WHO FOOD ADDITIVES SERIES: 57

Toxicological evaluation of certain veterinary drug residues in food

Prepared by the
Sixty-sixth meeting of the Joint FAO/WHO
Expert Committee on Food Additives (JECFA)

IPCS

International Programme on Chemical Safety
World Health Organization, Geneva

WHO FOOD ADDITIVES SERIES: 57

Toxicological evaluation of certain veterinary drug residues in food

Prepared by the sixty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

The summaries and evaluations contained in this book are, in most cases, based on unpublished proprietary data submitted for the purpose of the JECFA assessment. A registration authority should not grant a registration on the basis of an evaluation unless it has first received authorization for such use from the owner who submitted the data for JECFA review or has received the data on which the summaries are based, either from the owner of the data or from a second party that has obtained permission from the owner of the data for this purpose.

World Health Organization, Geneva, 2006

IPCS–International Programme on Chemical Safety

WHO Library Cataloguing-in-Publication Data

Toxicological evaluation of certain veterinary drug residues in food / prepared by the sixty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

(WHO food additives series ; 57)

1.Drug residues - toxicity. 2.Veterinary drugs - adverse effects. 3.Food contamination. 4.Risk assessment. 5.Colistin - toxicity. 6.Erythromycin - toxicity. 7.Tylosin - toxicity. I.Joint FAO/WHO Expert Committee on Food Additives. Meeting (66th : 2006: Rome, Italy) II.International Programme on Chemical Safety. III.Series.

ISBN 92 4 166057 0 (NLM classification: WA 701)
ISBN 978 92 4 166057 0
ISSN 0300-0923

CONTENTS

PREFACE

The monographs contained in this volume were prepared at the sixty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met at FAO headquarters in Rome, Italy, 22–28 February 2006. These monographs summarize the data on the safety of residues in food of selected veterinary drugs reviewed by the Committee.

The sixty-sixth report of JECFA has been published by the World Health Organization as WHO Technical Report No. 939. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication; a summary of the conclusions of the Committee is given in Annex 4. Some of the substances listed in Annex 4 were considered at the meeting for residue evaluation only.

Residue monographs that were developed at the sixty-sixth meeting of JECFA will be published in the FAO JECFA Monographs No. 2. The toxicological monographs in the present publication should be read in conjunction with the residue monographs and the report.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives and Contaminants and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The toxicological monographs contained in the volume are based on working papers that were prepared by Temporary Advisers. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers.

Many proprietary unpublished reports are unreferenced. These were voluntarily submitted to the Committee by various producers of the veterinary drugs under review and in many cases represent the only data available on those substances. The Temporary Advisers based the working papers they developed on all the data that were submitted, and all of these reports were available to the Committee when it made its evaluation. The monographs were edited by M. Sheffer, Ottawa, Canada.

The preparation and editing of the monographs included in this volume were made possible through the technical and financial contributions of the Participating Organizations of the International Programme on Chemical Safety (IPCS), which supports the activities of JECFA.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in the IPCS concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers

or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of the compounds evaluated in this publication should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Expert Committee on Food Additives, International Programme on Chemical Safety, World Health Organization, Avenue Appia 20, 1211 Geneva 27, Switzerland.

RESIDUES OF VETERINARY DRUGS

COLISTIN

First draft prepared by

Dr Gladwin Roberts,[1] Dr Carl Cerniglia,[2] and Dr Kevin Greenlees[3]

[1] Consultant Toxicologist, Greenway, ACT, Australia

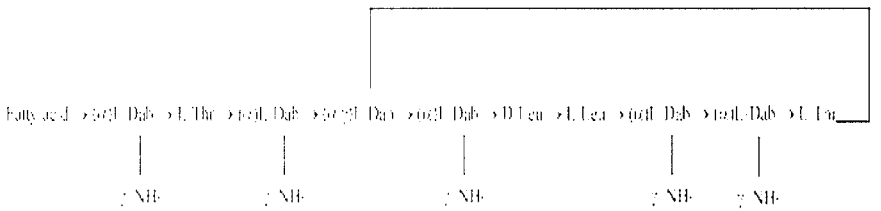
[2] Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, Arkansas, USA

[3] Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, Maryland, USA

1. EXPLANATION

Colistin is an antibacterial agent with activity against a range of Gram-negative organisms. It is a cyclopeptide antibiotic, also known as polymyxin E, produced as a fermentation product of *Bacillus polymyxa* var. *colistinus*. Colistin is composed of a mixture of colistin A and colistin B, which differ only in the length of the acyl side chain. The structure of colistin is shown in Figure 1. One unit of colistin is defined as the minimum concentration of colistin that can inhibit the growth of *Escherichia coli* 95 I.S.M. in 1 ml of broth at pH 7.2. Pure colistin base has been assigned a potency of 30 000 IU/mg (Schwartz, 1964). An International Standard for colistin sulfate is prepared to contain 20 500 IU/mg. Sodium colistin methanesulfonate should not have a potency less than 11 500 IU/mg.

Figure 1. Structure of colistin



Colistin has bactericidal activity as a result of binding to the outer cell membrane with subsequent disruption of the cell envelope. Colistin is used for the prevention and treatment of various infectious diseases caused by colistin-sensitive bacteria in target species such as swine, poultry, cattle, sheep, goats and rabbits. It is also used in human medicine. Colistin is available as the sulfate and the methanesulfonate. In both humans and animals, colistin is used orally (colistin sulfate) and parenterally (colistin sulfate, colistin methanesulfonate).

Colistin has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Colistin was placed on the agenda because it was on the priority list of the Codex Committee on Residues of Veterinary Drugs in Foods.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution, excretion and metabolism

(a) Mice, rats, rabbits and dogs

In a study of the microbiological effects of colistin sulfate, the drug was administered via drinking-water at concentrations of 0, 50 and 500 µg/ml to human flora-associated (HFA) mice for 3 weeks. These concentrations correspond to 0, 3.1 and 31 mg colistin base/kg body weight per day. Faecal drug concentrations, measured on days 2, 4, 9 and 15 of treatment, showed gradual increases to a maximum of 420 and 2159 µg/g faeces at day 15 for the 3.1 and 31 mg/kg body weight per day dose groups, respectively. Thereafter, the concentrations decreased to non-detectable levels within 5 days after the cessation of drug administration (Jeong et al., 2005).

Colistin sulfate and sodium colistin methanesulfonate were administered by the oral route in rats (0.16 mg colistin base), rabbits (0.33 mg colistin base) and dogs (1 mg colistin base), by the duodenal route in dogs (4.16 mg colistin base) and by the rectal route in rabbits (0.16 mg colistin base) and dogs (0.33 mg colistin base). In each case, no drug could be detected in blood over the subsequent 24 h (Anonymous, 1968).

It was reported that an oral dose of colistin sulfate or sodium colistin methanesulfonate (60 mg colistin base/kg body weight) in rabbits showed "poor and variable absorption". No data were presented (Schwartz et al., 1960).

Rats were injected either intramuscularly or intravenously with 10 or 30 mg sodium colistin methanesulfonate/kg body weight. Peak colistin concentrations in serum were achieved within 1 h, with lesser concentrations in most tissues. Kidneys showed higher drug levels than serum, as the majority of the compound was excreted in urine within 24 h (Yamada et al., 1974).

Rabbits were given an intravenous injection of sodium colistin methanesulfonate at 100 mg/kg body weight. The recovery of parent compound in a 24-h period was approximately 74% of the dose in urine and about 0.13% in the bile. One metabolite was detected, colistin *N*-glucuronide, which accounted for 1.7% and 6.7% of the dose in urine and bile, respectively (Abe et al., 1976).

Sodium colistin methanesulfonate was injected intramuscularly into rabbits (12–60 mg colistin base/kg body weight) and dogs (0.56–1.15 mg colistin base/kg body weight). Colistin sulfate was injected intramuscularly into rabbits at doses of 11–60 mg colistin base/kg body weight. In all cases, peak drug concentrations in serum were reached within 2 h (Schwartz et al., 1960).

Dogs were given intramuscular injections of either colistin sulfate or sodium colistin methanesulfonate at dose rates of 1.1–4.4 mg colistin base/kg body weight. The methanesulfonate resulted in 2-fold higher initial plasma levels but disappeared at a faster rate. Urinary recovery of drug was 7-fold higher in dogs treated with the methanesulfonate. Greater binding of colistin sulfate to plasma and tissue proteins was demonstrated and is the likely reason for the kinetic differences. Pretreatment with probenecid did not affect renal excretion, suggesting that glomerular filtration was the primary process of elimination. A comparison of the urinary recovery using a microbiological assay and a measurement of D-leucine content showed that a proportion of the drug material had no microbiological activity, suggesting that metabolism may have occurred (Al-Khayyat & Aronson, 1973).

(b) Chickens

Groups of five White Leghorn hens received single oral doses of 25 or 50 mg colistin base/kg body weight in the form of colistin sulfate. The hens were killed over an 8-h period after dosing. Low drug concentrations in serum (peaks of 1.0 and 8.3 µg colistin base/ml at 25 and 50 mg/kg body weight, respectively) and bile were detected, with "trace" amounts in the liver and kidneys. The drug was not detected in muscle, liver, lung, spleen, pancreas or ovary. Large amounts were, however, found in the contents of the digestive tract (Sato et al., 1972).

(c) Pigs

Seven gnotobiotic piglets received a single oral dose of colistin sulfate (16 mg colistin base/kg body weight). The animals were killed from 2 to 48 h after the

treatment. The drug could not be detected in blood or a wide range of systemic organs and tissues. High concentrations were found in the gastrointestinal contents, with levels up to 10 mg/g in tissues lining the gastrointestinal tract (Terakado et al., 1972).

Groups of four Landrace × Large Yorkshire piglets received single oral doses of 25 or 50 mg colistin base/kg body weight in the form of colistin sulfate. The pigs were killed over a period of 16 h after dosing. Low drug concentrations were detected in serum (peaks of 1.25 and 10.2 µg colistin base/ml at 25 and 50 mg/kg body weight, respectively) and bile, with "trace" amounts in the kidneys. The drug was not detected in muscle, liver, lung, spleen, pancreas, ovary or testes. Large amounts were found in the contents of the digestive tract. Further groups of six piglets were fed diets containing colistin sulfate for 16 weeks. The drug concentrations in the feed provided doses of 0.7, 3.0 and 6.0 mg colistin base/kg body weight. These animals were killed 1, 2, 4, 6, 10 or 16 weeks after the start of treatment. Very low levels of drug were detectable in serum and bile, but not in other systemic organs or tissues (Sato et al., 1972).

Colistin sulfate was administered orally to groups of three LW strain hogs at doses of 10 or 20 mg colistin base/kg body weight per day for 3 days. The animals were killed 72 h after the final dose. In one animal given the high dose, the plasma concentration was 0.8 µg colistin base/ml, 1 h after the third dose. Drug concentrations were below the limit of detection in all other plasma samples taken over a 24-h period after the final dose and in muscle, fat, liver, kidney, small intestine and bile obtained at termination (Fukuhara, 1990a).

(d) Cattle

Colistin sulfate was administered orally to groups of two female calves at doses of 5 or 10 mg colistin base/kg body weight per day for 3 days. The animals were killed 72 h after the third dose. Drug concentrations were below the limit of detection in plasma samples taken over a 24-h period after the final dose and in muscle, fat, liver, kidney, small intestine and bile obtained at termination (Fukuhara, 1990b).

Dairy cattle were given an oral dose of 3.33 mg colistin base/kg body weight in the form of colistin sulfate. During the next 24 h, the drug could not be detected in blood (Archimbault et al., 1980).

(e) Humans

Colistin was reported not to be absorbed from the gastrointestinal tract of adult humans following oral doses of colistin sulfate or sodium colistin methanesulfonate. Intramuscular and intravenous administration of sodium colistin methanesulfonate produced high peak serum concentrations within 2 h (Schwartz et al., 1960; Yamada et al., 1974).

In infants between 4 and 51 days of age, a single intramuscular injection of colistin (compound not specified) at 5 mg/kg body weight resulted in peak drug

concentrations in serum of about 15 µg/ml in 2 h. The half-life in serum was approximately 2.5 h, only slightly shorter than in adult humans (Yaffe & Rane, 1971).

Patients with cystic fibrosis were given an intravenous infusion of colistin methanesulfonate (12 740 IU/mg) every 8 h for at least 2 days. Patients weighing more than 50 kg were administered 160 mg, equivalent to 66.6 mg colistin base, and patients weighing less than 50 kg were administered 80 mg, equivalent to 33.3 mg colistin base. Plasma concentrations of colistin base and colistin methanesulfonate were measured by high-performance liquid chromatographic methods after 3–17 days of treatment. Colistin methanesulfonate hydrolyses to form colistin base in vitro, and this conversion was reflected in the plasma kinetics in this study. Plasma concentrations of both colistin methanesulfonate and colistin base decreased over a 7-h period; however, the mean half-life of the base was 2-fold greater than that of the methanesulfonate due to the continued conversion of the latter to the former (Li et al., 2003).

Sodium colistin methanesulfonate was injected intravenously into pregnant volunteers at a dose of 150 mg colistin base during labour. Monitoring of maternal serum drug levels throughout labour and drug levels in cord blood at delivery demonstrated significant passage of colistin across the placenta (MacAulay et al., 1967).

In a study of the effects of colistin sulfate on human faecal microflora, a daily oral dose of 0.45 g colistin base was given to six healthy adult volunteers for 3 consecutive days. Maximum faecal concentrations were found on the day after the final dose. The mean value was 42.6 µg colistin base/g faeces. Concentrations decreased to non-detectable levels 5 days later. Serum and urine were sampled 2 days after the final dose. The mean drug concentration in serum was 0.21 µg colistin base/ml, and no drug was found in urine at the detection limit of 0.1 µg/ml (Tancrede, 1993).

Orally administered colistin sulfate was excreted in the faeces of adults and children, mainly in "bound form". Daily oral doses of colistin sulfate at 5–20 mg/kg body weight have led to average faecal drug concentrations of 128 µg/g. Sodium colistin methanesulfonate injected intramuscularly and intravenously is principally excreted through the kidneys (Schwartz, 1964; Yamada et al., 1974).

2.2 Toxicological studies

The studies of acute and short-term toxicity generally did not comply with Good Laboratory Practice (GLP). A number of studies were also reported in summary form and with no supporting data. Colistin is not absorbed orally; therefore, studies using parenteral routes of administration have been reviewed to provide information on the toxicological potential should systemic exposure occur.

2.2.1 Acute toxicity

Results of studies of acute toxicity of colistin sulfate and sodium colistin methanesulfonate are summarized in Tables 1 and 2, respectively.

Table 1. Results of studies of acute toxicity of colistin sulfate

Species	Route	LD_{50} (mg base/kg body weight)	Reference
Mouse	Oral	720–725	Schwartz (1964)
Mouse, M&F	Oral	648–690	Morimoto et al. (undated a)
Mouse	Oral	432	Lin et al. (2005)
Mouse	Subcutaneous	48.6–54.0	Schwartz (1964)
Mouse	Intramuscular	38.7	Lin et al. (2005)
Mouse	Intraperitoneal	19.8–22.5	Schwartz (1964)
Mouse, M&F	Intraperitoneal	16–17	Morimoto et al. (undated a)
Mouse	Intravenous	5.4–7.6	Schwartz (1964)

F, female; M, male

Table 2. Results of studies of acute toxicity of sodium colistin methanesulfonate

Species	Route	LD_{50} (mg base/kg body weight)	Reference
Mouse	Oral	766.7–2000	Schwartz (1964)
Mouse	Subcutaneous	138–220	Schwartz (1964)
Mouse	Intraperitoneal	126.5–220	Schwartz (1964)
Mouse	Intravenous	220–405	Schwartz (1964)

Lethal doses of colistin compounds caused muscular incoordination, respiratory distress and occasionally strychnine-like convulsions in mice. Death occurred from asphyxia within 3 h (Schwartz, 1964; Morimoto et al., undated a).

