also exhibits activity against certain gram-negative bacteria.

Uses

As food additive :	Not used
In food environment :	Not used
As feed additive for growth purposes :	Used in some countries
In veterinary medicine :	All except leucomycin are generally used
In human medicine :	All except tylosin are used (see p. 20)

These antibiotics are used in the treatment of respiratory diseases of poultry, bovine mastitis, and gastroenteritis in pigs.

Residues

Absorption occurs readily ; no special feature of the residues has been noted.

Biological data

Direct biological effects

The members of this group are therapeutically important and their toxicity for man is low. The oral administration of erythromycin estolate, erythromycin stearate, or triacetyloleandomycin at a dosage of 1 g daily for 150-180 days caused only mild, transient, untoward effects. On the basis of present information, these antibiotics do not appear to constitute a direct public health problem when used at the recommended feed additive levels of 2-20 ppm.¹

Other biological effects

Allergic reactions. No serious allergic reactions, such as occur with penicillin and novobiocin, have been reported in man following the clinical use of any of these antibiotics.

¹ Ticktin, H. E. & Robinson, M. M. (1963) *Ann. N.Y. Acad. Sci.*, **104**, 1080.

Microbial resistance. Two types of resistance can result from the use of these compounds: (a) complete, in which the drug-induced resistance extends to all members of the class; and (b) partial, in which the drug-induced resistance is limited to fewer than all members.¹ Both types of resistance have been reported to be reversible.²

Evaluation and comments

Acceptable levels of residues in food

The following comments do not apply to leucomycin (see recommendation 3, below). If antibiotics of this group are used, they should not be allowed to give rise to detectable residues in human food. If the methods of analysis recommended by the Committee (see p. 4) are used, it will be possible to ensure that the residue levels in food for human consumption will be within the following limits (ppm):

<i>Antibiotic</i>	<i>Milk</i>	<i>Meat</i>	<i>Eggs</i>
erythromycin	0-0.04	0-0.3	0-0.3
oleandomycin	0-0.15	0-0.3	0-0.1
spiramycin	—	0-0.025	—

Effects of residues

Microbiological effects. Development of resistant organisms is not likely to be a problem with this group of antibiotics at the levels noted above.

Toxic effects. No acute toxic effects are likely. There is no reason to expect long-term effects, but the available evidence does not provide a satisfactory basis for evaluation.

Allergic effects. It is most unlikely that the levels noted above will cause allergic effects.

Recommendations

- (1) Macrolides should not be more extensively used than at present.
- (2) Adequate long-term studies of these antibiotics should be carried out and the results submitted to WHO within 5 years.
- (3) Additional data are required before leucomycin can be fully evaluated. The additional biological information should include the results of adequate toxicity studies and studies of bacterial resistance and cross resistance with other macrolide antibiotics.

¹ McGuire, J. M. et al. (1961) *Antibiot. and Chemother.*, **11**, 320; Desmyter, J. & Reybrouck, G. (1965) *Chemotherapy (Basel)*, **9**, 183.

² Ferrando, R. (1968) *Bibl. "Nutr. et Dieta" (Basel)*, **10**, 90.

Tylosin

Uses

As food additive :	Proposed ¹
In food environment :	Not used
As feed additive for growth purposes :	Used in some countries
In veterinary medicine :	Used in some countries
In human medicine :	Not used

Residues

Absorption occurs readily. No special feature of the residues has been noted.

Biological data

Direct biological effects

Biochemical aspects. In the rat, serum assay following single oral doses of 50 mg/kg of tylosin base showed peaks of 1 µg/ml or less after 1–2 hours. None was detected after 7 hours. Oral administration of 25 mg/kg to 2 dogs produced a low level of serum activity in one; about 2% of the dose was recovered from the urine of this dog. Intraduodenal administration of the same dose of tylosin base to 4 dogs produced a mean serum peak of 2 µg/ml in 30 min; urinary recovery averaged 7.2% in 5 hours. Following parenteral injection in rats and dogs, tylosin was found to be excreted in the bile and urine. The half-life of tylosin in the blood of the dog was 48 min. Tylosin base was non-antigenic in the guinea-pig (Anderson, R. C. et al., unpublished report, 1968).

Four adult human subjects given 2 or 5 mg/day of tylosin orally for 6 months showed no changes in the balance or antibiotic sensitivity of the faecal flora (Kuwahara, S., unpublished report, 1968). In 12 adult males given 20 mg/day of tylosin for 6 months, the balance and tylosin sensitivity of the faecal flora did not differ from those in a control group of 11 subjects given 20 mg/day of lactose (Malin, B. & Silliker, J. H., unpublished report, 1968).

Acute toxicity. The results of some studies of the acute toxicity of tylosin are outlined in Table 1.

Short-term studies. Three groups of 24 rats were fed a special canned cooked food containing 0, 1000, and 10 000 ppm of tylosin, respectively, for 130 days; control groups received a standard laboratory diet or an

¹ Since tylosin is active against clostridia and other spore-forming bacteria, it has been proposed that it be directly added to certain foods as an aid in processing.

TABLE 1
ACUTE TOXICITY OF TYLOSIN *

Antibiotic	Animal	Route of administration	LD ₅₀ (mg per kg of body weight)
tylosin tartrate	mouse	oral	> 6 200
	mouse	subcutaneous	1 354
	mouse	intravenous	589
	rat	oral	> 6 200
	rat	intravenous	695
tylosin hydrochloride	mouse	intravenous	582
tylosin lactate	mouse	intravenous	589
tylosin base	mouse	oral	> 5 000
	rat	oral	> 5 000
	dog	oral	> 800

* Data from Anderson, R. C. et al. (1966) *Food Cosmet. Toxicol.*, 4, 1.

uncooked special diet. No difference was seen in the survival, rate of weight gain, blood, urine, or hepatic tissues of the groups.¹

Groups of 8 dogs were given oral doses of 0, 1, 10, or 100 mg per kg of body weight per day for 2 years; additional groups of 4 dogs were given 200 or 400 mg/kg/day. Salivation and occasional vomiting were seen in those receiving 200 and 400 mg/kg/day. By the end of the test, BSP retention was high in one of the dogs receiving 400 mg/kg and in 2 of those receiving 100 mg/kg/day. Otherwise, no difference was seen between the groups in survival, appearance, maintenance of body weight, blood picture, faecal flora (studied periodically), or in gross and microscopic appearance of tissues (examined subsequent to the test). Serum antibiotic activity was assayed in the dogs during the test, immediately before and 2 hours after administration of the drug. No activity was detected in dogs receiving 1 mg/kg/day, and only rarely was any detected before administration of the drug in those receiving 10 mg/kg/day. After 2 years, pre-dosing levels in dogs receiving 10, 100, 200, and 400 mg/kg were less than 0.1, from less than 0.1 to 0.43, 0.19–0.75, and 0.26–0.97 µg/ml, respectively; post-dosing levels were from less than 0.01 to 1.45, from less than 0.10 to 14.00, 0.68–5.20, and 2.30–5.90 µg/ml, respectively.¹

Long-term studies. Groups of 5–14 male and female mice were fed diets containing 0%, 0.1%, 1%, and 10% of tylosin for 1½ years. Food refusal and weight loss were seen during the first 2 weeks in those receiving 10%

¹ Anderson, R. C. et al. (1966) *Food Cosmet. Toxicol.*, 4, 1.

tylosin, but the terminal body weights were no different from those of other groups. No effect of the compound was seen at any level on survival, tumour incidence, and organ histology (Tsubura, Y. et al., unpublished report, 1968).