Anaesthetized dogs were given an intravenous infusion of colistin sulfate (0.33–4.33 mg colistin base/kg body weight) or sodium colistin methanesulfonate (1.72–2.4 mg colistin base/kg body weight). A dose-related and marked depression of blood pressure was observed with colistin sulfate, but not with sodium colistin methanesulfonate (Schwartz et al., 1960).

2.2.2 Short-term toxicity

(a) Mice

Groups of 10 male and 10 female ICR-JCL mice were given intraperitoneal injections of sodium colistin methanesulfonate (purity unknown) at 0, 50, 100 or 300 mg/kg body weight per day for 30 days. At 300 mg/kg body weight per day, reduced body weight gain and spontaneous activity, convulsions and death of some animals of each sex were observed. The number of erythrocytes, haematocrit and haemoglobin were depressed and liver weight was increased in males at this dose. Toxicologically significant changes were not found in serum biochemistry parameters. It was reported that "reactive symptoms" were noted in liver, spleen and kidney (Tomizawa et al., 1973a).

(b) Rats

Colistin sulfate (18 000–20 000 IU/mg) was administered orally to rats at doses of 6.67, 20 or 60 mg colistin base/kg body weight per day for 90 days. It was reported that there were no adverse effects on growth, behaviour, haematology, urinalysis, liver function or pathology. However, no supporting data were presented (Schwartz et al., 1960).

Groups of 25 male and 25 female Wistar rats were fed diets containing colistin sulfate (505 µg colistin base/mg) at concentrations of 0, 40, 200 or 1000 mg/kg for a period of 26 weeks. The dietary concentrations were equal to 20.2, 101 and 505 mg colistin base/kg and equivalent to 2, 10 and 50 mg colistin base/kg body weight per day. Ten animals per sex were sacrificed after 9 weeks of treatment. There were no treatment-related deaths and no effect on food intake or body weight gain. Haematology, blood biochemistry, urinalysis and organ weights showed no toxicologically significant changes as a result of treatment. Histopathology of a range of tissues revealed no differences between groups. The no-observed-effect level (NOEL) was the highest dose, 505 mg/kg in feed, equivalent to 50 mg colistin base/kg body weight per day (Morimoto et al., undated b).

Groups of 10 male and 10 female Wistar rats were given intraperitoneal injections of sodium colistin methanesulfonate (purity unknown) at 0, 5, 10, 20 or 60 mg/kg body weight per day for 30 days. All high-dose animals died within 3 days. Reduced body weight gain, reduced spontaneous activity and convulsions were observed at higher doses. The number of erythrocytes, haematocrit and haemoglobin were depressed in males given 20 mg/kg body weight per day, and alanine aminotransferase was increased in females at this dose. It was reported that "reactive symptoms" were present in liver, with hyperaemia in the spleen (Tomizawa et al., 1973a).

Rats received intraperitoneal injections of sodium colistin methanesulfonate (11 500 IU/mg) at doses of 0.83, 2.5 or 7.5 mg colistin base/kg body weight per day for 60 days. It was reported that there were no significant adverse effects on growth, behaviour, haematology or urinalysis. Hyperaemia of the kidneys occurred

regularly at the highest dose and infrequently at the lower doses. However, no supporting data were presented (Schwartz et al., 1960).

Groups of 10 male and 10 female Wistar rats were given intraperitoneal injections of sodium colistin methanesulfonate (purity unknown) at 0, 5, 10, 20 or 30 mg/kg body weight per day for 6 months. Body weight gain was depressed in males at 30 mg/kg body weight per day and in females at 10 mg/kg body weight per day and above. Mortality was dose related at 10 mg/kg body weight per day and above, and reduced spontaneous activity and convulsions were observed (doses not provided). Significant biochemical changes included decreased aspartate aminotransferase and alanine aminotransferase and increased blood urea nitrogen in males at 20 and 30 mg/kg body weight per day and increased albumin/globulin ratio in females at 30 mg/kg body weight per day. Albuminuria was seen in both sexes at 20 and 30 mg/kg body weight per day. Haematology parameters and organ weights were not significantly altered. It was reported that "pathological changes" were noted in kidney, liver and spleen at 20 and 30 mg/kg body weight per day (Tomizawa et al., 1973b).

(c) Dogs

Colistin sulfate (18 000–20 000 IU/mg) was administered orally to dogs at doses of 6.67, 20 or 60 mg colistin base/kg body weight per day for 90 days. It was reported that there were no adverse effects on growth, behaviour, haematology, urinalysis, liver and kidney function or pathology. However, no supporting data were presented (Schwartz et al., 1960).

Dogs received intramuscular injections of sodium colistin methanesulfonate (11 500 IU/mg) at doses of 0.83, 2.5 or 7.5 mg colistin base/kg body weight per day for 60 days. It was reported that there were no significant adverse effects on growth, behaviour, haematology, urinalysis or pathology. However, no supporting data were presented (Schwartz et al., 1960).

2.2.3 Genotoxicity

The older (pre-1992) studies of genotoxicity did not comply with GLP, whereas those conducted in 1992 complied with appropriate standards. Results of genotoxicity tests using colistin salts are summarized in Table 3.

Chromatid breaks and fragments comprised the majority of the aberrations and were associated with a decrease in mitotic index and in the rate of cell division. Bromodeoxyuridine was added at the initiation of cultures in order to visualize sister chromatid exchanges. This is not normal practice in assays for the detection of chromosomal aberrations, and it is not known if there may have been an interaction with the added bromodeoxyuridine. The frequency of sister chromatid exchanges was not affected at any concentration.

Table 3. Results of tests for genotoxicity with colistin salts

End-point	Test object[a]	Concentration/dose[b]	Result	Reference
Reverse mutation[c]	*Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100, ±S9	0.086–1.37 µg/plate as colistin sulfate	Negative	Molinier (1992a)
Reverse mutation[d]	*Escherichia coli* strain Sd-4-73, –S9	Unknown compound and concentration	Negative	Szybalski (1958)
DNA damage[d]	SOS chromotest with *E. coli* strains PQ37, BR513, BR339, ±S9	>10 µg/disc (compound unknown)	Equivocal[e]	Mamber et al. (1986)
DNA repair[d]	Rec assay with *Bacillus subtilis* strains H17 Rec^+ and M45 Rec^-, ±S9	Unknown compound and concentration	Negative	Kada et al. (1980)
Forward mutation[f]	V79 Chinese hamster cells in culture, ±S9	20.6–2055 µg/ml as colistin sulfate	Negative	Marzin (1992)
Chromosomal damage in vitro[d]	Human lymphocytes in culture	2.3–7.1 µg/ml (compound unknown)	Positive[g]	Jaju et al. (1983)
Sister chromatid exchange in vitro[d]	Human lymphocytes in culture	2.3–7.1 µg/ml (compound unknown)	Negative[g]	Jaju et al. (1983)
Chromosomal damage in vivo[h]	Micronucleus formation in bone marrow from mice	Single intraperitoneal dose of 13.7 mg/kg body weight as colistin sulfate	Negative	Molinier (1992b)

[a] S9, 9000 × *g* supernatant of rat liver used for metabolic activation.
[b] Concentrations/doses are expressed as colistin base.
[c] Positive controls were sodium azide for TA1535 and TA100, 9-aminoacridine for TA1537 and 2-nitrofluorene for TA1538 and TA98 in the absence of S9; 2-anthramine for all strains in the presence of S9.
[d] No positive control was used.
[e] The equivocal finding was presented in a table of results, but with no supporting information.
[f] Positive controls were ethylmethanesulfonate in the absence of S9; benzo[*a*]pyrene in the presence of S9.
[g] The positive result in cultured human lymphocytes was concentration related and significant at 4.6 and 7.1 µg/ml.
[h] Positive control was cyclophosphamide by intraperitoneal injection.

2.2.4 Reproductive toxicity

(a) Fertility studies

(i) Mice

Groups of 25 male and 25 female ICR-JCL mice were given daily intravenous injections of sodium colistin methanesulfonate (12 000 IU/mg) at 0, 60, 125 or 250 mg/kg body weight per day. The doses were equivalent to 0, 24, 50 and 100 mg colistin base/kg body weight per day. Male mice were injected from day 63 prior to mating through to the end of the 2-week mating period. Females were treated from day 14 prior to the mating period through to day 7 of gestation. All pregnant females were killed on day 18 of gestation for examination of uteri and fetuses. An increase in spontaneous motor activity during the first 10 days was noted in males of the 50 and 100 mg/kg body weight per day groups. In these same groups, females gained more body weight during the gestation period, in association with higher fetal and placental weights. There were no effects on copulation, pregnancy and implantation rates, litter sizes or fetal abnormalities. The intravenous dose of 24 mg colistin base/kg body weight per day had no effect in this study (Saitoh et al., undated).

(ii) Rats

Groups of 22 male and 22 female Wistar rats were given daily intravenous injections of sodium colistin methanesulfonate (12 000 IU/mg) at 0, 6.25, 12.5 or 25 mg/kg body weight per day. The doses were equivalent to 0, 2.5, 5 and 10 mg colistin base/kg body weight per day. Male rats were injected for "more than 60 days" prior to mating through to the end of the 2-week mating period. Females were treated from day 14 prior to the mating period through to day 7 of gestation. All pregnant females were killed on day 21 of gestation for examination of uteri and fetuses. There were no effects on behaviour, copulation, pregnancy and implantation rates or litter sizes. The only finding on examination of fetuses was delayed ossification at some vertebral and phalangeal sites in the 10 mg/kg body weight per day group. Intravenous doses of 2.5 and 5 mg colistin base/kg body weight per day had no effect in this study (Tsujitani et al., undated c).

(b) Developmental studies

(i) Mice

Groups of mated ICR-JCL mice were given daily intraperitoneal injections of sodium colistin methanesulfonate (purity unknown) at 0, 15, 50 or 150 mg/kg body weight per day on gestation days 7–14. The body weight gain of females and fetal body weight were unaffected. Resorptions were slightly increased at 150 mg/kg body weight per day. Fetal abnormalities were not increased. Intraperitoneal doses of 15 and 50 mg/kg body weight per day had no effect in this study (Tomizawa & Kamada, 1973).

Groups of 20 mated ICR-JCL mice were given daily intravenous injections of sodium colistin methanesulfonate (purity unknown) at 0, 125, 250 or 500 mg/kg body weight per day on gestation days 6–15. Approximately two thirds of the females were killed on gestation day 18. There were no effects on body weight gain in females, numbers of resorptions or live fetuses, body weight of fetuses or external and visceral development. Reduced ossification was noted in cervical vertebrae of fetuses in the 250 and 500 mg/kg body weight per day groups. The remaining females were allowed to deliver naturally and raise the offspring. The duration of gestation, number of pups and body weight of pups were unchanged. Postnatal survival of offspring, growth and development were unaffected. The results of a range of neurological and behavioural tests identified a reduction in spontaneous activity in offspring of the 250 and 500 mg/kg body weight per day groups. The intravenous dose of 125 mg/kg body weight per day had no effect in this study (Saitoh et al., 1981).

(ii) Rats

Groups of 20 mated Wistar rats were given colistin sulfate (670 µg colistin base/mg) by gavage on gestation days 7–17. The doses were 0, 2.6, 65 and 130 mg colistin base/kg body weight per day. All animals were killed on gestation day 20. There were no differences between groups in the body weight gain of dams, the number of resorptions or the body weight of fetuses. The number of dead fetuses was slightly but non-significantly increased at 130 mg/kg body weight per day. Examination of fetuses revealed a slightly increased number of proximal phalanges in all treated groups, but with no dose relationship. Visceral and external development was unaffected. There were no toxicologically significant findings. Therefore, the NOEL is the highest dose, 130 mg colistin base/kg body weight per day (Morimoto et al., 1984).

Groups of mated Wistar rats were given daily intraperitoneal injections of sodium colistin methanesulfonate (purity unknown) at 0, 5, 20 or 40 mg/kg body weight per day on gestation days 8–15. Maternal body weight gain was slightly increased while fetal body weight was slightly decreased in groups given 20 and 40 mg/kg body weight per day. The numbers of resorptions and fetal abnormalities were similar between groups. The intraperitoneal dose of 5 mg/kg body weight per day had no effect in this study (Tomizawa & Kamada, 1973).

Groups of 30 mated Wistar rats were given daily intravenous injections of sodium colistin methanesulfonate (12 000 IU/mg) at 0, 6.25, 12.5 or 25 mg/kg body weight per day on gestation days 7–17. The doses were equivalent to 0, 2.5, 5 and 10 mg colistin base/kg body weight per day. Twenty females were killed on gestation day 21. There were no effects on body weight gain in females, numbers of resorptions or live fetuses, body weight of fetuses or external and visceral development. Reduced ossification was noted in certain sites in the vertebrae and hind paws of fetuses in the 10 mg/kg body weight per day group. The remaining 10 females were allowed to deliver naturally and raise the offspring. The duration of gestation, number of pups and body weight of pups were unchanged. Postnatal survival of offspring was reduced in litters at 10 mg/kg body weight per day, but

growth and development were unaffected. The results of a range of neurological and behavioural tests were similar between groups. At the age of 10 weeks, two males and two females from each dam were mated, avoiding within-litter coupling, and the females were killed on gestation day 21. No effects on pregnancy or implantation rates, numbers of resorptions or live fetuses, body weight or external development of fetuses were observed. Intravenous doses of 2.5 and 5 mg colistin base/kg body weight per day had no effect in this study (Tsujitani et al., 1981a, undated a).

(iii) Rabbits

Sodium colistin methanesulfonate (12 000 IU/mg) was injected intravenously into groups of 10–12 mated New Zealand White rabbits at doses of 0, 50, 63 or 80 mg/kg body weight per day on gestation days 6–18. The doses were equivalent to 0, 20, 25 and 32 mg colistin base/kg body weight per day. All females were killed on gestation day 29. There were no meaningful effects on body weight gain in females, numbers of resorptions or live fetuses, body weight of fetuses or external, skeletal and visceral development. Intravenous doses of up to 32 mg colistin base/kg body weight per day had no effect in this study (Tsujitani et al., 1981b, undated b).

(c) Peri- and postnatal studies

(i) Rats

Groups of 20 pregnant female Wistar rats were given daily intravenous injections of sodium colistin methanesulfonate (purity unknown) at 0, 6.25, 12.5 or 25 mg/kg body weight per day from gestation day 17 to day 21 post-parturition. There were no toxic effects observed in the dams. The duration of gestation, number of live births, body weight of pups and external malformation rate were similar between groups. Postnatal survival of offspring, growth and development were unaffected. The results of a range of neurological and behavioural tests were similar between groups. Approximately 40–45 males and females from each group were mated, and the females were allowed to litter naturally. No effects were observed on pregnancy or implantation rates, numbers of resorptions or live fetuses, body weight or external development of fetuses. Intravenous doses of 6.25–25 mg/kg body weight per day had no effect in this study (Tsujitani et al., 1981c).

2.2.5 Special studies

(a) Kidney toxicity

Groups of five Sprague-Dawley rats were given single subcutaneous injections of sodium colistin methanesulfonate at 8 or 24 mg/kg body weight. Serum concentrations reached 39 µg/ml 90 min after injection of the higher dose. The animals were killed after 48 h when blood urea levels and histopathology of the kidney were found to be unaffected (Lawson et al., 1972).

(b) Microbiological effects

(i) Pure cultures of human gut flora

Minimum inhibitory concentrations (MICs) for colistin sulfate (685 µg colistin base/mg) were determined in 10 strains of *E. coli*, *Bifidobacterium* spp. and *Bacteroides fragilis* isolated from the faeces of healthy adult humans. Microbiological activity was measured using bacterial suspensions at cell densities of 10^7 bacteria/ml. The lowest concentration that completely inhibited the growth of 50% of a microbial culture (MIC_{50}) was 0.1 µg colistin base/ml for *E. coli*. *Bacteroides fragilis* and *Bifidobacterium* spp. were less sensitive, with MIC_{50} values of 10.7 µg colistin base/ml and 32 µg colistin base/ml, respectively (Richez, 1992).

In a more comprehensive study, pooled human faecal samples from three volunteers who had not received any medical treatments 3 months prior to faecal collection were used. Ten predominant human faecal flora species were isolated, and 10 strains of each bacterial species were grown for use in the MIC testing with colistin sulfate (15 000 IU/mg). *Bacteroides fragilis*, *Bacteroides* spp., *Fusobacterium* spp., *Bifidobacterium* spp., *Eubacterium* spp., *Clostridium* spp., *Peptococcus* spp., *Peptostreptococcus* spp., *Enterococcus* spp., *Lactobacillus* spp. and *E. coli* were tested at cell densities of 10^5 and 10^9 colony-forming units (CFU)/ml. Of the species tested, *E. coli* exhibited the lowest MIC_{50} of 1 µg colistin base/ml for the faecal isolates and 0.5 µg colistin base/ml for the ATCC-type culture at 1×10^5 CFU/ml (Table 4). Using higher-density inocula of 1×10^9 CFU/ml, the MIC_{50} for *E. coli* was 8 µg colistin base/ml (Table 5) (Jeong et al., 2005).

(ii) Chemostat model of human colonic microflora

Anaerobic chemostat systems following the protocols of Carman & Woodburn (2001) were used, with a control culture and test human faecal cultures exposed to colistin sulfate (15 000 IU/mg) at 5, 50 or 500 µg/ml. These concentrations are equal to 2.5, 25 and 250 µg colistin base/ml. Total aerobic and anaerobic bacterial counts were determined. Reductions in total anaerobes and the *B. fragilis* group were observed for the 250 µg/ml cultures. *E. coli* dropped to non-detectable levels at concentrations of 25 and 250 µg/ml, but were unchanged for the 2.5 µg/ml cultures. Data for the other bacterial groups were inconclusive. The susceptibility of the bacteria to colistin sulfate before and after addition of the drug to the cultures was tested. No change was detected for the anaerobes, but a decrease in colistin sulfate susceptibility was observed in the total aerobe cultures at all three concentrations. An increase in resistant *E. coli* was seen at 2.5 µg/ml. Since molecular characterization and serotyping of the *E. coli* isolates were not undertaken, the study could not determine whether the increase in resistance was due to the selection of less susceptible strains or the induction of resistance. In enzyme activity studies, reductions in glucosidase and glucuronidase activities were seen at the highest concentration. A transient increase in azoreductase activity was also observed at all three concentrations, but this appeared to have begun in at least one of the cultures even before the addition of colistin sulfate. Some modest changes in short-chain fatty acid ratios were also demonstrated.

The available information indicates that the no-observed-effect concentration (NOEC) is less than 2.5 µg/ml (Jeong et al., 2005).

Table 4. MIC of colistin sulfate on human microflora (1×10^5 CFU/ml)

Microflora[a]	MIC (µg colistin base/ml)					
	Faecal samples			ATCC samples		
	MIC_{50}	MIC_{90}	GM MIC[b]	MIC_{50}	MIC_{90}	GM MIC[b]
Bacteroides fragilis	128	128	53.8	256	256	256
Bacteroides spp.	>256	>256	>256	>256	>256	>256
Fusobacterium spp.	>256	>256	>256	256	256	203.2
Bifidobacterium spp.	128	128	103.9	64	64	50.8
Eubacterium spp.	256	>256	219.5	>256	>256	>256
Clostridium spp.	>256	>256	>256	>256	>256	>256
Peptococcus and *Peptostreptococcus* spp.	128	256	160.9	256	256	203.2
Enterococcus spp.	>256	>256	>256	256	256	256
Lactobacillus spp.	>256	>256	>256	>256	>256	>256
Escherichia coli	1	1	1	0.5	0.5	0.5

[a] 10 isolates per organism (total 100 isolates) were used.
[b] Geometric mean of MIC.

(iii) Human flora-associated mice

These studies were conducted using a protocol similar to that of Perrin-Guyomard et al. (2001). Colistin sulfate (15 000 IU/mg) was administered to HFA mice via drinking-water at concentrations of 0, 50 or 500 µg/ml for 3 weeks, commencing 10 days after the inoculation of human faecal flora. These concentrations correspond to 0, 3.1 and 31 mg colistin base/kg body weight per day, respectively. The microbiological end-points measured were total aerobic and anaerobic counts, susceptibility to colistin sulfate, bacterial enzyme activity and short-chain fatty acid ratios. Reductions in *E. coli* numbers at both doses were significant, but the other bacterial population changes were not significant. No change in colistin sulfate susceptibility was observed. A small decrease in glucuronidase activity was observed at both doses, whereas azoreductase levels were initially higher, followed by a drop below the control levels. No data were given to indicate that the enzyme levels were comparable before the addition of colistin sulfate. No changes in short-chain fatty acid ratios were observed. It was not possible to identify a NOEL (Jeong et al., 2005).

Table 5. MIC of colistin sulfate on human microflora (1×10^9 CFU/ml)

Microflora[a]	MIC (µg colistin base/ml)					
	Faecal samples			ATCC samples		
	MIC_{50}	MIC_{90}	GM MIC[b]	MIC_{50}	MIC_{90}	GM MIC[b]
Bacteroides fragilis	256	>256	238.8	>256	>256	>256
Bacteroides spp.	>256	>256	>256	>256	>256	>256
Fusobacterium spp.	>256	>256	>256	256	256	256
Bifidobacterium spp.	>256	>256	>256	>256	>256	>256
Eubacterium spp.	>256	>256	>256	>256	>256	>256
Clostridium spp.	>256	>256	>256	>256	>256	>256
Peptococcus and *Peptostreptococcus* spp.	256	256	207.9	256	256	203.2
Enterococcus spp.	>256	>256	>256	>256	>256	>256
Lactobacillus spp.	>256	>256	>256	>256	>256	>256
Escherichia coli	8	8	6	4	8	5

[a] 10 isolates per organism (total 100 isolates) were used.
[b] Geometric mean of MIC.

(iv) Human studies

In a study of the effects of colistin sulfate on human faecal microflora, a daily oral dose of 0.45 g colistin base was given to six healthy adult volunteers for 3 consecutive days. Changes in total enterobacteriaceae and anaerobes and colistin-resistant enterobacteriaceae and anaerobes were monitored in faecal samples before and throughout treatment. In addition, faecal samples were evaluated for the above targeted groups 4 days after treatment was withdrawn. There was no decrease in the total numbers of anaerobes; however, the enterobacteriaceae were eliminated in all volunteers between 24 and 48 h after treatment started, with the exception of one volunteer carrying *Proteus mirabilis*, which persisted throughout the treatment. All six volunteers were progressively recolonized by colistin-sensitive enterobacteriaceae in the days that followed the withdrawal of treatment. This study provides little useful information with which to determine a NOEL (Tancrede, 1993).

2.3 Observations in humans

In humans, colistin sulfate is considered to be of higher potential toxicity than sodium colistin methanesulfonate and therefore is used only in oral or topical preparations. Systemic toxicity following oral doses of colistin sulfate has not been

observed, presumably because of the negligible absorption. Side-effects after intramuscular injections of sodium colistin methanesulfonate include kidney toxicity, such as elevated blood urea nitrogen, suggesting acute renal failure, and neurotoxicity, such as numbness, tingling, dizziness and ataxia. However, these toxic effects are reversible, rare and generally observed following the use of higher than recommended doses or in persons with already impaired renal function (Schwartz, 1964; Goodwin, 1970).

In a prospective study, 288 hospitalized patients were monitored during 317 courses of therapy with sodium colistin methanesulfonate. The daily dosage ranged between <99 and >400 mg colistin base/day, given by intramuscular injection. The overall frequency of adverse events was 25.1%. Kidney toxicity, mostly renal insufficiency based on biochemical measurements, was observed at a frequency of 20.2%. Tubular necrosis was seen in six patients. Neurological disorders, which occurred at a frequency of 7.3%, included mainly paraesthesias, nausea, vomiting and dizziness. Respiratory insufficiency and apnoea leading to death in six patients were considered to be associated with neuromuscular blockade. Allergic reactions were seen at a frequency of 2.2%. The majority of the adverse effects were noted within 4 days of instituting therapy and appeared to be dose related (Koch-Weser et al., 1970).

Fourteen patients with severe *Klebsiella* chest and/or urinary tract infections were treated with sodium colistin methanesulfonate. Therapy was continued for a mean duration of 9.7 days using daily doses of 333 mg colistin base by intramuscular injection, 333 mg colistin base by intravenous injection or 200 mg colistin base by aerosol inhalation. Serum colistin levels were 22–82 µg/ml, with urinary levels between 85 and 1074 µg/ml. Acute renal failure was diagnosed in all patients on the basis of reduced creatinine clearance and increases in blood urea and serum creatinine and the appearance of urinary casts in some cases. Necropsy was carried out on six of the eight patients who died during or soon after the end of treatment. Definite tubular necrosis was seen in five cases, with an early form of necrosis in the sixth case. It was concluded that renal failure contributed to death in some cases, but in the majority death was due to the primary illness (Price & Graham, 1970).

Patients with cystic fibrosis and with pulmonary infections were treated with 3–8 mg colistin sulfate/kg body weight per day by intravenous infusion for at least 5 days. Of the 19 patients, increased serum creatinine, proteinuria and casts were noted in only one case (5.3%). Paraesthesia and/or ataxia were observed in six cases (31.6%) within a few days, but with no increase in severity on continued treatment (Bosso et al., 1991).

Seventeen patients who received intravenous colistin (compound unknown) for more than 4 weeks for the treatment of multidrug-resistant infections were evaluated. The mean dose was 146.7 mg colistin base/day, for a mean duration of 43.4 days. The median serum creatinine concentration increased during treatment but returned to near baseline values at the end of dosing. There was no effect on blood urea or liver function, and signs of neurotoxicity were not observed (Falagas et al., 2005a).

In a prospective study, 21 patients who received intravenous sodium colistin methanesulfonate for at least 7 days were assessed. The mean dose was 183.3 mg colistin base/day, for an average of 17.7 days. Increased serum creatinine was observed in three patients (14.3%) and was correlated with the cumulative dose (Falagas et al., 2005b).

Contact dermatitis in response to colistin salts is rare. In a survey of the literature, three possible reactions to sodium colistin methanesulfonate and two possible reactions to colistin sulfate were discovered (Sasaki et al., 1998).

3. COMMENTS

The Committee considered the results of studies on pharmacokinetics, acute and short-term toxicity, genotoxicity, fertility and developmental toxicity, renal toxicity, microbiological safety and effects in humans. Some of the genotoxicity studies were carried out according to appropriate standards. The majority of the other studies were performed prior to the establishment of standards for study protocol and conduct.

3.1 Biochemical data

Colistin, when administered orally as either the sulfate or methanesulfonate, was very poorly absorbed in humans. Drug concentrations in serum were either very low or non-detectable. Following oral administration, colistin was excreted in faeces. Following an injection of sodium colistin methanesulfonate, the drug hydrolysed to form colistin base. Excretion was principally in the urine.

Similarly, in rats, rabbits and dogs, colistin compounds were poorly absorbed in the gastrointestinal tract. When colistin compounds were injected into rats, there was wide distribution; with the exception of the kidney, however, drug concentrations were lower in tissues than in serum. The majority of injected drug was excreted in urine within a 24-h period. In rabbits, approximately 7% of the dose was recovered in bile.

In humans, intramuscular administration of colistin methanesulfonate resulted in therapeutic drug concentrations in serum that persisted for 6 h. The elimination half-life for colistin sulfate was approximately twice that for colistin methanesulfonate, 4 h vs 2 h, following intravenous administration of the drugs to patients with cystic fibrosis.

After parenteral administration, there was some evidence for metabolism in rabbits and dogs. In rabbits injected with sodium colistin methanesulfonate, 8.4% of the dose was excreted as colistin *N*-glucuronide in urine and bile. Dogs injected with either the sulfate or methanesulfonate excreted an unquantified proportion of drug material in faeces, in a form that had no microbiological activity, suggesting biotransformation of colistin.

3.2 Toxicological data

The acute oral toxicities of both colistin sulfate and sodium colistin methanesulfonate were low in mice. The lowest LD_{50} values were 432 mg colistin base/kg body weight for the sulfate and 766 mg colistin base/kg body weight for the methanesulfonate. The sulfate was more toxic than the methanesulfonate when parenterally administered to mice. The LD_{50} values were generally below 50 mg/kg body weight for the sulfate given as subcutaneous, intramuscular, intraperitoneal or intravenous injection and between 126 and 405 mg/kg body weight for the methanesulfonate given as subcutaneous, intraperitoneal or intravenous injection. At lethal doses, toxic signs included muscular incoordination, respiratory distress and occasionally convulsions. Death occurred within 3 h from asphyxia.

Colistin sulfate was administered orally to rats at doses of 6.67, 20 or 60 mg colistin base/kg body weight per day for 90 days. It was reported that there were no adverse effects on growth, behaviour, haematology, urinalysis, liver function or pathology. The design of the study was inadequate to allow the full toxicological potential of colistin sulfate to be evaluated.

Rats were fed diets containing colistin sulfate at concentrations of 0, 40, 200 or 1000 mg/kg for a period of 26 weeks. The dietary concentrations were equivalent to 0, 20.2, 101 and 505 mg colistin base/kg. There were no toxicologically significant changes as a result of treatment. The NOEL was the highest dose, 505 mg/kg in feed, equivalent to 50.5 mg colistin base/kg body weight per day.

Colistin sulfate was administered orally to dogs at doses of 6.67, 20 or 60 mg colistin base/kg body weight per day for 90 days. It was reported that there were no adverse effects on growth, behaviour, haematology, urinalysis, liver and kidney function or pathology. The design of the study was inadequate to allow the full toxicological potential of colistin sulfate to be evaluated.

Mice were given intraperitoneal injections of sodium colistin methanesulfonate at 0, 50, 100 or 300 mg/kg body weight per day for 30 days. At 300 mg/kg body weight per day, reduced body weight gain, reduced spontaneous activity, convulsions and death of some animals of each sex were observed. The number of erythrocytes, haematocrit and haemoglobin were depressed and liver weight was increased in males at this dose. The authors reported that "reactive symptoms" were noted in liver, spleen and kidney.

In three separate studies, rats were given intraperitoneal injections of sodium colistin methanesulfonate. When rats were administered doses of 0, 5, 10, 20 or 60 mg/kg body weight per day for 30 days, reduced body weight gain, reduced spontaneous activity and convulsions were observed at higher doses, and all high-dose animals died within 3 days. The number of erythrocytes, haematocrit and haemoglobin were depressed in males given 20 mg/kg body weight per day, and alanine aminotransferase was increased in females at this dose. In a second study, at doses of 0.83, 2.5 or 7.5 mg colistin base/kg body weight per day for 60 days, the only observed effect was hyperaemia of the kidneys, which occurred frequently at the highest dose and infrequently at the lower doses. In the third study, at doses of 0, 5, 10, 20 or 30 mg/kg body weight per day for 6 months, body

weight gain was depressed in males at 30 mg/kg body weight per day and in females at 10 mg/kg body weight per day and above. Mortality was dose-related at 10 mg/kg body weight per day and above, and reduced spontaneous activity and convulsions were observed. Decreased aspartate aminotransferase and alanine aminotransferase and increased blood urea nitrogen were seen in males at 20 and 30 mg/kg body weight per day, and albumin/globulin ratios were increased in females at 30 mg/kg body weight per day. Albuminuria was seen in both sexes at 20 and 30 mg/kg body weight per day. It was reported that "pathological changes" were noted in kidney, liver and spleen at 20 and 30 mg/kg body weight per day.