Groups of 8 male and 8 female rats were fed diets containing 0%, 0.01%, 0.1%, 1%, and 10% of tylosin for 20 months without signs of toxicity or adverse effect on food consumption, survival, rate of weight gain, or the gross and microscopic appearance of major organs.¹

Groups of 20-50 rats were fed diets containing 0%, 0.001%, 0.01%, 0.1%, 1%, 2%, 5%, 10%, and 20% of tylosin base for 2 years. Few of the animals receiving 20% survived for 1 year; the rate of body weight gain was adversely affected in those receiving 10%, but no effect on the blood or on the gross and microscopic appearance of major organs was seen in those receiving up to and including 5%. Groups of 10 females and 5 males were selected from the 0 and 1% groups and mated throughout the 2-year period; second-litter animals were used in a concurrent three-generation reproduction trial at the same dosage level. No effect of the drug on the fertility, gestation, lactation, or viability of young rats was observed.²

Groups of 15 males and 10 females were fed diets containing 0.01% and 1% of tylosin, and groups of 30 males were fed diets containing 0.001%, 0.1%, and 10% for 500 days. The control group consisted of 30 males and 10 females. Rate of weight gain was adversely affected in those receiving 10%, with no effect on food consumption. Otherwise, no adverse effect of the drug was seen on peripheral blood, serum enzymes, survival, weight ratios of major organs, and post-test gross and microscopic appearance of tissues (Uraguchi, K., unpublished report, 1968).

Other biological effects

Allergic reactions. No information is available, since tylosin has not been used in man.

Microbial resistance. Although tylosin cross-resistance is not complete with all macrolides, the incidence of cross-resistance to therapeutically important macrolides is sufficiently significant³ to preclude the use of tylosin as a direct food additive unless it can be used to solve an extremely important problem.

Evaluation and comments

Acceptable levels of residues in food

If tylosin is used in animal feed or in veterinary medicine, it should not be allowed to give rise to detectable residues in human food. If the methods

¹ Aiso, K. et al. (1966) *Jap. J. Hyg.*, **20**, 383.

² Anderson, R. C. et al. (1966) *Food Cosmet. Toxicol.*, **4**, 1.

³ McGuire, J. M. et al. (1961) *Antibiot. and Chemother.*, **11**, 320; Zanella, A. (1966) *Arch. vet. ital.*, **17**, 465.

of analysis recommended by the Committee (see p. 4) are used, it will be possible to ensure that the residues in meat for human consumption will not exceed 0.2 ppm.

Effects of residues

Microbiological effects. Development of antibiotic-resistant organisms is not likely to occur at levels as low as that noted above, but it might be brought about if detectable levels of tylosin were to exist in food.

Toxic effects. No direct toxic effects are likely to occur.

Allergic effects. Allergic effects are most unlikely to be caused by levels as low as that noted above.

Recommendation

Tylosin should not be used for any purpose that might result in the presence of detectable residues in human food, unless its use is necessary to solve an extremely important problem that might modify the balance of benefit and risk.

PENICILLINS

Penicillins are a class of antibiotics produced by some members of the genus *Penicillium*. They are active primarily against gram-positive bacteria. Since penicillins are widely used in man and animals, careful consideration must be given to any potential hazards to the human consumer of animal by-products containing them.

Uses

	<i>Benzylpenicillin</i>	<i>Other penicillins</i>
As food additive :	Not used	Not used
In food environment :	Not used	Not used
In feeds for growth purposes :	Used	Not used
In veterinary medicine :	Used	Used
In human medicine :	Used	Used

Residues

Benzylpenicillin is not stable in solution and tends to be destroyed in the gastrointestinal tract. Penicillin G is readily excreted and rapidly passes out in urine, milk, and other secretions.

An important way in which penicillin may enter food for human consumption is the use of preparations containing penicillins for the treatment of mastitis. Penicillins may be consumed in milk that has not been withheld from the market until free of antibiotic activity.

Biological data

Direct biological effects

The direct toxicity of the penicillins now used in both man and animals does not appear to be serious, since penicillin is among the least toxic of antibiotics. In the treatment of subacute bacterial endocarditis, doses of 10 million units or more of benzylpenicillin have been given daily to human subjects for over 6 weeks without toxic reactions.¹

Other biological effects

Allergic reactions. Hypersensitivity reactions in man caused by penicillins are more frequently reported than those caused by any other antibiotic used in man or animals. (However, it should be pointed out that penicillins have received much wider use than any other antibiotic.) Low levels of orally administered penicillin have caused such reactions. For example, it has been reported that as little as 40 units (0.024 mg) of benzylpenicillin taken by mouth may elicit an allergic reaction in highly susceptible individuals,² and that fatal and near fatal reactions have resulted from the sucking of lozenges containing 20 000 units of penicillin.³ Allergic dermatitis was caused in two patients by the consumption of milk containing penicillin.⁴

Microbial resistance. Since benzylpenicillin, in the quantities added to animal feeds, has no inhibitory influence on gram-negative intestinal bacteria, the nonmedical use of this antibiotic is not likely to cause a preferential growth of such bacteria that carry R-factors. Ampicillin, however, does act against gram-negative bacteria and participates in the transfer of resistance in bacteria that carry R-factors,⁵ and the nonmedical use of this antibiotic should be approached more cautiously.

Evaluation and comments

Acceptable levels of residues in food

If penicillins are used, they should not be allowed to give rise to detectable residues in human food. If the methods of analysis recommended by the Committee (see p. 4) are used, it will be possible to ensure that the residue levels in food for human consumption will not exceed the following values (ppm): milk, 0–0.006; meat, 0–0.06; and eggs, 0–0.018.

¹ Goodman, L. S. & Gilman, A. (1965) *The pharmacological basis of therapeutics*, 3rd ed., New York, Macmillan.

² Siegel, B. B. (1959) *Bull. Wld Hlth Org.*, **21**, 703.

³ Rosenthal, A. (1958) *J. Amer. med. Ass.*, **167**, 1118.

⁴ Vickers, R.R. et al. (1958) *Lancet*, **1**, 351.

⁵ Anderson, E. S. & Datta, N. (1965) *Lancet*, **1**, 407; Lebek, G. (1967) *Path. et Microbiol. (Basel)*, **30**, 1015; Voogd, C. E. et al. (1968) *Antonie v. Leeuwenhoek*, **34**, 357.

Effects of residues

Microbiological effects. Since benzylpenicillin does not act against gram-negative organisms, it is not involved in the transfer of resistance. In general, it seems unlikely that residues of benzylpenicillin at the levels noted above will cause any problem. However, this is not necessarily true of the newer penicillins, such as ampicillin, which does have an effect on gram-negative micro-organisms and might, therefore, become involved in the transfer of resistance. Penicillin residues in milk may interfere with the manufacture of certain dairy products such as cheese and yoghourt.

Toxic effects. It is most unlikely that any toxic effects will be caused by levels as low as those noted above.

Allergic effects. Allergic effects are unlikely to be caused by levels as low as those noted above.

Recommendations

- (1) Benzylpenicillins should be considered acceptable as at present used.
- (2) The possible effects of ampicillin on the development of antibiotic resistance in micro-organisms should be further investigated.
- (3) Special precautions should be taken to prevent the contamination of milk with penicillin.

POLYENES

Nystatin

Nystatin is an antifungal antibiotic that is derived from *Streptomyces noursei*.

Uses

As food additive :	Not used
In food environment :	Used in some countries on banana skins
As feed additive for growth purposes :	Not generally used
In veterinary medicine :	Used as antifungal agent
In human medicine :	Used as antifungal agent

Some countries permit the use of nystatin for the treatment of banana skins and, under special circumstances, the surface of meat.