Dogs received intramuscular injections of sodium colistin methanesulfonate at doses of 0.83, 2.5 or 7.5 mg colistin base/kg body weight per day for 60 days. It was reported that there were no significant adverse effects on growth, behaviour, haematology, urinalysis or pathology.

Assays covering an adequate range of genotoxic end-points were conducted with colistin. An equivocal result was claimed in the SOS chromotest for DNA damage in *E. coli* strains. Colistin was tested as one of a range of compounds in a series of screening assays. However, it was not possible to assess the validity of this finding, as no primary data were provided. A concentration-related increase in chromosomal damage was observed in cultured human lymphocytes associated with decreases in the mitotic index and in the rate of cell division. Bromodeoxyuridine was added at the initiation of cultures in order to visualize sister chromatid exchanges. This is not normal practice in assays for the detection of chromosomal aberrations, and it is not known if there may have been an interaction with the added bromodeoxyuridine. Assays for reverse mutation in *S. typhimurium* and *E. coli*, DNA repair in *B. subtilis*, forward mutation in cultured Chinese hamster cells, sister chromatid exchange formation in cultured human lymphocytes and micronucleus formation in bone marrow of treated mice were clearly negative. The Committee concluded that, on the weight of evidence, colistin is unlikely to pose a genotoxic hazard.

No long-term studies of toxicity, including carcinogenicity, have been reported for colistin. Colistin has no significant genotoxic activity and is not chemically related to known carcinogens. Furthermore, colistin is poorly absorbed from the gastrointestinal tract, and no neoplastic or preneoplastic lesions were observed in 26-week studies in rats given repeated oral or parenteral doses. Despite the absence of studies on carcinogenicity, the Committee concluded that colistin compounds are unlikely to pose a carcinogenic risk.

Reproductive toxicity was evaluated in three separate studies in mice that were given intraperitoneal injections of sodium colistin methanesulfonate. The administration of 24–100 mg colistin base/kg body weight per day, from prior to mating through the first 7 days of gestation, caused no detrimental effects on reproductive capacity or on the fetuses. The administration of 15–150 mg/kg body weight per day during the period of organogenesis slightly increased resorptions at the highest dose. The administration of 125–500 mg/kg body weight per day during the period of organogenesis resulted in delayed ossification in fetuses at 250 and 500 mg/kg body weight per day. Postnatal monitoring identified a

reduction in spontaneous activity in offspring of the 250 and 500 mg/kg body weight per day groups. Maternal toxicity and fetal abnormalities were not observed in any study in mice.

Rats received colistin sulfate at doses of 0, 2.6, 65 or 130 mg colistin base/kg body weight per day by gavage on gestation days 7–17. There were no toxicologically significant findings on dams or fetuses. Therefore, the NOEL was the highest dose, 130 mg colistin base/kg body weight per day.

Reproductive toxicity was investigated in four separate studies in rats that were given parenteral doses of sodium colistin methanesulfonate. The intravenous administration of 2.5–10 mg colistin base/kg body weight per day, from prior to mating through the first 7 days of gestation, caused no detrimental effects on reproductive capacity, but delayed ossification was apparent in fetuses at the highest dose. The intraperitoneal administration of 5–40 mg/kg body weight per day during the period of organogenesis slightly decreased fetal body weight at 20 and 40 mg/kg body weight per day. The intravenous administration of 2.5–10 mg colistin base/kg body weight per day during the period of organogenesis resulted in reduced ossification in fetuses at 10 mg/kg body weight per day. Postnatal monitoring of offspring revealed reduced survival in litters of the 10 mg/kg body weight per day group. Maternal toxicity and fetal abnormalities were not observed in any study. The intravenous administration of 6.25–25 mg/kg body weight per day, from after organogenesis through the end of lactation, caused no effect on growth and development of the offspring in rats.

Rabbits received sodium colistin methanesulfonate at doses of 0, 20, 25 or 32 mg colistin base/kg body weight per day by intravenous injection on gestation days 6–18. There were no significant effects on dams or fetuses.

In a special study to assess renal toxicity in rats, blood urea nitrogen concentrations and kidney pathology were unaffected by single subcutaneous injections of sodium colistin methanesulfonate at 8 or 24 mg/kg body weight.

In humans, systemic toxicity following oral doses of colistin sulfate has not been observed, presumably because of its negligible absorption. Parenteral administration of colistin sulfate or sodium colistin methanesulfonate has been associated with kidney toxicity, such as elevated blood urea nitrogen, and neurotoxicity, such as numbness, tingling, dizziness and ataxia. Renal tubular necrosis and neuromuscular blockade have also occurred at lower frequencies. These toxic effects were generally observed following the use of higher than recommended doses or in persons with already impaired renal function, and in most cases they were reversible. Gastrointestinal disturbances have not been reported.

The most relevant study for determining a toxicological acceptable daily intake (ADI) is the 26-week study in rats given colistin sulfate in the diet. Studies in which colistin salts were given by parenteral injection are less relevant for assessing the acceptable intake of colistin in food. The NOEL for toxicity was 50.5 mg colistin base/kg body weight per day, the highest dose used in this study. A safety factor of 100 was considered appropriate in view of the absence of toxicity after oral administration and the poor absorption through the gastrointestinal

tract in all species tested. Therefore, an ADI of 0–500 µg/kg body weight could be established on the basis of the toxicological data.

3.3 Microbiological data

Colistin sulfate has been tested for its inhibitory activity against micro-organisms representative of the human colonic microflora. The most sensitive organism was *E. coli*, with a MIC_{50} of 0.1 µg colistin base/ml in one study and 1 µg colistin base/ml in a second, more comprehensive study. Other predominant human intestinal microflora were not susceptible to colistin sulfate.

Using anaerobic chemostat systems, human faecal cultures were exposed to colistin sulfate at concentrations of 2.5, 25 or 250 µg colistin base/ml. Reductions in total anaerobes and the *B. fragilis* group were observed at 250 µg/ml. *E. coli* levels were non-detectable at concentrations of 25 and 250 µg/ml. The susceptibility of the bacteria to colistin sulfate before and after addition of the drug to the cultures was unchanged for the anaerobes, but a decrease in susceptibility was observed in the total aerobe cultures at all three concentrations. An increase in resistant *E. coli* was seen at a concentration of 2.5 µg/ml. Reductions in glucosidase and glucuronidase activities were seen at the highest concentration, and a transient increase in azoreductase activity was observed at all three concentrations. Some modest changes in short-chain fatty acid ratios were also demonstrated. A NOEC was not identified in this study.

Colistin sulfate was administered in drinking-water at concentrations of 0, 50 or 500 µg/ml to HFA mice for 3 weeks, commencing 10 days after the inoculation of human faecal flora. These concentrations correspond to 0, 3.1 and 31 mg colistin base/kg body weight per day, respectively. Reductions in *E. coli* numbers at both doses were significant, but other bacterial population changes were not significant. No change was observed in susceptibility to colistin sulfate. A small decrease in glucuronidase activity was observed at both doses, whereas azoreductase levels were initially higher, followed by a drop to below the control levels. No changes in short-chain fatty acid ratios were observed. A NOEL was not identified in this study.

In a study of the effects of colistin sulfate on human faecal microflora, a daily oral dose of 0.45 g colistin base was given to six healthy adult volunteers for 3 consecutive days. There was no decrease in the total numbers of anaerobes in faeces; however, the enterobacteriaceae were eliminated in all volunteers between 24 and 48 h after treatment started, with the exception of one individual carrying *Proteus mirabilis*, which persisted throughout the treatment. All six volunteers were progressively recolonized by colistin-sensitive enterobacteriaceae in the days that followed the withdrawal of treatment. A NOEL was not identified in this study.

A decision-tree for evaluating the potential effect of veterinary drug residues on human intestinal microflora was developed by the Committee at its fifty-second meeting (Annex 1, reference *140*). At its present meeting, the Committee used the decision-tree to answer the following questions in its assessment of colistin:

1. *Does the ingested residue have antimicrobial properties?*

Yes, but the spectrum of activity against the major groups of intestinal microflora is limited. The mechanism of action of colistin involves binding with the anionic lipopolysaccharide molecules by displacing calcium and magnesium from the outer cell membrane of Gram-negative bacteria, leading to permeability changes in the cell envelope, leakage of cell contents and cell death. Colistin is active against many Gram-negative aerobic bacilli, including *Acinetobacter* species, *Pseudomonas aeruginosa*, *Klebsiella* species, *Enterobacter* species, *E. coli*, *Salmonella* species, *Shigella* species, *Citrobacter* species, *Yersinia pseudotuberculosis*, *Morganella morganii* and *Haemophilus influenza*, and it has considerable activity against *Stenotrophomonas* species. Colistin is not active against some Gram-negative aerobic bacilli (including *Pseudomonas mallei*, *Burkholderia cepacia*, *Proteus* species, *Providencia* species, *Serratia* species, *Edwardsiella* species and *Brucella* species), Gram-negative and Gram-positive aerobic cocci, Gram-positive aerobic bacilli, anaerobes, fungi and parasites (Falagas & Kasiakou, 2005).

2. *Does the drug residue enter the lower bowel?*

Yes. Colistin is very poorly absorbed from the intestinal tract of humans and laboratory animals after oral administration. Studies in humans given oral doses of colistin sulfate have shown high concentrations of colistin in faeces. Therefore, oral doses of colistin compounds are available to the gastrointestinal tract microflora.

3. *Is the ingested residue transformed irreversibly to inactive metabolites by chemical transformation, by metabolism mediated by the host or intestinal microflora in the bowel and/or by binding to intestinal contents?*

No specific information was available on the metabolism of colistin by intestinal microflora. However, it has been reported that a significant proportion of the drug excreted in human faeces is in bound form, which may result in inactivation of microbiological activity.

4. *Do data on the effects of the drug on the colonic microflora provide a basis to conclude that the ADI derived from toxicological data is sufficiently low to protect the intestinal flora?*

No. Colistin is very poorly absorbed from the gastrointestinal tract, and therefore the compound exhibits low toxicity in humans and laboratory animals. Microbiological studies have indicated that colistin sulfate may have adverse effects on the intestinal microflora.

5. *Do clinical data from the therapeutic use of the class of drugs in humans or data from in vitro or in vivo model systems indicate that effects could occur in the gastrointestinal tract?*

Yes. In vitro MIC data indicated that *E. coli* was sensitive to colistin sulfate. In addition, in a short-term, high-dose study in humans, the enterobacteriaceae were eliminated within 48 h of treatment. All subjects in the study group were recolonized by colistin-sensitive enterobacteriaceae in the days that followed the

withdrawal of treatment. It was noted that, based on the spectrum of activity and mechanism of action of colistin, gastrointestinal effects such as nausea, vomiting, abdominal cramps and diarrhoea would not be expected and are not commonly reported.

6. *Determine the most sensitive adverse effect(s) of the drug on the human intestinal microflora.*

An increase in resistant *E. coli* was seen in an in vitro study. However, emergence of resistance does not appear to be the microbiological end-point of concern for colistin in the case of the predominant anaerobes in the human gastrointestinal tract. The available data indicate that disruption of the colonization barrier may be a minor concern with colistin compounds, but it is the most sensitive adverse effect on the human gastrointestinal microflora.

7. *If disruption of the colonization barrier is the concern, determine the MIC of the drug against 100 strains of predominant intestinal flora and take the geometric mean MIC of the most sensitive genus or genera to derive an ADI using the formula discussed at the forty-seventh meeting of the Committee (Annex 1, reference 125). Other model systems may be used to establish a NOEC to derive an ADI.*

The Committee considered that the MIC data were the most appropriate to use in determining a microbiological ADI. An evaluation of the MIC_{50} values for relevant gastrointestinal microflora provides a figure of 1 µg colistin base/ml for *E. coli*. This value can be used to calculate a microbiological ADI, as follows:

$$\text{Upper limit of ADI} = \frac{MIC_{50} \times MCC}{FA \times SF \times BW}$$

where:

MIC_{50} = minimum inhibitory concentration for 50% of strains of the most sensitive relevant organism. The MIC_{50} for the most sensitive relevant genus of the gut flora was 1 µg colistin base/ml (1 µg/g) for *E. coli*.

MCC = mass of colonic contents: a value of 220 g, determined by the Committee at its forty-seventh meeting (Annex 1, reference *125*), was used in the calculation.

FA = available fraction of the dose: the microbiologically active residue is colistin. Colistin is very poorly absorbed from the gastrointestinal tract in humans and laboratory animals. However, measurement of colistin concentrations in human faeces did not account for the administered dose. It has been reported that a significant proportion of the drug excreted in human faeces is in bound form. Therefore, a conservative estimate of the fraction of the dose available to the gastrointestinal microflora is 50%. Hence, the value in the equation is 0.5.

SF = safety factor: the magnitude of the safety factor depends on the quality and quantity of the microbiological data available. A value of 1 is appropriate when extensive relevant microbiological data are available, as is the case in the current assessment. Thus, the safety factor should be 1.

BW = body weight: a value of 60 kg has been adopted for an adult.

Hence,

$$\text{Upper limit of ADI} = \frac{1\ \mu g/g \times 220\ g}{0.5 \times 1 \times 60\ kg}$$

$$= 7\ \mu g/kg \text{ body weight}$$

4. EVALUATION

The Committee considered that microbiological effects were more relevant than toxicological effects for the establishment of an ADI for colistin. Therefore, the Committee established an ADI of 0–7 µg/kg body weight on the basis of the MIC_{50} for *E. coli*. The upper bound of this ADI is significantly lower than the upper bound of the toxicological ADI of 500 µg/kg body weight.

A toxicological monograph was prepared.

5. REFERENCES

Abe, M., Shimizu, K., Ouchi, M. & Matsumoto, T. (1976) [Studies on sodium colistin methanesulfonate (CL-M) on metabolism in the urine and bile of rabbits.] *Chemotherapy*, **24**, 1592–1596 (in Japanese).

Al-Khayyat, A.A. & Aronson, A.L. (1973) Pharmacologic and toxicologic studies with polymyxins. II. Comparative pharmacologic studies of the sulfate and methanesulfonate salts of polymyxin B and colistin in dogs. *Chemotherapy*, **19**, 82–97.

Anonymous (1968) Étude physique, chimique, pharmacodynamique et thérapeutique des antibiotiques. *Thérapie*, **23**, 127–174.

Archimbault, P., Boutier, C., Fellous, R. & Muscat, G. (1980) Étude pharmacocinétique de la colistine chez les bovines. *Rec. Med. Vet. Ec. Alfort*, **156**, 621–626.

Bosso, J.A., Liptak, C.A., Seilheimer, D.K. & Harrison, G.M. (1991) Toxicity of colistin in cystic fibrosis patients. *DICP*, **25**, 1168–1170.

Carman, R.J. & Woodburn, M.A. (2001) Effects of low levels of ciprofloxacin on a chemostat model of the human colonic microflora. *Reg. Toxicol. Pharmacol.*, **33**, 276–284.

Falagas, M.E. & Kasiakou, S.K. (2005) Colistin: The revival of polymyxins for the management of multidrug-resistant Gram-negative bacterial infections. A review. *Clin. Infect. Dis.*, **40**, 1333–1341.

Falagas, M.E., Rizos, M., Bliziotis, I.A., Rellos, K., Kasiakou, S.K. & Michalopoulos, A. (2005a) Toxicity after prolonged (more than four weeks) administration of intravenous colistin. *BMC Infect. Dis.*, **10**, 1.

Falagas, M.E., Fragoulis, K.N., Kasiakou, S.K., Sermaidis, G.J. & Michalopoulos, A. (2005b) Nephrotoxicity of intravenous colistin: a prospective evaluation. *Int. J. Antimicrob. Agents*, **26**, 504–507.