Residues

Nystatin is poorly absorbed and is unlikely to give rise to tissue residues. However, it may find its way into food for human consumption if it is used indiscriminately for veterinary purposes.

Biological data*Direct biological effects*

Nystatin appears to be poorly absorbed from the gastrointestinal tract. Information on its biological breakdown was not available to the Committee.

Nystatin is lethal to *Candida albicans* at a concentration of 5–20 units/ml, but does not affect white blood cells at 30 units/ml or other nonfungal micro-organisms at 250 units/ml (1 µg of nystatin is equivalent to 2.8 units).

In man, occasional gastrointestinal symptoms, including nausea, have been observed when high therapeutic doses of 1–2 million units have been administered daily for a week or more.¹

Other biological effects

Microbiological effects. Resistance to nystatin did not occur *in vitro* or in fungi isolated from patients during or after treatment. No cross resistance with other antifungal antibiotics was observed.¹

Evaluation and comments*Acceptable levels of residues in food*

Since nystatin is used only externally and not in food itself, only trace amounts are likely to be present in food for human consumption. If the methods of analysis recommended by the Committee (see p. 4) are used, it will be possible to ensure that the residue levels in such food will not exceed the following levels (ppm): milk, 0–1.1; meat, 0–7.1; and eggs, 0–4.3.

Effects of these residues

Microbiological effects. The development of resistant fungi is unlikely.

Toxic effects. No toxic effects are likely.

Allergic effects. No allergic effects have been reported.

Recommendations

- (1) The present restricted external use of nystatin should be permitted.

¹ Stewart, G. T. (1956) *Brit. med. J.*, **1**, 658.

(2) The use of nystatin should not be extended until more adequate studies on its breakdown and toxicology have been carried out.

Pimaricin

Pimaricin is a polyene macrolide antibiotic derived from cultures of *Streptomyces natalensis*.

Uses

As food additive :	Not used, but has been proposed
In food environment :	Used in some countries
As feed additive for growth purposes :	Not used
In veterinary medicine :	Not used
In human medicine :	Limited topical use

Pimaricin is applied to cheese rind to control unwanted fungal growth. It does not penetrate into the cheese, but it is retained in the outer 1 mm of the rind, which is not normally eaten. After a period of 5–10 weeks the pimaricin disappears; during this time, the rind of the cheese hardens, becoming much less susceptible to fungal infection. It seems unlikely that more than trace amounts of pimaricin will find their way into food.

The use of pimaricin for other purposes—e.g., the prevention of fungal growth on the surface of sausages—has been proposed, and it seems that this antibiotic may have wide and useful application in food technology. However, further information is needed before such other uses can be considered. The notes and evaluation that follow apply only to pimaricin as used on the rind of hard cheese.

Biological data

Direct biological effects

Biochemical aspects. Since pimaricin appears to be rather unstable, much more information on its breakdown would be needed before its use as a direct food additive could be contemplated.

Acute toxicity. The results of some studies of the acute toxicity of pimaricin are given in Table 2.

Acute toxicity studies have also been carried out on some breakdown products of pimaricin, which appear to have slightly greater toxicity in the mouse than pimaricin itself; however, adequate comparison is not possible. Skin and eye studies have revealed no irritant or sensitising action of pimaricin.¹

¹ Levinskas, G. J. et al. (1966) *Toxicol. appl. Pharmacol.*, **8**, 97.

TABLE 2
ACUTE TOXICITY OF PIMARICIN *

Animal	LD ₅₀ (mg per kg of body weight)
mouse	1500 <i>a, b</i> >2500 <i>a</i>
rat male female	1500 <i>a, b</i> 2730 <i>c</i> 4670 <i>c</i>
guinea-pig	450 <i>a, b</i>
rabbit	1420 <i>c</i>
dog	>1000 <i>a</i>

* The drug was administered orally in all studies.

a Royal Netherlands Fermentation Industries, unpublished report, 1965.

b Struyk, A. P. et al. (1958) *Antibiot. Ann.*, 1957-58, p. 878.

c Levinskas, G. J. (1966) *Toxicol. appl. Pharmacol.*, 8, 97.

Short-term studies. Oral administration of 50–70 mg of pimaricin per kg of body weight daily for 5–10 weeks had no effect on the growth, blood, or tissues of rats. A daily oral dose of 150 mg per kg for 9 weeks caused some growth inhibition, and a daily dose of 500 mg per kg caused 30% of the rats to die within 2 weeks.¹

In a study of the effects of pimaricin on reproduction, groups of 20 male and 40 female rats being fed diets containing 0 or 1000 ppm of pimaricin were mated after 48, 184, and 260 days. The treated rats were comparable to or better than the controls in fertility, gestation, lactation, and viability indices. Weanling weights were reduced in the pimaricin-treated rats. The offspring of the group mated after 184 days (F_{1b} generation) were weaned to the diets of their parents, and 4 females on each diet were mated. The fertility index was low in those receiving pimaricin (although the significance of this result is doubtful in view of the small number of animals involved), and the weanling weights were again reduced. The type and incidence of foetal abnormalities were similar in the two groups.²

Groups of 3 male and 3 female dogs were fed diets containing 0, 125, 250, or 500 ppm of pimaricin for 2 years. Apart from a slight impairment of body-weight gain in those receiving 500 ppm, no pimaricin-related difference in the groups was noted with respect to food acceptance, blood

¹ Struyk, A. P. et al. (1958) *Antibiot. Ann.*, 1957-58, p. 878.

² Levinskas, G. J. (1966) *Toxicol. appl. Pharmacol.*, 8, 97.

cells, blood alkaline phosphatase, blood glucose, blood urea nitrogen, BSP retention, or organ weights. There were no significant gross or microscopic lesions attributed to pimaricin.¹

In man, nausea, vomiting, and diarrhoea have occasionally been caused by oral doses of 300–400 mg daily; no changes in the peripheral blood have been observed (Royal Netherlands Fermentation Industries, unpublished report, 1966). More than 2500 persons have received pimaricin without developing sensitivity or adverse reactions (Lubbers, G. S., unpublished report, 1967; Mezzadra, G., unpublished report, 1965). Human subjects have received as much as 400–600 mg of pimaricin orally daily without adverse effects. Doses above 600 mg/day have caused temporary nausea or flatulence (Lubbers, op. cit.; Germeraad, W. F., unpublished report, 1966). In a group of 10 patients with systemic mycoses who received oral doses of 50–1000 mg/day for 13–180 days, nausea, vomiting, and diarrhoea occurred in those receiving 600–1000 mg/day.²

Long-term studies. Groups of 35–40 male and 35–40 female rats were fed diets containing 0, 125, 250, 500, and 1000 ppm of pimaricin for 2 years. At the highest level both male and female rats showed growth inhibition. Food intake was reduced in the early part of the study, but overall food intake during the 2-year period was not affected. Apart from the findings already mentioned, there were no adverse effects on body weight, food consumption, survival, organ weights, blood, or tissues. The distribution of tumours was such that pimaricin was not considered to be connected with their development.¹

Other biological effects

Allergic effects. No history of adverse reaction was found in 73 workers engaged in the manufacture of pimaricin. No allergic reaction was obtained when cutaneous or intradermal challenge doses were administered to 71 workers and to 37 persons not engaged in the manufacture of pimaricin (Malten, K. E., unpublished report, 1967).

Microbial resistance. Apart from the experimental isolation of two strains of *Candida albicans* that had become partially resistant as a result of adaptation, no evidence of the development of fungi resistant to pimaricin has been obtained (Royal Netherlands Fermentation Industries, 1965). The use of pimaricin for the treatment of fungal diseases in man has not resulted in the appearance of resistant fungi or yeasts (Goslings, W. R. O., unpublished report, 1965). No cross resistance has been found between amphotericin and pimaricin.³ No change in sensitivity to erythromycin was

¹ Levinskas, G. J. et al. (1966) *Toxicol. appl. Pharmacol.*, **8**, 97.

² Newcomer, V. D. et al. (1960) *Ann. N.Y. Acad. Sci.*, **89**, 240.

³ Sorensen, L. J. et al. (1959) *Antibiot. Ann.*, 1958–59, p. 920.

demonstrated after 10 transplants of 10 different bacterial strains, all sensitive to erythromycin, when pimarcin was included in the culture media.

Evaluation and comments

Acceptable levels of residues in food

On the basis of available toxicological data, a conditional ADI of 0-0.25 mg of pimarcin per kg of body weight is established. On this basis, 15 ppm in cheese would be acceptable, assuming that a man would eat no more than 1 kg of cheese per day. If the method of analysis recommended by the Committee (see p. 4) is used, such a level would not be detectable in a homogenate of whole cheese and rind.

However, a better estimate of the amount of pimarcin present in a given cheese could be obtained by weighing and homogenizing the cheese and rind separately and determining the pimarcin in aliquots of each homogenate. From the results, the concentration in the cheese as a whole could be calculated.

Effects of residues

Microbiological effects. There has been no demonstration of significant resistance in fungi.

Toxic effects. No toxic effects occur at the level noted above.

Allergic effects. No allergic effects occur at the level noted above.

Recommendations

(1) The external application of pimarcin to hard cheese should be considered acceptable provided it does not result in daily intakes by human beings exceeding 0.25 mg per kg of body weight, a figure that is suggested as a conditional ADI.

(2) Further information on breakdown products and their possible biological effects should be obtained before consideration is given to extending the use of pimarcin.

POLYPEPTIDES

Bacitracin

Bacitracin, an antibiotic produced by *Bacillus subtilis*, is active principally against gram-positive micro-organisms.

Uses

As food additive :	Not used
In food environment :	Not used

As feed additive for growth purposes :	Used in some countries
In veterinary medicine :	Used
In human medicine :	Used topically

Residues

Bacitracin residues are readily absorbed in small amounts and may appear in the urine.

Biological data

Direct biological effects

Biochemical aspects. After 4 consecutive daily doses of 5 mg of zinc-¹⁴C-bacitracin were given to each of a group of male rats, about 90% of the radioactivity was eliminated in the faeces during the 96 hours following the last dose (Craig, G. et al., unpublished report, 1967). The administration of bacitracin to dogs at a level of 10 000 units¹ per kg of body weight per day resulted in plasma levels of up to 0.085 units/ml and urinary levels of up to 0.22 units/ml.² Administration of bacitracin orally to human beings at doses of 60 000–180 000 units did not produce detectable levels in the blood, but the antibiotic was detected in concentrations of 0.125–1.0 unit/ml in the urine.³

Acute toxicity. The results of some studies of the acute toxicity of bacitracin are given in Table 3.

TABLE 3
ACUTE TOXICITY OF BACITRACIN

Antibiotic	Animal	Route of administration	LD ₅₀ (mg per kg of body weight)
bacitracin	mouse	oral intraperitoneal	>3 750 ^a 200–650 ^a
	rat	intraperitoneal	190 ^a
bacitracin methylene disalicylate	rat	oral	>10 000 ^b

^a Scudi, J. V. & Anapol, W. (1947) *Proc. Soc. exp. Biol. (N.Y.)*, **64**, 503.

^b Radomski, J. L. et al. (1954) *Antibiot. and Chemother.*, **4**, 304.

¹ The international unit has been defined as follows: 42 000 IU = 1 g of bacitracin.

² Bond, G. C. et al. (1948) *Proc. Soc. exp. Biol. (N.Y.)*, **68**, 395.

³ Longacre, A. B. & Waters, R. M. (1951) *Surg. Gynec. Obstet.*, **92**, 213.

Short-term studies. Groups of mice were given daily oral doses of 0, 31 250, 62 500, and 125 000 units per kg of body weight for 30 days without gross or microscopic hepatic, renal, or other effects.¹

Groups of 5 male and 5 female rats were fed diets containing 0%, 0.11%, or 0.5% of bacitracin methylene disalicylate for 3 months; control groups were fed diets containing 0.1% or 0.5% of methylene disalicylic acid. None of the test group showed any adverse effect on survival, body-weight gain, or gross and microscopic appearance of major organs.² Groups of 29 male and 29 female rats were fed diets containing 0, 20, 200, or 1000 ppm of zinc bacitracin for 12 weeks without ill effect; no differences were observed in the blood, urine, hepatic function, and microscopic structure of major organs in the different groups, and no antibiotic activity was detected in the livers of the test animals (Kämmerer, K., unpublished report, 1967).

Dogs were given daily oral doses of 0, 5 000, 10 000, or 25 000 units per kg of body weight; no gross or microscopic changes in the kidney, liver, spleen, or gastrointestinal tract were observed.¹

Long-term studies. No data on long-term studies are available.

Other biological effects

Allergic reactions. Guinea-pigs are sensitive to bacitracin.³ Dermal sensitivity to bacitracin has been observed in 7.8% of 17 500 patients.⁴

Microbial resistance. Some resistance to bacitracin may occur in gram-positive micro-organisms. There is low cross-resistance to other antibiotics.⁵

Evaluation

Acceptable levels of residues in food

If bacitracin is used, it should not be allowed to give rise to detectable residues in food for consumption by humans. If the methods of analysis recommended by the Committee (see p. 4) are used, it will be possible to ensure that the residues in food will not exceed the following limits: milk, 0–1.2 IU/ml; meat, 0–0.7 IU/g; and eggs, 0–4.8 IU/g (1 mg of bacitracin = 42 IU).

Recommendations

- (1) Bacitracin should be considered acceptable as at present used.

¹ Payne, H. G. et al. (1951) *Antibiot. and Chemother.*, **1**, 387.

² Radomski, J. L. et al. (1954) *Antibiot. and Chemother.*, **4**, 304.

³ Epstein, S. & Wenzel, F. J. (1962) *Arch. Derm.*, **86**, 183.

⁴ Pirila, V. et al. (1967) *Acta dermat.-venereol. (Stockh.)*, **47**, 419.

⁵ Szybalski, W. (1953) *Antibiot. and Chemother.*, **3**, 1095.

(2) Long-term studies of the effects of low-level bacitracin residues should be carried out and the results submitted to WHO within 5 years.

Nisin

Nisin is a polypeptide antibiotic produced by *Streptococcus lactis* of the Lancefield group N.¹

Uses

As food additive :	Used in some countries
In food environment :	Not used
As feed additive for growth purposes :	Not used
In veterinary medicine :	Not used
In human medicine :	Not used

In some countries, nisin has been accepted for use as a direct food additive in the production of cheese. It is particularly useful for preventing the growth of clostridia and consequent "blowing". The use of nisin in certain other foods is also permitted in some countries. The level recommended for use in foods is 20 units² per g, although greater amounts are sometimes used. Nisin occurs naturally in milk and milk products.