Fukuhara, S. (1990a) *Residual study of colistin sulfate in pigs*. Unpublished report of Test No. 89-215 from Research Institute for Animal Science in Biochemistry and Toxicology, Sagamihara-city, Kanagawa-prefecture, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Fukuhara S (1990b) *Residual study of colistin sulfate in cattle*. Unpublished report of Test No. 89-214 from Research Institute for Animal Science in Biochemistry and Toxicology, Sagamihara-city, Kanagawa-prefecture, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Goodwin, N.J. (1970) Colistin and sodium colistimethane. *Med. Clin. North Am.*, **54**, 1267–1276.

Jaju, M., Jaju, M. & Ahuja, Y.R. (1983) Cytogenetic effect of colistin on human lymphocytes in vitro: Chromosome aberrations, sister chromatid exchanges, mitotic index, cell cycle kinetics, and acrocentric associations. *Teratog. Carcinog. Mutagen.*, **3**, 515–526.

Jeong, S.-H., Song, Y.-K. & Cho, J.-H. (2005) *Evaluation of acceptable daily intake of colistin sulfate based on microbiological toxicity on human intestinal microflora*. Unpublished report from Toxicology Laboratory, National Veterinary Research and Quarantine Service, Anyang, Korea. Submitted to WHO by National Veterinary Research and Quarantine Service, Korea.

Kada, T., Hirano, K. & Shirasu, Y. (1980) Screening of environmental chemical mutagens by the Rec-assay system with *Bacillus subtilis*. *Chem. Mutagens*, **6**, 149–173.

Koch-Weser, J., Sidel, V.W., Federman, E.B., Kanarek, P., Finer, D.C. & Eaton, A.E. (1970) Adverse effects of sodium colistimethane. Manifestations and specific reaction rates during 317 courses of therapy. *Ann. Intern. Med.*, **72**, 857–868.

Lawson, D.H., Macadam, R.F., Singh, H., Gavras, H., Hartz, S., Turnbull, D. & Linton, A.L. (1972) Effect of furosemide on antibiotic-induced renal damage in rats. *J. Infect. Dis.*, **126**, 593–600.

Li, J., Coulthard, K., Milne, R., Nation, R.L., Conway, S., Peckham, D., Etherington, C. & Turnidge, J. (2003) Steady-state pharmacokinetics of intravenous colistin methanesulphonate in patients with cystic fibrosis. *J. Antimicrob. Chemother.*, **52**, 987–992.

Lin, B., Zhang, C. & Xiao, X. (2005) Toxicity, bioavailability and pharmacokinetics of a newly formulated colistin sulfate solution. *J. Vet. Pharmacol. Ther.*, **28**, 349–354.

MacAulay, M.A., Charles, D. & Burgess, F.M. (1967) Placental transmission of colistimethane. *Clin. Pharmacol. Ther.*, **8**, 578–586.

Mamber, S.W., Okasinski, W.G., Pinter, C.D. & Tunac, J.B. (1986) The *Escherichia coli* K-12 SOS chromotest agar spot test for simple, rapid detection of genotoxic agents. *Mutat. Res.*, **171**, 83–90.

Marzin, D. (1992) *Gene mutation assay at the HPRT locus in V79 Chinese hamster cell culture (6-thioguanine resistance) carried out with the compound colistin sulfate.* Unpublished Report No. 920401 from Genetic Toxicology Laboratory, Institut Pasteur de Lille, Lille, France. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Molinier, B. (1992a) *Colistin sulfate. Reverse mutation assay by the Ames test.* Unpublished report of Study No. 8492 MMO from Centre International de Toxicologie, Miserey, Evreux, France. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Molinier, B. (1992b) *Colistin sulfate. Micronucleus test by intraperitoneal route in mice.* Unpublished report of Study No. 8494 MAS from Centre International de Toxicologie, Miserey, Evreux, France. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Morimoto, H., Noro, H., Shimizu, T., Hanazumi, M., Hayashi, Y. & Yonemochi, C. (1984) *Teratogenicity study on colistin sulfate.* Unpublished report from Japan Scientific Feeds Association, Narita-city, Chiba, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Morimoto, H., Noro, H. & Ohtaki, H. (undated a) *Study on acute toxicity of colistin sulfate in mice.* Unpublished report from Japan Scientific Feeds Association, Narita-city, Chiba, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Morimoto, H., Noro, H., Ohtaki, H. & Shimizu, T. (undated b) *Study on chronic toxicity of colistin sulfate.* Unpublished report from Japan Scientific Feeds Association, Narita-city, Chiba, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Perrin-Guyomard, A., Cottin, S., Corpet, D.E., Boisseau, J. & Poul, J.M. (2001) Evaluation of residual and therapeutic doses of tetracycline in the human-flora-associated (HFA) mice model. *Reg. Toxicol. Pharmacol.*, **34**, 125–136.

Price, D.J.E. & Graham, D.I. (1970) Effects of large doses of colistin sulphomethane sodium on renal function. *Br. Med. J.*, **4**, 525–527.

Richez, P. (1992) *Antibacterial activity of colistin sulphate against human gut microflora.* Unpublished report of Study No. MJ1001 from Laboratoire Vétérinaire Départemental de Montpellier, Montpellier, France. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Saitoh, T., Tsujitani, M., Okuchi, M. & Matsumoto, T. (1981) [Reproduction studies of sodium colistin methanesulfonate. II. Teratogenicity study in mice.] *Chemotherapy*, **29**, 1051–1061 (in Japanese).

Saitoh, T., Tsujitani, M., Okuchi, M. & Matsumoto, T. (undated) *Reproduction studies of colistin. I. Fertility study in mice.* Unpublished report from Pharmacological Research Center, Kayaku Antibiotic Research Co. Ltd, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Sasaki, S., Mitsuhashi, Y. & Kondo, S. (1998) Contact dermatitis due to sodium colistimethane. *J. Dermatol.*, **25**, 415–417.

Sato, H., Ouchi, M. & Koumi, J. (1972) [Studies on the distribution of colistin sulfate in the body. Distribution and change with time in chickens and pigs by oral administration.] *Jpn. J. Antibiot.*, **25**, 239–245.

Schwartz, B.S. (1964) The polypeptides of the polymyxin group. In: Schnitzer, R. & Hawking, F., eds. *Experimental chemotherapy*. New York, Academic Press, pp. 217–270.

Schwartz, B.S., Warren, M.R., Barkley, F.A. & Landis, L. (1960) Microbiological and pharmacological studies of colistin sulfate and sodium colistin methanesulfonate. *Antibiot. Annu.*, **1959–1960**, 41–60.

Szybalski, W. (1958) Special microbiological systems. II. Observations on chemical mutagenesis in microorganisms. *Ann. N. Y. Acad. Sci.*, **76**, 475–489.

Tancrede, C. (1993) *The effect of oral colistin on faecal flora in man*. Unpublished report from Institut Gustave-Roussy, Villejuif, France. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Terakado, S., Azechi, H., Omae, K., Koyama, T., Ninomiya, K. & Kashiwazaki, M. (1972) [*Distribution of colistin sulfate and changes with time in intestinal* E. coli *count in pigs following oral administration*.] 73rd Congress of Japan Society of Veterinary Medicine. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan (in Japanese).

Tomizawa, S. & Kamada, K. (1973) [Colistin sodium methanesulfonate on fetuses of mice and rats.] *Pharmacometrics*, **7**, 1047–1060 (in Japanese).

Tomizawa, S., Okamoto, R., Kawamura, K. & Iida, T. (1973a) [Colistin sodium methanesulfonate. Subacute toxicity of colistin sodium methanesulfonate in mice and rats.] *Pharmacometrics*, **7**, 915–928 (in Japanese).

Tomizawa, S., Okamoto, R., Kawamura, K. & Iida, T. (1973b) [Colistin sodium methanesulfonate. Chronic toxicity of colistin sodium methanesulfonate in rats.] *Pharmacometrics*, **7**, 947–956 (in Japanese).

Tsujitani, M., Kawaguchi, Y., Takada, H., Ohuchi, M., Saitoh, T. & Matsumoto, T. (1981a) [Reproduction studies of sodium colistin methanesulfonate. II. Teratogenicity study in rats.] *Chemotherapy*, **29**, 149–163 (in Japanese).

Tsujitani, M., Ohuchi, M., Saitoh, T. & Matsumoto, T. (1981b) [Reproduction studies of sodium colistin methanesulfonate. Teratogenicity study in rabbits.] *Chemotherapy*, **29**, 300–305 (in Japanese).

Tsujitani, M., Oyama, M., Kadoya, K., Ohuchi, M., Saitoh, T. & Matsumoto, T. (1981c) [Reproduction studies of sodium colistin methanesulfonate. III. Perinatal and postnatal study in rats.] *Chemotherapy*, **29**, 289–299 (in Japanese).

Tsujitani, M., Kawaguchi, Y., Takada, H., Ohuchi, M., Saitoh, T. & Matsumoto, T. (undated a) *Reproduction studies of colistin. II. Teratogenicity study in rats*. Unpublished report from Pharmacological Research Center, Kayaku Antibiotic Research Co. Ltd, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Tsujitani, M., Ohuchi, M., Saitoh, T. & Matsumoto, T. (undated b) *Reproduction study of colistin. Teratogenicity study in rabbits*. Unpublished report from Pharmacological

Research Center, Kayaku Antibiotic Research Co. Ltd, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Tsujitani, M., Oyama, M., Ohuchi, M., Saitoh, T. & Matsumoto, T. (undated c) *Reproduction study of colistin. I. Fertility study in rats.* Unpublished report from Pharmacological Research Center, Kayaku Antibiotic Research Co. Ltd, Japan. Submitted to WHO by Meiji Seika Kaisha Ltd, Tokyo, Japan.

Yaffe, S.J. & Rane, A. (1971) Developmental aspects of pharmacokinetics. *Acta Pharmacol. Toxicol.*, **29** (Suppl. 3), 240–249.

Yamada, S., Mayahara, T., Mitsuhashi, N., Wakabayashi, K., Hiratsuka, K., Sadaoka, K., Nakazawa, S., Watanabe, O., Sato, H., Matsumoto, T. & Noda, I. (1974) [Fundamental studies on colistin methanesulfonate (Colimycin (CL-M)). I. On the blood level, distribution, and excretion of CL-M.] *Jpn. J. Antibiot.*, **27**, 8–14 (in Japanese).

ERYTHROMYCIN

First draft prepared by

Dr Sang-Hee Jeong,[1] Professor Arturo Anadón,[2] and Dr Carl Cerniglia[3]

[1] Toxicology and Chemistry Division, National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, Anyang City, Republic of Korea

[2] Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain

[3] Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, Arkansas, USA

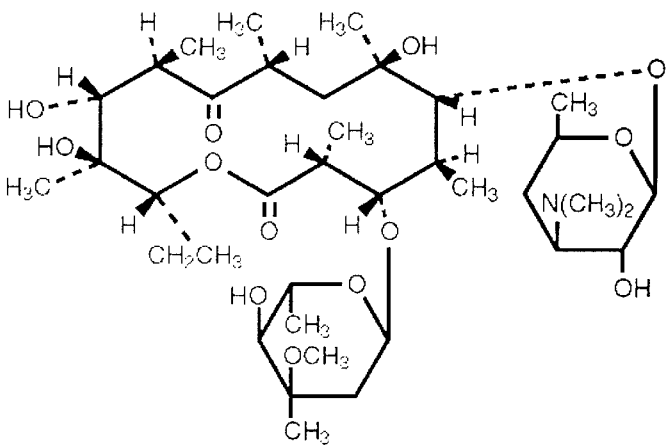
1. EXPLANATION

Erythromycin is a 14-membered macrocyclic lactone produced by the streptomycete *Saccharopolyspora erythraea*, a soil-borne organism. It is a mixture of

three compounds, named A, B and C, produced during fermentation. The principal product is erythromycin A, with small portions of B (≤5%) and C (≤5%).

Erythromycin consists of two sugars, desosamine and cladinose, which are attached to erythronolide, a macrocyclic lactone (Figure 1). It is slightly hygroscopic and slightly soluble in water (0.2%). Erythromycin is more active at pH 8 than at neutral pH and is unstable in acid media (Huber, 1977; Sweetman, 2002).

Figure 1. Structural formula of erythromycin



Erythromycin	Mol. Formula	Mr	R1	R2
A	$C_{37}H_{57}NO_{13}$	734	OH	CH_3
B	$C_{37}H_{57}NO_{12}$	718	H	CH_3
C	$C_{38}H_{55}NO_{13}$	720	OH	H

Erythromycin has antibacterial effects by inhibition of protein synthesis. It interferes with the translocation step by binding to the 50S ribosomal subunit (Burrows, 1980; Champney et al., 2003). There are no studies available on the emergence of resistance to erythromycin in human intestinal microflora. Cross-resistance has been noted between erythromycin and oleandomycin (Huber, 1977). Erythromycin may lead to the development of resistant organisms via methylation or mutation in 23S ribosomal RNA or ribosomal proteins or via alteration of *mef* genes found in a variety of Gram-positive genera (Courvalin et al., 1985; Roberts et al., 1999; Tait-Kamradt et al., 2000; Davydova et al., 2002; Leclerc, 2002; Bartkus et al., 2003; Farrell et al., 2003; Edelstein, 2004; O'Connors et al., 2004; Payot et al., 2004; Haanpera et al., 2005).

Erythromycin is effective against many Gram-positive organisms and is useful in the treatment of staphylococcal infections that are resistant to penicillin therapy. *Mycoplasma*, *Staphylococcus*, *Streptococcus*, *Neisseria*, some strains of *Haemophilus*, *Corynebacterium*, *Listeria*, *Pasteurella multocida*, *Brucella*, *Rickettsia* and *Treponema* are sensitive to erythromycin. It has a structure similar to that of its derivatives, such as azithromycin, clarithromycin and roxithromycin (Huber, 1977).

Erythromycin has been widely used as an antimicrobial compound against a variety of infectious microorganisms in animals and humans. Erythromycin is available in the form of estolate, ethylsuccinate, gluceptate, lactobionate and stearate. In veterinary medicine, erythromycin is used for the treatment of clinical and subclinical mastitis in lactating cows, for the treatment of infectious diseases, including digestive and respiratory diseases, due to erythromycin-sensitive bacteria in cattle, sheep, swine and poultry and for the treatment of chronic diseases due to mycoplasma in poultry. Erythromycin is used alone or in combination with other antimicrobial agents, such as ampicillin, colistin sulfate, sulfonamides and tylosin. It can be added to drinking-water or feed or administered via intramuscular or intramammary injection. The maximum recommended therapeutic dose in veterinary use is 20 mg/kg body weight per day as erythromycin base.

Erythromycin was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its twelfth meeting in 1968 (Annex 1, reference *17*). At this meeting, no acceptable daily intake (ADI) was established. Erythromycin was re-evaluated on priority request of the Codex Committee on Residues of Veterinary Drugs in Foods. The Committee was requested to establish an ADI for erythromycin.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Rats

In a study that was not conducted according to Good Laboratory Practice (GLP), 50 rats (strain not stated) received orally a single dose of propionyl erythromycin ester lauryl sulfate (PELS) at 25 mg/kg body weight. The major absorption site was the small intestine, and only a small amount was absorbed in the stomach. The peak serum concentration (approximately 0.27 µg/ml) was observed 2 h after dosing. When the same dose of propionyl erythromycin was given to 50 rats, the maximum serum concentration (≤1 µg/ml) was reached 1 h after dosing (Anderson et al., 1959).