Biological data

Direct biological effects

Biochemical and in vitro studies. No antibiotic was detected in human saliva 10 min after the consumption of chocolate milk containing 200 units/ml of nisin.³ Nisin is readily inactivated by pancreatin at pH 8.0 when held at a temperature of 37°C for 15–30 min.⁴

Crude nisin had no effect on the mobility of leucocytes and did not cause haemolysis at a concentration of 1 : 250.¹ Purified nisin caused no haemolysis in 22 h at a concentration of 20 000 units/ml.⁵

Acute toxicity. The results of some studies of acute toxicity resulting from the administration of nisin are given in Table 4.

¹ Mattick, A. T. R. & Hirsch, A. (1947) *Lancet*, **2**, 5.

² The unit has been redefined by Tramer & Fowler (*J. Sci. Fd Agric.*, 1964, **15**, 522) in terms of a standard preparation; it is approximately the same as that described by Berridge (*Biochem. J.*, 1949, **45**, 486).

³ Claypool, L. et al. (1966) *J. Dairy Sci.*, **49**, 314.

⁴ Heinemann, B. & Williams, R. (1966) *J. Dairy Sci.*, **49**, 312.

⁵ Mattick, A. T. R. & Hirsch, A. (1949) *Lancet*, **2**, 190.

TABLE 4
ACUTE TOXICITY OF NISIN

Animal	Route of administration	LD ₅₀ (units per kg of body weight)
rat	oral	> 1 000 000 ^a
	intraperitoneal	> 1 000 000 ^a
rabbit	intramuscular	800 000 ^b
	subcutaneous	1 000 000 ^b

^a Frazer, A. C. et al. (1962) *J. Sci. Fd Agric.*, **13**, 32.

^b Mattick, A. T. R. & Hirsch, A. (1949) *Lancet*, **2**, 190.

Short-term studies. Groups of 10 weanling rats were fed for 12 weeks on diets containing 20%, 30%, or 40% of cheese containing either no nisin or about 100 000 units of nisin per g; this cheese was the sole source of protein. The nisin intakes were 1.2×10^6 , 1.8×10^6 , and 2.4×10^6 units, respectively, per kg of body weight. No significant differences were observed in the rate of weight gain in the control and the experimental groups. No changes were observed in the clinical state or behaviour of the animals, and no abnormalities were found at autopsy or on histological study of the main organs. A hydrolysate of nisin was similarly studied with the same result: 3.3×10^6 units of either nisin or nisin hydrolysate were added to each kg of the diet; a control group received no nisin. No differences were found between the control and experimental groups.¹

Long-term studies. Groups of 15 male and 30 female rats were fed diets containing 0, 33 300, or 3 330 000 units of nisin per kg of body weight for their life-span (2 years). In the first series of experiments, some depression of the rate of weight gain was observed in the male animals. Since it was thought that this effect might be related to the antibiotic, a hydrolysate of nisin was also studied. (However, subsequent studies in which 3 300 000 units of nisin per kg of body weight, or the equivalent amount of nisin hydrolysate, were administered, showed no reduction of the rate of weight gain.) No differences were found in the survival and reproductive performance of the control and experimental groups, or in the autopsy and histological findings. A significant increase in kidney size was observed in the animals on the higher dosage of nisin when they were caged individually, but not when the animals were caged in fives. Detailed studies showed no differences in hepatic, renal, or gastrointestinal function in the control and experimental groups.¹

¹ Frazer, A. C. et al. (1962) *J. Sci. Fd Agric.*, **13**, 32.

Other biological effects

Allergic effects. Guinea-pigs were sensitized to nisin by intraperitoneal injection ; no sensitization occurred after oral administration. Nisin caused sensitization in rabbits when administered parenterally, but not when given by mouth. The toxicity of a hydrolysate has also been studied.¹ There is no evidence of sensitization to nisin in human subjects.

Microbial resistance. Extensive microbiological studies have not shown any cross-resistance in micro-organisms that might affect the therapeutic use of other antibiotics. No effects could be demonstrated on the intestinal flora ; the antibiotic activity is rapidly destroyed by proteolytic digestion in the upper part of the gastrointestinal tract.

Evaluation and comments*Acceptable levels of residues in food*

The available evidence indicates that a level of 3 300 000 units of nisin per kg of body weight has no adverse effect. This finding permits an unconditional ADI to be set at 33 000 units per kg of body weight.

Effects of residues

At the level noted above, nisin has no microbiological, toxic, or allergic effects.

Recommendation

The use of nisin should be considered acceptable, the unconditional ADI being 0-33 000 units per kg of body weight.

Polymyxin B

Polymyxin B is one of a group of polypeptide antibiotics that are produced by strains of *Bacillus polymyxa*. These antibiotics are bactericidal and are generally active against gram-negative micro-organisms.

Uses

As food additive :	Not used
In food environment :	Not used
As feed additive for growth purposes :	Used in some countries

¹ Frazer, A. C. et al. (1962) *J. Sci. Fd Agric.*, **13**, 32.

In veterinary medicine :	Used
In human medicine :	Used topically

Polymyxin B is mainly used in combination with other agents for the treatment of mastitis in cattle and for the treatment of other infections with gram-negative organisms, particularly *Pseudomonas*.

Residues

Polymyxin B is not readily absorbed from the gastrointestinal tract. Only trace residues are found in milk or other foods of animal origin obtained 72 hours after administration.

Biological data

Direct biological effects

Biochemical and in vitro studies. No polymyxin could be demonstrated by bioassay in the blood or faeces of rabbits up to 48 hours after single oral doses of 4 mg/kg.¹ In groups of mice maintained for 1 week on diets containing a daily dose of 1 mg of polymyxin, it was estimated that about 1% of the dose was excreted in an active form in the faeces.²

No polymyxin was detectable in the blood of rabbits given oral doses of 10 mg/kg twice daily, of dogs given 50 mg/kg twice daily, or of adult humans given 100 mg every 4 hours. In mice given lethal doses, the blood concentrations at death were found to be about 15 µg/ml; the LD₅₀ was about 1000 mg/kg. Small amounts of polymyxin were detectable in the bloodstream following oral administration to new-born guinea-pigs and calves. On the second day of treatment, small amounts of polymyxin were detected in the faeces of rabbits given 10 mg/kg twice daily, and 128 µg/kg were found by the third day.³

Acute toxicity. The results of a study⁴ of acute toxicity resulting from the administration of polymyxin B are given in Table 5.

Short-term studies. Polymyxin B, administered by subcutaneous injection in single doses of 10 mg/kg to rats or in 4 daily doses of 3 mg/kg and 1 mg/kg to rabbits and dogs, respectively, did not affect the urinary

¹ Clinical dosages of polymyxin are stated in terms of international units. However, in the literature dosages have frequently been referred to in terms of what is believed to be the weight of pure base equivalent, and it should be noted that 1 mg is equivalent to 10 000 international units.

² Brownlee, G. & Bushby, S. R. M. (1948) *Lancet*, **1**, 127.

³ Brownlee, G. et al. (1952) *Brit. J. Pharmacol.*, **7**, 170.

⁴ Bacharach, A. L. et al. (1959) *J. Pharm. Pharmacol.*, **11**, 737.

TABLE 5
ACUTE TOXICITY OF POLYMYXIN B

Animal	Route of administration	LD ₅₀ (mg per kg of body weight)
mouse		
male	oral	713
female	oral	1050
male	intraperitoneal	24
female	intraperitoneal	24

excretion of protein, while identical doses of polymyxin A caused marked proteinuria associated with extensive renal tubular damage.¹

Long-term studies. No data from long-term studies are available.

Observations in man. The usual adult oral dose is 75–100 mg 4 times a day.

No antibiotic was detected by bioassay in the blood of human subjects following oral administration of 100 mg every 4 hours for short periods.² No adverse reactions or signs of neurotoxicity were seen in 9 children who received a daily oral dose of 15–20 mg per kg of body weight for 10 days.³ Kutscher et al., in a review⁴ of published results of clinical trials, have listed the main adverse reactions that may occur following parenteral administration of polymyxin B.

Other biological effects

Microbiological effects. The development of resistance to polymyxin B in *Salmonella typhi* has been demonstrated *in vitro* under certain conditions,⁵ and cross-resistance with colistin has been produced in *Escherichia coli*.⁶ The development of low or moderate resistance to polymyxin B *in vitro* by *Mycobacterium ranae* was accompanied by the development of a moderate degree of cross-resistance to circurlin and licheniformin and a striking degree of cross-resistance to streptomycin.⁷

¹ Brownlee, G. et al. (1949) *Ann. N.Y. Acad. Sci.*, **51**, 952.

² Brownlee, G. et al. (1952) *Brit. J. Pharmacol.*, **7**, 170.

³ Liebermann, D. & Jawetz, E. (1951) *Pediatrics*, **8**, 249.

⁴ Kutscher, A. H. et al. (1954) *J. Allergy*, **25**, 135.

⁵ Brownlee, G. & Bushby, S. R. M. (1948), *Lancet*, **254**, 127.

⁶ Monnier, J. J. & Bourse, R. (1961) *Ann. Inst. Pasteur*, Suppl. 4, p. 59.

⁷ Szybalski, W. & Bryson, V. (1954) *Amer. Rev. Tuberc.*, **69**, 267.

Evaluation*Acceptable levels of residues in food*

If polymyxin B is used, it should not be allowed to give rise to detectable residues in human food. If the methods of analysis recommended by the Committee (see p. 4) are used, it will be possible to ensure that the residues in food will not exceed the following limits: milk, 0-2 IU/ml; meat, 0-5 IU/g; eggs, 0-5 IU/g.

Recommendations

- (1) Polymyxin B should be considered acceptable as at present used.
- (2) Long-term studies of the effects of low residue levels should be carried out and the results submitted to WHO within 5 years.

TETRACYCLINES

Chlortetracycline, Oxytetracycline, and Tetracycline

The tetracyclines have a broad spectrum of antibacterial activity. Although as many as seven different tetracyclines are available for medical use, only three of them—chlortetracycline, oxytetracycline, and tetracycline—have been widely used as animal-feed additives or as preservatives added to the food environment (e.g., the ice used to chill fish).

Uses

As food additives:	Not used, but has been suggested ¹
In food environment:	Used for fish and poultry
As feed additives for growth purposes:	Used
In veterinary medicine:	Used
In human medicine:	Used

Residues

Tetracyclines are absorbed from the gastrointestinal tract and may become firmly attached to bones and teeth. Such residues in bones and teeth cannot be readily leached out into water. Tetracyclines are inactivated by heat, but the properties of the breakdown products are not fully known. A residue of 7 ppm may be reduced to less than 1 ppm by cooking.

The addition of tetracyclines to animal feedstuffs in amounts of 5-20 ppm does not seem to create a residue problem. However, such a problem is

¹ Limited use has been made of chlortetracycline and oxytetracycline hydrochloride as an aid in the preservation of certain foods.

likely to occur when these drugs are used for food preservation. Improper use of the drug in veterinary medicine may also give rise to tissue residues.

Biological data

Direct biological effects

There is no essential difference in the toxic properties of tetracycline, oxytetracycline, and chlortetracycline. The binding of the tetracyclines to calcium may result in their deposition during mineralization in bone and teeth tissues, which may lead to inhibition of skeletal growth and to discolouration and even hypoplasia of the deciduous and permanent teeth. Many reports have been made in different countries of the damage caused to the teeth of children by tetracycline therapy or prophylaxis instituted at any time between the fourth month of pregnancy and the seventh or eighth year of life.¹ Detectable amounts of tetracycline have been found in the bones of pigs, calves, and chickens fed diets containing 5-80 ppm of the drug; tetracycline was detected in the bones of chickens that received only 5 ppm of the drug for no more than 3 days.² Other workers³ found 5.5 ppm of tetracycline in the bones of chickens receiving 9.2 ppm in the feed for 56 days, 0.52 ppm in the bones of swine fed 30 mg per day for 96 days, and 1.79 ppm in the bones of calves fed 60 mg per day for 56 days. The antibiotic was not leached out of bone even after boiling for 90 minutes at different pH values.³

Since the resorption of bone tissue and replacement by new calcifying materials is a continuous process, the inhibitory action of tetracycline upon the growth of bones in young animals and children is not permanent, and growth usually returns to normal when the administration of tetracycline ceases. However, deposits in teeth remain, and if discolouration occurs it may appear only some years later, after eruption of the teeth.⁴

Since little is known about the effect of continuous administration of small quantities of tetracycline (e.g., 1-2 ppm in the diet) during pregnancy and early childhood, there is no certainty that it does not present a hazard. The Committee believe that at present the use of tetracyclines in the preservation of fish, poultry, meat, and other fresh foods is a matter of particular concern, since it has been shown that this practice may lead to the presence of 1-2 ppm in meat.⁵

¹ Meyler, L. (1968) *Side effects of drugs*, 6th ed., Amsterdam, Excerpta Medica Foundation.

² Brüggemann, J. et al. (1966) *Zbl. Vet.-Med.*, **13**, 59.

³ Marten, von G. et al. (1966) *Z. Tierphysiol. Tiernähr. Futtermittelk.*, **21**, 183.

⁴ Hilton, H. B. (1962) *J. clin. Path.*, **15**, 112; Stauffer, U. G. (1967) *Schweiz. med. Wschr.*, **97**, 291.

⁵ *Wld Hlth Org. techn. Rep. Ser.*, 1963, No. 260.

Groups of 20 male and 20 female rats were fed diets containing 0, 100, or 1000 ppm of oxytetracycline or tetracycline, and groups of about 100 males were fed diets containing 0, 100, 1000, or 3000 ppm for 2 years. No adverse effect was found on growth, food consumption, survival, blood, gross or microscopic appearance of organs, or tumour incidence.¹

Other biological effects

Allergic reactions. Allergy to the tetracyclines has been described,² but it appears to be very rare. It is known that chlortetracycline may cause photo-allergy or photo-toxicity, but so far these have only been observed in humans treated with therapeutic or prophylactic doses.

Microbial resistance. The administration of tetracyclines to animals in amounts of 5–20 ppm may induce resistance in Enterobacteriaceae. The possible public health implications of extra-chromosomal tetracycline resistance in *Escherichia coli*, *Salmonella*, and other intestinal bacteria have already been evaluated (p. 12).

Evaluation and comments

Acceptable levels of residues in food

If tetracyclines are used as feed additives or for therapy, they should not give rise to detectable residues. If the methods of analysis recommended by the Committee are used, it will be possible to ensure that the residues present in human food will not exceed the following limits (ppm):

<i>Antibiotic</i>	<i>Milk</i>	<i>Meat</i>	<i>Eggs</i>
tetracycline (calculated as base)	0–0.1	0–0.5	0–0.3
chlortetracycline (calculated as base)	0–0.02	0–0.05	0–0.05
oxytetracycline (calculated as base)	0–0.1	0–0.25	0–0.3

The low levels noted above might well be exceeded as a result of environmental or food additive use. Environmental use might give rise to residues as high as 7 ppm, although this might be substantially reduced by subsequent cooking.