In a non-GLP study, propionyl erythromycin and PELS were administered to rats (strain not stated) orally at a single dose of 100 mg/kg body weight. Following the administration of propionyl erythromycin and PELS, erythromycin activity was found in the following organs: lung (2.1–10.8 µg/ml) > spleen (1.3–6.6 µg/ml) >

liver (0.9–6.0 µg/ml) > heart (0.6–5.5 µg/ml) > kidney (0.5–4.4 µg/ml); however, erythromycin (at 0.12–0.32 µg/ml) was detected in the brain 2 and 7 h after the administration of PELS only (Anderson et al., 1959).

In a non-GLP study in which 20 female rats (strain not stated) were given 100 mg erythromycin base/kg body weight orally, erythromycin concentrations higher than 10 µg/g wet weight were detected in the liver, submaxillary glands, spleen, adrenals, lungs and kidneys 2 h after administration. Large amounts of erythromycin (mean 4.3–6.0 µg/g wet weight) were also found in the thymus, skin, muscle, reproductive organs and heart (Lee et al., 1953).

In another non-GLP study, rats (strain not stated) were intravenously administered 10 mg radiolabelled erythromycin (*N*-methyl-^{14}C-erythromycin, 0.3 MBq). Erythromycin was excreted mainly into the bile; 15.1% of the dose was found in the bile 2 h after the injection. Twenty hours after the administration, 37–43% of the administered dose was recovered in the intestinal tract plus faeces, 27–36% in the urine and 21–29% in the expired air (Lee et al., 1956a).

(b) Dogs

In a non-GLP study, dogs (strain not stated) were intravenously administered erythromycin at 10 mg/kg body weight. Within 8 h after the administration, 5.4% of the dose was found in the bile (Wilson & van Boxtel, 1978).

Peak tissue erythromycin concentrations exceeded serum concentrations in normal dogs (strain, administration route and dose not stated). The ratios of peak serum to peak tissue or fluid concentrations in lung, liver, spleen, kidney, prostate gland, milk, bile, bronchial fluid, pleural fluid, prostatic secretions, urethral secretions and vaginal secretions were larger than 1, especially for lung, milk, liver, spleen and kidney, where the ratios ranged from 3 to 5. The ratios for saliva, pancreatic secretion, cerebrospinal fluid, muscle and fetal tissues were less than 1 (range 0.1–0.5). The elimination half-life for erythromycin was 60 min, and the apparent volume of distribution in the body was in excess of 2 l/kg (Burrows, 1980).

(c) Cattle

Seven calves (breed not stated) were given erythromycin by a single intramuscular injection at a dose of 5 mg erythromycin activity/kg body weight. A maximum plasma concentration of 0.652 µg/ml was observed after 1.95 h, with a good bioavailability (bioavailable fraction 95%). The elimination half-life and the volume of distribution were 3.77 h and 3.24 l/kg per hour, respectively (Bligny, 1988a).

Ten calves (breed not stated) that received erythromycin by a single intramuscular injection at a dose of 5 mg/kg body weight showed average serum levels of erythromycin ranging from 0.48 to 0.74 µg/ml between 2 and 10 h after injection and 0.05 µg/ml at 24 h after injection. Erythromycin levels were higher in the lungs than in the serum. Average pulmonary levels increased from 1.71 µg/ml at 2 h to

2.58 µg/ml at 6 h post-injection and then decreased to 0.34 µg/ml at 24 h after administration (Bligny, 1988a).

Three Friesian calves that received five consecutive intramuscular injections of erythromycin at a dose of 5 mg/kg body weight at 24-h intervals showed no erythromycin accumulation. Five days after the last injection, liver, kidney, fat and muscle were free of antibiotic residues, except at the injection site (neck, 0.3 µg/g); 7 days after the last injection, all the tissues, including the injection site, were negative (Bligny, 1988a).

Cows (breed not stated) were administered intramuscularly a single dose of 8.3 mg erythromycin anhydrate/kg body weight. Kidney, muscle and liver levels ranged from 0.11 to 0.92 µg/g, with the highest levels being in the liver at 5 h post-injection. The ratio of the maximum concentration in serum to that in milk was 0.2, and the appearance time in milk was 0.2 h. The percentages of the dose of erythromycin in the serum and tissue compartments were 6% and 19%, respectively, and 75% of the dose was eliminated by 6 h after administration (Ziv, 1980a).

Five calves (breed not stated) were administered intravenously a single injection of erythromycin phosphate at a dose of 5 mg erythromycin/kg body weight. A large apparent volume of distribution (1.95 l/kg during the β-phase), a short mean residence time (2.36 h), an efficient ability of the organism to remove the drug (clearance = 0.77 l/kg per hour) and a fast elimination half-life (1.48–2.03 h in the β-phase) were observed (Bligny, 1988a).

Holstein-Friesian cows were given 12.5 mg erythromycin/kg body weight intravenously. The elimination half-life of erythromycin was about 3 h, and the tissue level of erythromycin reached a peak of 43% of the dose at 67 min post-injection (Baggot & Gingerich, 1976).

Cows (breed not stated) received 600 mg erythromycin by intramammary infusion. The first-order disappearance rate and elimination half-life of erythromycin in the milk were 0.14/h and 2.07 h, respectively (Baggot & Gingerich, 1976).

Cows were given 1200 mg erythromycin base by a single intramammary application. Erythromycin was well distributed in the body, and the mean erythromycin concentrations in renal cortex, muscle and liver ranged from 0.09 to 0.14 µg/g tissue 16 h after application (Nouws & Ziv, 1979; Ziv, 1980b).

(d) Chickens

Adult broiler chickens (strain not stated; n = 168) were given erythromycin thiocyanate at a dose of 27 mg/kg body weight per day in their drinking-water (a water consumption of 235 ml/chicken on average) for 3 consecutive days, with the treated water changed every 24 h. The average serum level of erythromycin ranged from 0.108 to 0.22 µg/ml 30 min after the beginning of a repeated administration and then declined to less than 0.04 µg/ml 8 h after the last administration. Erythromycin was detected in the lungs from 6 h after the beginning of the treatment, and the average pulmonary level (0.08–1.14 µg/g) was higher than the average serum level (0.02–0.29 µg/g) during the whole treatment period, from

6 h after the beginning of administration. In samples collected 12 h after the end of the medication, pulmonary levels were less than the detection limit (approximately 0.2 µg/g) (Bligny, 1988b).

In 40 laying hens given erythromycin thiocyanate at 25 mg/kg body weight per day in their drinking-water for 7 consecutive days, the erythromycin concentration in whole eggs ranged from 0.07 to 0.17 µg/ml during 3–7 days of medication and from 0.06 to 0.16 µg/ml during 1–4 days post-medication. The erythromycin concentration then decreased below the detection limit (0.06 µg/ml) 6 days after the end of medication (Bligny, 1988b).

(e) Humans

Erythromycin is rather slowly absorbed after oral administration in humans. Oral doses of 250 mg of erythromycin base or erythromycin stearate given to adults produced a peak serum concentration of 0.4 µg/ml within 2–4 h (Wilson & van Boxtel, 1978).

The peak serum concentrations were 2.8 and 4.8 µg/ml at 1–6.3 h following oral administration of 3 g erythromycin ethylsuccinate and 1.5 g erythromycin stearate, respectively, to healthy adults.

About 70–90% of erythromycin stearate is destroyed by gastric acid within 15 min after the shell of the tablet starts to rupture, except if administered with a protective enteric coating. Absorption occurs mainly in the duodenum. The oral bioavailability of unprotected erythromycin base and salts is less than 50% of the dose, and the plasma elimination half-life is from 1.2 to 4 h (DiSanto & Chodos, 1981; Periti et al., 1989). In vitro studies have demonstrated that erythromycin stearate is rapidly destroyed in gastric acid, retaining only 2% of its antibiotic activity. Food reduces the absorption of erythromycin (Griffith & Black, 1970; Boggiano & Gleeson, 1976; Wilson & van Boxtel, 1978; Reynolds, 1989; Fiese & Steffen, 1990; ASHP, 1991).

An estimated 0.1% of a daily dose of erythromycin appears in the breast milk of lactating women. About 10% of erythromycin is estimated to cross the placenta, and fetal blood levels are not higher than 10% (usually closer to 2%) of those present in the maternal circulation (Wilson & van Boxtel, 1978; AMA, 1986; Zhang et al., 1997). Premature infants given erythromycin estolate at a dose of 10 mg/kg body weight showed serum levels of 0.5 µg/ml or above within 1 h (Wilson & van Boxtel, 1978).

Erythromycin is highly bound to plasma proteins, and only 10% remains in unbound form (Wilson & van Boxtel, 1978). Erythromycin is bound mainly to alpha-1 acid glycoprotein in plasma and to a minor extent to albumin (Reynolds, 1989). The portion of an erythromycin dose excreted in the urine varies from 0.02% to 20%, and the elimination half-life may be prolonged in renal disease (Wilson & van Boxtel, 1978; Periti et al., 1989). Fifteen per cent of an administered dose was excreted in the bile, and 10% of the serum level was detected in saliva (Griffith & Black, 1970; Wilson & van Boxtel, 1978). Erythromycin and PELS pass through the faeces, mainly by way of bile, but in part also by direct passage

through the intestinal wall. An enterohepatic recirculation may also contribute to the high concentrations of erythromycin in faecal samples (Kroboth et al., 1982).

2.1.2 Biotransformation

Erythromycin is rapidly metabolized in the liver, mainly through *N*-demethylation, in rats, dogs, ruminants and humans and in the liver microsomal fraction of rabbits (Lee et al., 1956a, 1956b; Wilson & van Boxtel, 1978; Tsubaki & Ichikawa, 1985; Wrighton et al., 1985; Pineau et al., 1990).

Des-*N*-methyl-erythromycin is the major and the only microbiologically active metabolite of erythromycin. This metabolite accounted for approximately one third of the total bile radioactivity during the 2-h period following isotopic erythromycin administration. Seven additional metabolites of erythromycin are also formed; two of these are excreted in the bile, three in the urine and two in the faeces, and the latter are produced from metabolism of erythromycin by intestinal bacteria. Des-*N*-methyl-erythromycin is excreted into the bile and eliminated through the faeces. Erythromycin is absorbed in the small intestine as erythromycin base (Lee et al., 1956a, 1956b; Wilson & van Boxtel, 1978).

The hepatic cytochrome P-450 isozymes that catalyse erythromycin demethylation in rat are highly similar to the form of liver cytochrome P-450 present in mouse, rabbit, hamster and gerbil; this may also extend to humans, as human liver contains a protein equivalent to the rat cytochrome P-450 (Villa et al., 1984; Wrighton et al., 1985). Cytochrome P-450 3A is the most abundant cytochrome P-450 expressed in human liver and mainly catalyses erythromycin (Fujita, 2006). A high degree of similarity was also found between the ovine cytochrome P-450 involved in *N*-demethylation of erythromycin and the form isolated in rabbits (Pineau et al., 1990). In cattle, a form of cytochrome P-450 isozyme exhibiting a high catalytic activity for *N*-demethylation was found (Tsubaki & Ichikawa, 1985).

2.2 Toxicological studies

2.2.1 Acute toxicity

The acute toxicity of erythromycin, as both the base and the salts, has been determined using different routes of administration in various laboratory animals whose strains were not indicated. The studies of acute toxicity did not comply with GLP. The LD_{50} by the oral route was generally higher than 3000 mg/kg body weight, and that by the subcutaneous route was higher than 1500 mg/kg body weight. Intraperitoneal and intravenous LD_{50} values ranged from 180 to 490 mg/kg body weight (Tables 1–5). Death was preceded by clonic convulsions, prostration and respiratory depression (Anderson et al., 1952, 1955, 1959).

Table 1. Results of studies of acute toxicity of erythromycin base

Species	Route	LD_{50} (mg base/kg body weight)	Reference
Mouse	Oral	3112	Anderson et al. (1952)
Mouse	Subcutaneous	>2500	Anderson et al. (1952)
Mouse	Intramuscular	394	Anderson et al. (1952)
Mouse	Intraperitoneal	463	Anderson et al. (1952)
Mouse	Intravenous	426	Anderson et al. (1952)
Rat	Oral	>3000 or 9272	Anderson et al. (1952)
Rat	Subcutaneous	>2000	Anderson et al. (1952)
Rat	Intraperitoneal	374–425	Anderson et al. (1952)
Rat	Intravenous	209	Anderson et al. (1952)
Hamster	Oral	3018	Anderson et al. (1952)
Guinea-pig	Intraperitoneal	413	Anderson et al. (1952)

Table 2. Results of studies of acute toxicity of erythromycin hydrochloride

Species	Route	LD_{50} (mg base/kg body weight)	Reference
Mouse	Oral	2927	Anderson et al. (1952)
Mouse	Subcutaneous	1849	Anderson et al. (1952)
Mouse	Intraperitoneal	490	Anderson et al. (1952)
Mouse	Intravenous	426	Anderson et al. (1952)
Rat	Oral	>2000	Anderson et al. (1952)
Rat	Subcutaneous	1442	Anderson et al. (1952)
Rat	Intravenous	209	Anderson et al. (1952)
Rabbit	Intravenous	183	Anderson et al. (1952)
Dog	Intravenous	>140	Anderson et al. (1952)

Table 3. Results of studies of acute toxicity of erythromycin glucoheptonate

Species	Route	LD_{50} (mg/kg body weight)	Reference
Mouse	Intravenous	453	Anderson et al. (1955)
Rat	Intravenous	288	Anderson et al. (1955)
Rabbit	Intravenous	>300	Anderson et al. (1955)

Table 4. Results of studies of acute toxicity of erythromycin estolate (PELS)

Species	Route	LD_{50} (mg/kg body weight)	Reference
Mouse	Oral	>6450	Anderson et al. (1959)
Rat	Oral	>6450	Anderson et al. (1959)
Rat	Subcutaneous	>6450	Anderson et al. (1959)

Table 5. Results of studies of acute toxicity of erythromycin propionate

Species	Route	LD_{50} (mg/kg body weight)	Reference
Mouse	Oral	2850	Anderson et al. (1959)
Rat	Oral	>5000	Anderson et al. (1959)

2.2.2 Short-term toxicity

(a) Mice

In an experiment that complied with GLP, 8- to 9-week-old male and female B6C3F1 mice (five mice of each sex and dose) were fed diets containing 0, 3125, 6250, 12 500, 25 000 or 50 000 mg erythromycin stearate/kg feed (equivalent to approximately 0, 580, 1160, 2300, 2800 or 5000 mg/kg body weight per day) for 14 consecutive days. Feed consumption, body weight change, clinical signs and histopathological examination were performed. Two of the female mice that received 5000 mg/kg body weight per day died before the end of the studies. None of the dosed groups of male or female mice gained weight. Feed consumption by mice that received 2800 and 5000 mg/kg body weight per day was notably less than that by controls. Lethargy and rough coats were observed at all but the lowest dose. Hydration of the cornea was observed in mice that received 1160, 2300 and 2800 mg/kg body weight per day. Mice that received 5000 mg/kg body weight had hyperaemic jejunum and caecum, haemorrhagic spleen or haemorrhagic intestine, which was caused mainly by the death of intestinal bacteria due to erythromycin (NTP, 1988). The no-observed-effect level (NOEL) was 580 mg/kg body weight per day based on lethargy and hydration of the cornea.

In an experiment that complied with GLP, male and female B6C3F1 mice (10 mice per sex per dose group) were given diets containing 0, 1250, 2500, 5000, 10 000 or 20 000 mg erythromycin stearate/kg feed (equivalent to 0, 150, 300, 600, 1300 or 2600 mg/kg body weight per day) for 13 weeks. Examination of clinical signs, body weight change, feed consumption and histopathological change and haematological analysis were performed. None of the mice died before the end of the studies. Final mean body weights of mice that received 1300 or 2600 mg/kg body weight per day were, respectively, 15% or 19% lower than those of controls for males and 5% or 14% lower for females. Estimated feed consumption by dosed groups was comparable with that by the controls. No compound-related clinical signs, haematological effects or microscopic pathological effects were observed (NTP, 1988). The NOEL was 600 mg/kg body weight per day based on decreased body weight.