The no-effect level in the rat has been found to be 3000 ppm in the diet, equivalent to 150 mg per kg of body weight. Cumulative toxicity in man has been said to occur at a daily intake of 15–40 mg per kg of

¹ Deichmann, W. B. et al. (1964) *Industr. Med. Surg.*, **33**, 787.

² Weinstein, H. I. & Welch, H. (1959) *Antibiot. Ann.*, 643.

body weight, and administration of tetracyclines has been reported to increase the hazard in certain types of renal failure.¹ These reports provide one reason for conservatism in setting an ADI.

A temporary ADI of 0-0.15 mg per kg of body weight is established. This evaluation will be reviewed in 1970.

Effects of residues

Microbiological effects. Tetracyclines can give rise to transferred resistance. It is unlikely that residue levels below 1 ppm will cause difficulties to the consumer, but a level of 5-7 ppm might do so. The levels present in feeds might also cause resistance problems in animals.

Toxic effects. Residue levels below 1 ppm are unlikely to cause acute toxic effects. The Committee considered that the available evidence was not adequate for assessment of long-term effects.

Allergic effects. At the levels noted above, tetracyclines are unlikely to cause allergic effects.

Recommendations

(1) The use of tetracyclines in feeds for growth purposes should be considered acceptable at the levels at present approved.

(2) The veterinary use of tetracyclines should be adequately controlled so as to ensure that residues are not detectable in food for human consumption.

(3) The use of tetracyclines as intentional food additives should not be permitted until the results of long-term studies (including information on the effects of breakdown products) are available so that the hazards may be more fully assessed and an unconditional ADI established.

(4) The use of tetracyclines in the environment of food should not be extended until further information is available on their long-term effects and on the possibility that microbial resistance may develop as a result of exposure to residue levels of 1-7 ppm.

OTHER ANTIBIOTICS

Chloramphenicol

Chloramphenicol is effective against both gram-negative and gram-positive bacteria. It is important in medicine principally because of its activity against gram-negative bacteria, particularly the *Salmonella* species that cause typhoid and paratyphoid fevers.

¹ Shils, M. E. (1963) *Ann. intern. Med.*, **58**, 389.

Uses

As food additive :	Not used
In food environment :	Not used
As feed additive for growth purposes :	Not used
In veterinary medicine :	Used
In human medicine :	Drug of choice for infections with <i>S. typhi</i> and <i>S. paratyphi</i>

Residues

Chloramphenicol is readily absorbed and freely distributed throughout the body. It may enter food for human consumption if used to treat bovine mastitis or certain other infectious diseases of various food-producing animals. It might also enter food if it were to be used as a feed additive.

Biological data*Direct biological effects*

Chloramphenicol can produce dangerous effects in man, including blood dyscrasias, such as granulocytopenia ;¹ aplastic anaemia, which is frequently fatal ;² liver damage ;³ optic neuritis ;⁴ and grey syndrome in the newborn infant.⁵

Other biological effects

Microbial resistance. The development of resistance to chloramphenicol takes place in a series of steps. It may also occur by means of episomal (R-factor) transfer between pathogenic and nonpathogenic enterobacteria and members of other genera.⁶

Evaluation*Acceptable levels of residues in food*

None.⁷

¹ Kleint, W. et al. (1965) *Pädiat. Grenzgeb.*, **4**, 239.

² Dameshek, W. (1960) *J. Amer. med. Ass.*, **174**, 1853.

³ Gjone, E. & Orning, O. M. (1966) *Acta hepato-splenol. (Stuttg.)*, **13**, 288.

⁴ Cocke, J. G. et al. (1966) *J. Pediat.*, **68**, 27.

⁵ Ory, E. M. & Yow, E. M. (1963) *J. Amer. med. Ass.*, **185**, 273.

⁶ Mitsuhashi, S. & Harada, K. (1962) *Nature (Lond.)*, **195**, 517 ; Watanabe, T. (1963) *Bact. Rev.*, **27**, 87.

⁷ Although severe chloramphenicol toxicity is rare, it may well be fatal and the mechanism of its occurrence remains obscure. It is possible that resistance to chloramphenicol may be transferred from normal inhabitants of the gut to *S. typhi* or *S. paratyphi*.

Recommendation

Chloramphenicol should not be used for any purpose that might result in the presence of residues in food for human consumption.

Novobiocin

Novobiocin is usually produced by the growth of *Streptomyces niveus* or related organisms. It is active against gram-positive and some gram-negative bacteria.

Uses

As food additive :	Not used
In food environment :	Not used
As feed additive for growth purposes :	Not used
In veterinary medicine :	Used
In human medicine :	Used

Novobiocin is used as a therapeutic agent, either alone or in combination with other antibiotics, in the treatment of mastitis in dairy animals and staphylococcal infections in poultry. Its most valuable use may be the treatment of staphylococcal infections that are resistant to other antibiotics.

Biological data

Direct biological effects

The direct toxicity of novobiocin is well documented. Gastrointestinal upsets, hepatic dysfunction, and yellow discolouration of the skin have been reported.^{1, 2}

Other biological effects

Allergic reactions. Reports of hypersensitive reactions are frequent. According to one report,³ a macular or papular rash occurred in 89 (8.4%) of 1037 patients treated with novobiocin.

Microbial resistance. Bacterial resistance to novobiocin emerges rapidly.⁴ In cultures it has been shown that sensitivity can decrease more than 10-fold and sometimes more than 30-fold after one passage.¹ There appears to be no cross-resistance with other known antibiotics.⁴

¹ Macey P. E. & Spooner, D. F. (1964) *Novobiocin*. In: Schnitzer, R. J. & Hawking, F. (ed.), *Experimental chemotherapy*, vol. 3, New York, Academic Press.

² Welch, H. et al. (1956) *Antibiot. Med.*, 3, 27; Cox, R. P. et al. (1959) *New Engl. J. Med.*, 261, 139.

³ Grater, W. C. (1962) *Ann. Allergy*, 20, 480.

⁴ Kirby, W. M. M. et al. (1956) *Arch. intern. Med.*, 98, 1.

Evaluation and comments

Acceptable levels of residues in food

When novobiocin is used, it should not be allowed to give rise to detectable residues in food for human consumption. Use of the methods recommended by the Committee (see p. 4) will make it possible to ensure that the residues in food will not exceed the following limits (ppm): milk, 0-0.15; meat, 0-0.5; and eggs, 0-0.1.

Effects of residues

Microbiological effects. Resistance to novobiocin occurs rapidly, but there is no cross resistance to other antibiotics. However, resistance does not present a problem, since novobiocin should be used only for special purposes in veterinary and human medicine.

Toxic effects. Much higher levels than those noted above would be required to cause toxic effects in humans.

Allergic reactions. At levels higher than those noted above, novobiocin is likely to cause allergic reactions.

Recommendation

Novobiocin should be reserved for the treatment of resistant staphylococcal infections under expert supervision.

OTHER CHEMOTHERAPEUTIC AGENTS

The original list of substances submitted to the Committee for consideration included 32 antiprotozoan and antibacterial compounds other than antibiotics, that are used in agriculture (mainly for poultry raising) and the use of which might give rise to residues in human food. The Committee recognized the importance of studying such substances, but in some cases was unable to consider their significance as residues in food or to evaluate them toxicologically, owing to the lack of evidence.¹ Further data on substances that may give rise to significant residues in human food are required, and the attention of those concerned with supplying such data to FAO or WHO is drawn to earlier reports that describe the type of information that is required (see Annex 1). It should be particularly noted that adequate information on the breakdown products of such substances and on their effects in different mammalian species (including the

¹ However, tentative specifications for the following substances were drawn up and are available, on request, from FAO: amprolium, dimetridazole, ethopabate, furazolidone, nicarbazine, nithiazide, nitrofurazone, and 3-nitro-4-hydroxyphenylarsonic acid.

results of long-term studies) are usually necessary. It is hoped that national and international regulatory authorities will, whenever possible, make relevant chemical and biological data in their possession available to the Committee.