(b) Rats

In an experiment that complied with GLP, 8- to 9-week-old male and female F344/N rats (five rats per sex per dose) were fed diets containing 0, 3125, 6259, 12 500, 25 000 or 50 000 mg erythromycin stearate/kg feed (equivalent to 0, 360, 720, 1160, 1400 or 2250 mg/kg body weight per day) for 14 consecutive days. Feed consumption, body weight change, clinical signs and histopathological change were examined. None of the rats died before the end of the studies. Feed consumption of rats that received 1400 or 2240 mg/kg body weight per day was notably lower than that of controls. Final mean body weights of male rats that received 1160, 1400 or 2250 mg/kg body weight per day were 10%, 30% or 36% lower than that of control, respectively, and in females, 10%, 12% or 32% lower than that of control, respectively. Lethargy and rough coats were observed at 2250 mg/kg body weight per day. Two male rats that received 1400 mg/kg body weight per day had hyperaemic intestine (NTP, 1988). The NOEL was 720 mg/kg body weight per day based on decreased body weight.

In a non-GLP experiment, 90 male Wistar rats were given daily oral doses of erythromycin base, PELS or erythromycin lactobionate equivalent to 800 mg erythromycin base/kg body weight for 6 weeks. Compared with controls, a significantly smaller body weight increase was observed in PELS-treated animals. Mortality was significantly higher for erythromycin base (50%) and for erythromycin lactobionate and PELS (both 34%) than for the control group (17%). The cause of death could not be traced by histological and histochemical examination. Surviving animals showed no significant changes in pathological examination of kidneys and adrenals and in the levels of alanine aminotransferase (serum glutamic pyruvic transaminase). However, alkaline phosphatase was increased by erythromycin lactobionate, and incipient intrahepatic cholestasis was observed in the bile ducts of animals treated with erythromycin lactobionate and PELS. Marked atrophy of hair follicles was found in PELS-treated animals (Kaltiala et al., 1967).

In another non-GLP experiment, 12 rats (strain not stated) per dose group were given erythromycin estolate orally at 0, 50, 100, 250 or 500 mg/kg body weight for 3 months. Animals fed erythromycin estolate at 100–500 mg/kg body

weight per day produced retardation of growth related to a decrease in food intake (presumably due to the lack of palatability of the diet), but no visceral changes attributable to erythromycin were observed (Anderson et al., 1959).

In a non-GLP study, five female rats (strain not stated) per group were fed diets containing 0, 0.05, 0.1 or 0.20% of erythromycin base (equivalent to approximately 0, 90, 180 and 360 mg/kg body weight per day) for 13 weeks. Erythromycin did not alter the body weight gain. Weekly haematological studies revealed no abnormalities. One rat fed 90 mg/kg body weight per day died after 64 days, and one rat in the control group died after 30 days. All others that survived the 3-month treatment revealed no abnormalities in gross and histopathological studies at autopsy (Anderson et al., 1952). The NOEL was 360 mg/kg body weight per day, the highest dose administered.

In an experiment that complied with GLP, male and female F344/N rats (10 rats per sex per dose group) were given diets containing erythromycin stearate at 0, 1250, 2500, 5000, 10 000 or 20 000 mg/kg feed (equivalent to 0, 60, 120, 240, 480 or 1000 mg/kg body weight per day) for 13 weeks. Body weight, feed consumption, clinical signs and pathological change were examined. None of the rats died before the end of the studies. Final mean body weights of the 1000 mg/kg body weight groups were 12% lower than that of control in males and 7% lower in females. Feed consumption by dosed and control groups was comparable except for males at 1000 mg/kg body weight per day. All dosed rats except the 60 mg/kg body weight per day group exhibited lethargy. Males that received 480 or 1000 mg/kg body weight per day had rough coats. Multinucleated syncytial hepatocytes were observed in all males that received 1000 mg/kg body weight per day (NTP, 1988). The NOEL was 60 mg/kg body weight per day based on the incidence of lethargy in rats.

In a non-GLP experiment, Sprague-Dawley rats (50 rats per sex per dose group) received feed containing 2, 20 or 200 g of erythromycin thiocyanate per 907 kg of feed for 68 weeks (equivalent to 0.12, 1.2 or 12 mg/kg body weight per day). The control group consisted of 100 males and females that received untreated feed. No abnormalities attributable to the administration of erythromycin thiocyanate were observed in weight gain, feed intake, mortality, tumour incidence, haematology, urinalysis and histopathology at any dose level, except a minimal or small follicular hyperplasia in the thyroid from 3 of 10 rats treated with erythromycin thiocyanate at 12 mg/kg body weight per day. However, it was not certain that the follicular hyperplasia of the thyroid was causally related to feeding erythromycin because of the limited number of animals investigated in a non-GLP system (Frost et al., 1965). The NOEL was 12 mg/kg body weight per day, the highest dose tested.

(c) Cats

Six cats (strain not stated) received daily 50 mg of erythromycin base by the intramuscular route for 69 days to investigate the effects of erythromycin on the eighth cranial nerve. Periodically, all animals were tossed to a height of 1 m. All those receiving erythromycin were able to right themselves at all times before

landing. There was no nystagmus. Three cats sacrificed after 69 doses of erythromycin had fibrosis of the muscles at the sites of injection. Otherwise, there was no apparent visceral damage (Anderson et al., 1952).

(d) Dogs

In a non-GLP study, beagle dogs (two dogs per dose group) weighing between 8.3 and 13.7 kg were given erythromycin estolate orally in gelatin capsules at a dose of 0, 50, 100 or 220 mg/kg body weight per day for 10 weeks. The body weight of all dogs remained relatively constant throughout the experiment. No apparent side-effects were observed in any dogs. After 10 weeks of treatment, animals showed no abnormality in gross or histopathological examination for the main organs (Anderson et al., 1959). The NOEL was 220 mg/kg body weight per day, the highest dose tested.

In another non-GLP experiment, three female mongrel dogs received intravenously 170 mg erythromycin glucoheptonate/kg body weight twice per day for 3 months. During the early days of the study, the dogs had mild tremors and emesis soon after each injection. However, these reactions disappeared, and no other side-effects were noted. All dogs survived. Gross and microscopic examination revealed no histological abnormalities. Terminal bone marrow counts were also unaltered (Anderson et al., 1955).

In a non-GLP-compliant study, 20 female mongrel dogs (5 dogs per dose group) were given erythromycin base at a dose of 0, 50, 75 or 100 mg erythromycin/kg body weight via oral capsule daily for 13 weeks. All treated dogs survived throughout the study period. After 13 weeks, 11 dogs (3 dogs from each treated group and 2 controls) were sacrificed and necropsied. No pathological changes in the viscera had any relationship with the treatment. In addition, the counts of myeloid, erythroid and lymphoid cells in bone marrow were comparable to those of the control dogs, as were the blood and the urine analysis (Anderson et al., 1952). The NOEL was 100 mg/kg body weight per day, the highest dose tested.

A chronic toxicity study in dogs was conducted by Anderson et al. (1955) in a non-GLP system. Six female mongrel dogs (two dogs per dose) were treated orally for 1 year with erythromycin: for 3 months at 50, 75 or 100 mg/kg body weight per day and then for an additional 9 months at 100 mg/kg body weight per day. One dog was allocated as a control. Body weight gain, haematological and clinical chemistry and urinalysis were examined. One year after the start of the treatment, six dogs and the one untreated control were sacrificed and submitted for necropsy. Gross and microscopic studies revealed no abnormalities in heart, lungs, liver, spleen, kidneys, stomach, intestines, thymus, pancreas, thyroid or adrenal glands. In addition, bone marrow studies were made at the same time and revealed that myeloid, erythroid and lymphoid cell counts were comparable with those found in untreated dogs.

(e) Monkeys

Three rhesus monkeys received by stomach tube approximately 75 mg erythromycin base/kg body weight per day for 64 days. Complete blood studies, urinalysis and blood marrow studies on these animals gave no indication that erythromycin produced any toxic effect. Differential counts of the bone marrow were not significantly different from normal. This study was reported in a summary with data (Anonymous, undated a).

2.2.3 Long-term toxicity and carcinogenicity

(a) Mice

In an experiment that complied with GLP, groups of 50 male and female B6C3F1 mice received diets containing 0, 2500 or 5000 mg/kg feed (equivalent to about 0, 270 and 545 mg/kg body weight for males and 0, 250 and 500 mg/kg body weight for females) for 2 years. Clinical signs and histopathological changes were examined. Mean body weights and average daily feed consumption of dosed and control mice were generally comparable throughout the studies. No compound-related deaths or clinical signs were observed. Inflammation of the glandular stomach was observed at increased incidences in dosed male mice (control, 1/49; low dose, 4/50; high dose, 6/50). Lymphoid hyperplasia of the urinary bladder was observed at increased incidences in dosed female mice (control, 1/50; low dose, 9/47; high dose, 7/48), but it did not show a dose-dependent increase (NTP, 1988). The NOEL could not be identified.

(b) Rats

In a GLP-compliant experiment, diets containing 0, 5000 or 10 000 mg erythromycin stearate/kg feed (equivalent to 0, 180 or 370 mg/kg body weight per day for males and 0, 210 or 435 mg/kg body weight per day for females) were fed to 300 F344/N rats (50 per sex per dose group) for 2 years (NTP, 1988). Clinical signs and histopathological changes were examined.

Mean body weights of the high-dose male rats were 6% lower than those of controls throughout the studies. Mean body weights of high-dose female rats were 5–10% lower than those of controls from week 35 to the end of the studies. Mean body weights of low-dose male and female rats were comparable to those of controls throughout the studies. The average daily feed consumption by low- and high-dose rats was comparable to that by controls. No compound-related deaths or clinical signs were observed.

A slight incidence of oral cavity squamous cell papillomas was observed in the dosed groups of female rats (overall incidence rate of 1/50, 2/50 and 3/50 at 0, 210 and 435 mg/kg body weight per day, respectively). Although oral cavity tumours are uncommon in female rats, they are not considered to be related to erythromycin stearate, because the incidence of the dosed groups is not statistically significant.

Pheochromocytomas of adrenal glands in female rats occurred with a positive trend (overall incidence rate of 1/50, 4/49 and 6/50 at 0, 210 and 435 mg/kg body weight per day, respectively), but the increases are marginal in normal animals. The incidence of interstitial cell tumours of the testis in high-dose male rats was greater (43/50) than that in the controls (32/50). However, it is not considered to be a biologically important observation, as this is a commonly occurring tumour and the incidences are in the historical control range (Davis, 1995; Nold, 2001).

Granulomas in the liver were observed at an increased incidence ($P < 0.01$) in dosed rats (male: 1/50, 1/50, 10/50; female: 18/50, 27/50, 43/50; for control, low dose and high dose, respectively). The granulomas seen in dosed rats were generally larger than those observed in controls, and they consisted of focal aggregates of macrophages surrounded by various numbers of lymphocytes. Granulomatous inflammation or granulomas in the spleen were observed in high-dose female rats (control, 0/48; low dose, 1/49; high dose, 3/50). Reticulum cell hyperplasia in bone marrow was observed at increased incidences in dosed female rats (control, 10/50; low dose, 14/50; high dose, 25/50).

A possible mechanism for the granulomas may be the absorption of bacteria or bacterial products from the intestine through the disruption of the colonization barrier by erythromycin. Also, granulomas could be exacerbated by the potential immunomodulatory effects (increased leukocyte migration) of erythromycin (Anderson et al., 1984; Fraschini et al., 1986; Ras & Anderson, 1986). Estrogens are known to cause some immunosuppression (Luster et al., 1984; Dieter et al., 1987), which may explain the sex difference in the observed effect.

(c) Conclusion

In conclusion, erythromycin was not carcinogenic in mice or rats. However, non-neoplastic effects, granulomas of liver or spleen and reticulum cell hyperplasia of bone marrow were observed in rats. A NOEL was not established in this study, and the lowest-observed-effect level (LOEL) for non-tumorigenic effects was 210 mg/kg body weight per day in female rats, based on granulomas of the liver and reticulum cell hyperplasia of the bone marrow.

2.2.4 Genotoxicity

In assays performed in compliance with GLP, the genotoxic effects of erythromycin stearate were investigated with a range of genotoxic end-points in vitro (Table 6). Erythromycin was not mutagenic in the *Salmonella typhimurium* assay with strains TA98, TA100, TA1535 or TA1537 at 0.3–100 µg/plate, in a sister chromatid exchange test at 5–500 µg/ml or in the chromosomal aberration test (Chinese hamster ovary cells) at 16–500 µg/ml, both with and without S9 activation.

Erythromycin stearate showed equivocal mutagenicity in the mouse L5178Y lymphoma cell assay in the absence of metabolic activation, with a 1.6-fold increase above control in expression of mutant fraction only at or just below concentrations that caused precipitation (at 80, 100, 125, 140 or 150 µg/ml). It was

concluded that erythromycin stearate has no mutagenic properties, as the increase was noted near the level that caused precipitation, the increase of mutant fraction was not dose related and the effect was not observed in the presence of metabolic activation (NTP, 1988). In conclusion, erythromycin stearate is unlikely to pose a genotoxic hazard.

Table 6. Results of tests for genotoxicity with erythromycin stearate

End-point	Test object[a]	Concentration/dose	Result
Reverse mutation[b]	*S. typhimurium* strains TA100, TA1535, TA1537, TA98, ±S9	0.3–100 µg/plate	Negative
Forward mutation[c]	Mouse L5178Y lymphoma cells, ±S9	6.25–1000 µg/ml	Negative
Sister chromatid exchange in vitro[d]	Chinese hamster ovary cells, ±S9	5–500 µg/ml	Negative
Chromosomal damage in vitro[d]	Chinese hamster ovary cells, ±S9	16–500 µg/ml	Negative

[a] S9, 9000 × *g* supernatant of male Syrian hamster liver or Sprague-Dawley rat liver used for metabolic activation.

[b] Positive controls were 4-nitro-*o*-phenylenediamine for TA98, sodium azide for TA100 and TA1535, and 9-aminoacridine for TA1537 in the absence of S9; 2-aminoanthracene for all strains in the presence of S9.

[c] Positive controls were ethyl methanesulfonate in the absence of S9 and methylcholanthrene in the presence of S9.

[d] Positive controls were mitomycin C in the absence of S9 and cyclophosphamide in the presence of S9.

2.2.5 Reproductive toxicity

(a) Multigeneration studies

(i) Rats

In a non-GLP-compliant reproduction study, two groups of 21 rats (strain not stated) received 0 or 21 mg erythromycin thiocyanate/kg feed (equivalent to 0 and 1.05 mg/kg body weight per day) for 100 days before mating. Females of the parent generation (F0) were then mated three times, and the female offspring of these three matings were followed through three subsequent generations (F1, F2 and F3). No statistically detectable differences between the control and erythromycin groups could be found in the mean values for fertility and fetotoxicity. No malformation was observed in the rats (Anonymous, undated b).

Non-GLP reproductive toxicity studies in male and female rats (strain not stated) using oral erythromycin base at levels up to 2.5 g/kg feed (equivalent to

125 mg/kg body weight per day) prior to and during mating, during gestation and through weaning of two successive litters did not reveal evidence of teratogenicity or impaired fertility (ASHP, 1991). The NOEL was the highest dose, 125 mg/kg body weight per day.

(b) Fertility studies

(i) Rats

Rats (strain, administration route and duration not stated) were given erythromycin base at a therapeutic dose of 3.3 mg/kg body weight per day. The frequency of mitotic division in testes was decreased during medication but recovered 18 days after the end of treatment (Schlegel et al., 1991).