GENERAL RECOMMENDATIONS

The Committee makes the following general recommendations on the use of antibiotics for any purpose that might result in their presence, or the presence of their breakdown products, in human food.

Use of antibiotics as direct (intentional) food additives

(1) Only antibiotics that have no important therapeutic use, and that do not give rise to cross resistance or any other form of interference with the therapeutic use of other antibiotics in human or veterinary medicine, should be considered for use as direct (intentional) food additives. However, exceptions may be justified to solve certain important problems.

(2) An antibiotic that alters the ecological pattern of microbial spoilage of food, resulting in possible danger to the consumer, should not be used as a direct (intentional) food additive.

(3) In considering the use of a given antibiotic as an intentional food additive, information on its effects on the normal body flora of the consumer should be obtained.

(4) No lowering of the usual standards of food hygiene should be permitted because of the use of an antibiotic as a food additive.

Use of antibiotics as feed additives

For growth purposes

(1) Antibiotics should not be added to feeds in greater amounts than are necessary to produce the desired effect.

(2) The use of antibiotics in feeds should be controlled by regulations. If an approved level is not effective, it should not be increased without thorough investigation of the causes of the lack of effectiveness and of the possible consequence of increased levels of intake.

(3) A combination of two or more antibiotics should not be added to a feed without adequate study of the possible consequences of using the mixture.

(4) An antibiotic should not be used as a feed additive if the proposed levels of use involve any risk of microbiological effects that might be

prejudicial to the welfare of animals or the wholesomeness of foods of animal origin.

For other purposes

(1) Antibiotics should not be added to the feed of laboratory animals for any purpose without the knowledge of the investigator who intends to use the animals.

(2) Antibiotics that might give rise to residues in eggs should not be added to feeds for laying birds if the eggs are intended for human consumption or are likely to be used for research purposes.

(3) Antibiotics that give rise to residues in milk should not be administered to lactating animals. However, if the use of such antibiotics is unavoidable, the milk should be discarded until the residue can no longer be detected by methods of analysis such as those recommended by the Committee (see p. 4).

Use of antibiotics in veterinary medicine

(1) Antibiotics should not be used for prophylactic or curative purposes in animals from which human food may be derived except on the advice—and preferably under the control—of a veterinarian.

(2) Residues of antibiotics in human food that may result from the prophylactic or curative use of these agents should be as low as possible.

(3) Adequate information about each antibiotic should be printed on the label and in promotional literature. Since there is a risk that undue reliance on the hoped-for prophylactic effects of antibiotics may lead to faulty practices, all concerned should be fully informed of the dangers of misuse.

(4) A satisfactory procedure should be devised to ensure disclosure of the continued presence of antibiotic residues in slaughtered animals, particularly those that result from the use of depot preparations. The possible use of markers for antibiotics should be further studied.

(5) If antibiotic residues are unavoidable, evidence should be available, including appropriate life-span studies, to permit adequate assessment of the long-term hazards.

Medical and veterinary co-ordination

Co-ordination is desirable in the use of antibiotics in veterinary and medical practice.

RECOMMENDATIONS TO FAO AND WHO

(1) A Joint FAO/WHO Expert Committee on Food Additives should be convened as soon as practicable to draw up specifications for, and to make a toxicological and microbiological evaluation of, certain anti-protozoal chemotherapeutic agents that might leave residues in foods of animal origin.

(2) FAO should gather information on the proper use of antibiotics and other chemotherapeutic agents in food-producing animals, and disseminate it (in a readily understood form) to all concerned.

(3) In the interest of public health, WHO should consider the possibility of promoting (a) research on the transfer of antibiotic resistance under the practical conditions of agricultural and veterinary use of antibiotics and (b) long-term studies of the effects of antibiotic residues that are likely to occur in human food.

Annex

REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. General Principles Governing the Use of Food Additives : First Report, *FAO Nutrition Meetings Report Series*, 1956, No. 11 : *Wld Hlth Org. techn. Rep. Ser.*, 1956, 129.
2. Procedures for the Testing of International Food Additives to Establish their Safety for Use ; Second Report, *FAO Nutrition Meetings Report Series*, 1958, No. 17 ; *Wld Hlth Org. techn. Rep. Ser.*, 1958, 144.
3. Specifications for Identity and Purity of Food Additives (Antimicrobial Preservatives and Antioxidants) : Third Report. These specifications were subsequently revised and published as *Specifications for Identity and Purity of Food Additives*, vol. I. *Antimicrobial Preservatives and Antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962.
4. Specifications for Identity and Purity of Food Additives (Food Colours) : Fourth Report. These specifications were subsequently revised and published as *Specifications for Identity and Purity of Food Additives*, vol. II. *Food Colors*, Rome, Food and Agriculture Organization of the United Nations, 1963.
5. Evaluation of the Carcinogenic Hazards of Food Additives : Fifth Report, *FAO Nutrition Meetings Report Series*, 1961, No. 29 ; *Wld Hlth Org. techn. Rep. Ser.*, 1961, 220.
6. Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants : Sixth Report, *FAO Nutrition Meetings Report Series*, 1962, No. 31 ; *Wld Hlth Org. techn. Rep. Ser.*, 1962, 228.
7. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Emulsifiers, Stabilizers, Bleaching and Maturing Agents : Seventh Report, *FAO Nutrition Meetings Report Series*, 1964, No. 35 ; *Wld Hlth Org. techn. Rep. Ser.*, 1964, 281.
8. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Food Colours and Some Antimicrobials and Antioxidants : Eighth Report, *FAO Nutrition Meetings Report Series*, 1965, No. 38 ; *Wld Hlth Org. techn. Rep. Ser.*, 1965, 309.
- * 9. Specifications for Identity and Purity and Toxicological Evaluation of some Antimicrobials and Antioxidants, *FAO Nutrition Meetings Report Series*, 1965, No. 38A ; WHO/Food Add/24.65.
- *10. Specifications for Identity and Purity and Toxicological Evaluation of some Food Colours, *FAO Nutrition Meetings Report Series*, 1966, No. 38B ; WHO/Food Add/66.25.

* These documents can be obtained on request from : Food Additives, World Health Organization, Avenue Appia, 1211 Geneva, Switzerland, or : Food Science and Technology Branch, Food and Agriculture Organization of the United Nations, 00100 Rome, Italy.

11. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-treatment Agents, Acids and Bases : Ninth Report, *FAO Nutrition Meetings Report Series*, 1966, No. 40 : *Wld Hlth Org. techn. Rep. Ser.*, 1966, 339.
- *12. Toxicological Evaluation of Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-Treatment Agents, Acids and Bases, *FAO Nutrition Meetings Report Series*, 40 A, B, C ; WHO/Food Add/67.29.
13. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Emulsifiers and Stabilizers and Certain Other Substances : Tenth Report, *FAO Nutrition Meetings Report Series*, 1967, No. 43 ; *Wld Hlth Org. techn. Rep. Ser.*, 1967, 373.
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* These documents can be obtained on request from : Food Additives, World Health Organization, Avenue Appia, 1211 Geneva, Switzerland, or : Food Science and Technology Branch, Food and Agriculture Organization of the United Nations, 00100 Rome, Italy.

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