Female Sprague-Dawley rats (24 per group) were given a single dose of erythromycin lactobionate at 0, 70 or 280 mg/kg body weight via transcervical administration. Twenty rats per group were mated, and four animals per group were sacrificed without mating 21 days after the administration. Fourteen days after mating, numbers of ovarian corpora lutea, total uterine implants and embryos were evaluated; for unmated animals, uterine sections were examined for fibrosis and lumen closure. Erythromycin lactobionate did not alter numbers of corpora lutea but decreased pregnancy rate and number of implantations (increased pre-implantation loss) in a dose-related fashion. In erythromycin-treated animals, fibrosis and lumen closure were observed with increased extent and severity from 21 to 35 days after the administration (Fail et al., 2000).

(ii) In vitro

Short-term exposure of sperm from humans as well as ram, bull, rabbit and horse spermatozoa to erythromycin at high doses induced impaired motility or spermicidal effects. Erythromycin at 1000 IU depressed the motility of frozen thawed bovine spermatozoa (Berndtson & Foote, 1976).

In an in vitro study, the effects of erythromycin on human sperm movement characteristics, viability and the ability of spermatozoa to undergo the acrosome reaction were investigated. Erythromycin significantly increased sperm movement rapidity, mean path velocity, straight-line velocity and curvilinear velocity only at concentrations higher than 100 µg/ml when spermatozoa were cultured for 24 h. The mean lateral head displacement and viability were significantly reduced at 1 mg/ml, but the ability of spermatozoa to undergo the acrosome reaction was not affected (Hargreaves et al., 1998).

(c) Developmental studies

(i) Mice

ddY mice (number not stated) treated orally with erythromycin at 2 g/kg body weight per day during gestation days 8–13 showed a significant decrease in maternal body weight at gestation day 19 and in fetus body weight at delivery

time, but no gross, organ or skeletal malformations were observed (Moriguchi et al., 1972). The NOEL was 2 g/kg body weight per day, the only dose tested.

2.2.6 Special studies

(a) Immune responses

Male ddY mice given 250 mg erythromycin/kg body weight per day by the intraperitoneal, intravenous, subcutaneous or oral route for 7 days produced greater amounts of thymocyte-activating factors and interleukin 1 and 6. This suggests that erythromycin may have immunostimulating activity (Hirakata et al., 1992).

(b) Microbiological effects

Several studies were available on the effects of orally administered erythromycin on the intestinal microflora of humans and experimental animals.

Oral daily doses of 3 g of erythromycin given to healthy human volunteers for 3 weeks caused a decrease in enterobacteria counts ($<10^2$ enterobacteria per gram of faeces) after 2 days of treatment. The population of enterobacteria in faecal flora returned to pretreatment levels after cessation of treatment (Tancrede et al., 1980).

Treatment of 18 human volunteers with orally administered doses of erythromycin at 1, 2 or 3 g/day for 5 days caused a 1000-fold reduction in enterobacteria per gram of faeces sampled in 17 of the 18 volunteers. All doses were equally effective. The number of enterobacteriaceae returned to pretreatment levels 4 days after cessation of dosing (Andremont & Tancrede, 1981).

In gnotobiotic mice inoculated with human faecal flora, the effect of erythromycin on intestinal resistance to colonization was studied after the mice were challenged with six microbial strains that are potentially pathogenic for immunocompromised patients. Faecal samples from 11 untreated human donors were inoculated either in untreated germ-free mice or in germ-free mice given erythromycin (10 g/kg diet). Total intestinal microflora bacterial counts were not significantly different in the human donor and in the recipient mice and were not affected by erythromycin treatment. When the effect of erythromycin on microbial antagonism against challenge strains in the recipient mice was studied, erythromycin induced suppression of sensitive strains, including all enterobacteria. However, erythromycin did not disturb the predominant microflora to a great extent. Erythromycin treatment did not reduce colonization resistance against *Candida albicans*, *Clostridium perfringens* or erythromycin-sensitive *Escherichia coli*. However, it reduced some colonization resistance against *Pseudomonas aeruginosa*, *Clostridium difficile* and erythromycin-resistant *E. coli* (Andremont et al., 1983, 1986).

Minimum inhibitory concentrations (MICs) of erythromycin have been reported for a range of bacterial species. Some of the bacteria tested are usually considered the most relevant microorganisms of the human gut.

Several studies evaluated the activity of erythromycin against anaerobic clinical isolates (Sutter & Finegold, 1976; Finegold, 1977; Barlam & Neu, 1984). The susceptibility of 492 strains of anaerobic bacteria isolates to erythromycin is shown in Table 7. Erythromycin inhibited most of the *Eubacterium*, *Bifidobacterium* and *Lactobacillus* at 0.5 µg/ml or less. In *Clostridium* species, 64% and 84% of the strains were sensitive to 0.5 and 1.0 µg/ml, respectively. Erythromycin was less active against *Bacteroides fragilis* and other *Bacteroides* species.

One-hundred and twenty-two strains of *Bifidobacterium* and *Lactobacillus* species isolated from the faeces of eight healthy individuals were screened for erythromycin susceptibility (Delgado et al., 2005). Most of the tested strains showed low MIC values (<1 µg/ml); however, a few highly resistant strains were found in *Lactobacillus* (MIC >1024) and *Bifidobacterium* (MIC >128 µg/ml) isolates (Table 8).

Antimicrobial susceptibility of 37 strains of *Bifidobacterium*, including *B. bifidum*, *B. longum* and *B. infantis*, to erythromycin indicated that most of the bifidobacteria showed a MIC value of <0.19 µg/ml (Lim et al., 1993).

Eighteen strains representing 10 species of *Bifidobacterium* were tested for their susceptibility to erythromycin in a disc diffusion assay. At suspensions of 10^8 cells/ml, 15 µg of erythromycin inhibited the growth of all the test organisms (Yazid et al., 2000). Lower concentrations of erythromycin were not tested.

2.3 Observations in humans

2.3.1 Immune responses

Hypersensitivity reactions to erythromycin are rare in humans, and mild clinical symptoms, such as rash, pruritus, urticaria and angio-oedema, are observed in less than 0.5% of treated patients (Midtvedt, 1981; Keller & Follath, 1988; Dewdney et al., 1991).

Skin allergic reactions to erythromycin were reported in humans, but the incidence was low (Raab, 1977; Pradalier et al., 1980). The presence of immunoglobulin E (IgE) and non-IgE antibodies against erythromycin in the serum of a patient who ingested two tablets (approximately 15 mg/kg body weight) of erythromycin stearate suggests that an allergic reaction of types 1 and 3 participated in the course of an acute respiratory response (Abramov et al., 1978). Administration of erythromycin stearate in 500-mg tablets daily for 3 days improved cell migration in humans with persistent abnormal polymorphonuclear leukocyte migration (Ras & Anderson, 1986).

An erythromycin-induced haemolytic anaemia was observed in a patient, and the presence of an IgM anti-erythromycin antibody was demonstrated in the patient's serum (Wong et al., 1981). Erythromycin may, in exceptional cases, be responsible for hepatic injuries caused by a specific allergic response to macrolide metabolite-modified hepatic cells (Dewdney et al., 1991).

Table 7. Activity of erythromycin against anaerobic bacteria

Bacteria	No. of strains tested	Cumulative percentage susceptible to indicated concentration (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	34	3	6	15	24	47	71	88	94	100		
Bacteroides melaninogenicus	9	44	89	100								
Other *Bacteroides* and *Selenomonas* species	51	10	45	78	86	96		98				100
Fusobacterium nucleatum	10					30	70	90		100		
Other *Fusobacterium* species	4				25	50	75		100			
Peptococcus and *Gaffkya* species	42		5	36	71	83						100
Peptostreptococcus species	13	77	85	92	100							
Anaerobic and microaerophilic streptococci	4		100									
Gram-negative cocci	19		5	32	58	79	90	100				
Eubacterium species	9	44	100									
Arachnia propionica	1			100								
Propionibacterium species	8	88		100								
Bifidobacterium species	5	60	100									
Lactobacillus species	8	63	88									100

Table 7 (Contd)

Bacteria	No. of strains tested	Cumulative percentage susceptible to indicated concentration (μg/ml)									
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Clostridium perfringens	1			100								
Other *Clostridium* species	7	14	71	100								

[a] Includes all strains identified as subspecies *of B. fragilis.*

Source: Sutter & Finegold (1976).

Table 8. Distribution of MIC of erythromycin for Bifidobacterium *and* Lactobacillus *species in humans*

Strains	MIC (μg/ml)											
	No. of strains	≤1	2	4	8	16	32	64	128	256	512	≥1024
B. longum	47	31	2	1	4	4	4	1				
B. bifidum	16	16										
B. pseudocatenulatum	11	8			2				1			
B. catenulatum	2	1			1							
L. gasseri	20	19	1									
L. delbrueckii	8	8										
L. casei/paracasei	7	5		2								
L. rhamnosus	5	3										2
L. acidophilus	2											2
L. planatrum	1	1										
L. parabuchneri	1	1										
L. brevis	1				1							
L. vaginalis	1				1							

Source: Delgado et al. (2005).

2.3.2 Gastrointestinal effects

In humans, gastrointestinal effects are the most common adverse effects of oral erythromycin; abdominal pain occurs frequently, and nausea, vomiting and diarrhoea also occur, especially after high doses (Oliver et al., 1972; Hughes & Cunliffe, 1987; ASHP, 1991). High doses of greater than 2 g/day can induce gastrointestinal problems in 5–30% of patients. Usually, the problems disappear 24–48 h after cessation of treatment (Heusghem & Lechat, 1973; AMA, 1986; Bessard, 1987; Keller & Follath, 1988; Reynolds, 1989).

2.3.3 Hepatotoxicity

Hepatotoxicity occurs mainly in adults and is most likely to appear in patients who receive erythromycin at 1 g/day for longer than 10 days or in repeated courses of therapy (ASHP, 1991). Although half the cases may be asymptomatic, the frequency of this complication has been reported to be 12% in patients who received 1 g erythromycin estolate/day for longer than 10–16 days (Braun, 1969; Shulman & Sellers, 1971).

In children, elevated transaminases were noted when daily doses of 1.2 g, but not 0.6 g, of erythromycin were given (Keller & Follath, 1988). In pregnant women, an increase in aspartate aminotransferase (serum glutamic oxaloacetic transaminase) level was observed in 14 of 97 women who took erythromycin estolate for more than 3 weeks during pregnancy. All levels of aspartate aminotransferase returned to normal after cessation of treatment (McCormack et al., 1977).

Reversible cholestatic jaundice may occur with all forms of erythromycin (dose not stated), including the base, propionate, stearate, ethylsuccinate and estolate, but the occurrence of the cholestatic reaction seems to be more frequent with erythromycin estolate (Keller & Follath, 1988).

2.3.4 Ototoxicity

High parenteral doses of erythromycin have resulted in transient perceptive deafness (Keller & Follath, 1988). Ototoxic reactions have been observed in patients receiving erythromycin stearate, erythromycin propionate and erythromycin ethylsuccinate. They are reversible within a few days after cessation of treatment and have occurred more frequently in older patients receiving large doses or with renal failure (AMA, 1986; ASHP, 1991).

2.3.5 Reproductive toxicity

A study of the association of maternal erythromycin use with a risk for cardiac defects in the offspring was performed, with cases (cardiovascular defects without known chromosome anomalies, n = 5015) and controls (n = 57 730) all being infants. Associations between maternal erythromycin use and infant cardiovascular defects were identified, but some of these associations are probably due to

confounding from underlying disease or inadequate statistical power (Kallen & Otterblad Olausson, 2003).

A reproductive cohort study including 210 799 mother/infant pairs was performed to assess the association between prenatal erythromycin and infantile hypertrophic pyloric stenosis of infants. There was no association with prenatal erythromycin prescription and infantile hypertrophic pyloric stenosis either after 32 weeks' gestation or at any time during pregnancy (Cooper et al., 2003).

Another epidemiological study was performed to evaluate the human teratogenic potential of oral erythromycin treatment during pregnancy. Of 22 865 pregnant women who had newborns or fetuses with congenital abnormalities, 113 (0.5%) had been treated with erythromycin. This case–control pair analysis did not indicate a teratogenic potential of erythromycin during the 2nd through 3rd months of gestation (Czeizel et al., 1999).

Women who had taken erythromycin in early pregnancy and their infants were studied for the risk of cardiac malformation in infants. The risks for cardiovascular malformation and pyloric stenosis after erythromycin therapy were increased after exposure to erythromycin in early pregnancy. These malformations may be linked to the fact that erythromycin inhibits a specific cardiac potassium channel (Kallen et al., 2005).

2.3.6 Other adverse reactions

Erythromycin has been associated with complications such as confusion, nightmares and visual hallucinations (Reynolds, 1989).

3. COMMENTS

The present Committee considered data on the pharmacokinetics, metabolism, acute toxicity, short-term and long-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity, immunotoxicity, epidemiological findings and microbiological effects of erythromycin. Many of the studies were conducted prior to the development of GLP. Data and publications from the open literature on pharmacokinetic and metabolic studies in experimental animals and humans were submitted for evaluation by the Committee.

3.1 Biochemical data

Erythromycin administered orally to rats at a dose of 25 mg/kg body weight was absorbed mainly in the small intestine. The peak serum concentration was reached after 1–2 h. Twenty hours after an intravenous administration of 10 mg of erythromycin (*N*-methyl-^{14}C-erythromycin; 0.3 MBq), approximately 37–43% of the radiolabelled dose was recovered in the intestinal tract and faeces, 27–36% in the urine and 21–29% in the expired air. Erythromycin was rapidly metabolized in the liver, mainly through a demethylation process, and excreted into the bile mainly as des-*N*-methyl-erythromycin; the major metabolite was present only in the bile and

in the intestinal contents. The isotopic methyl group was eliminated in the expired air as carbon dioxide.

In dogs (route of administration and dose not stated), peak erythromycin concentrations in most tissues exceeded the serum concentration, except for saliva, pancreatic secretion, cerebrospinal fluid, muscle and fetal tissues. After intravenous administration of 10 mg/kg body weight, approximately 5% of the dose was excreted into the bile after 8 h. The elimination half-life was 60 min, and the apparent volume of distribution in the body was higher than 2 l/kg.

In cattle, the elimination half-life ranged from approximately 1 to 4 h following intravenous or intramuscular administration. Following a single intramuscular dose of 5 mg erythromycin/kg body weight, a plasma concentration of 0.652 µg/ml was reached after 2 h. In cows after intramammary infusion, the elimination half-life in the milk was 2 h.

Chickens dosed with 27 mg erythromycin/kg body weight in drinking-water for 3 days showed average serum levels of 0.1–0.2 µg/ml 30 min after the repeated administration. Erythromycin appeared in the lungs within 6 h of the last administration, and the concentration was higher in lungs than in serum. Twelve hours after its administration, pulmonary levels were less than the detection limit. In laying hens given 25 mg erythromycin thiocyanate/kg body weight per day in drinking-water for 7 consecutive days, the erythromycin concentration in whole eggs ranged from 0.07 to 0.17 µg/ml during 3–7 days of medication and from 0.06 to 0.16 µg/ml during 1–4 days post-medication; levels then decreased below the detection limit 6 days after the end of medication.

In humans, erythromycin is slowly absorbed after oral administration. Peak serum concentrations occurred 1–6.3 h after dosing and ranged from 0.1 to 4.8 µg/ml according to the formulation of erythromycin administered. Erythromycin is sensitive to degradation by gastric acid, and the oral absorption is less than 50%. An estimated 0.1% of a daily dose appears in breast milk. Fetal blood levels are less than 10% of those present in the maternal circulation. Erythromycin is absorbed in the small intestine as erythromycin base.

3.2 Toxicological data

The acute oral toxicity of erythromycin was very low. The LD_{50} in mice and rats was greater than 2000 mg/kg body weight. Signs of toxicity included clonic convulsion, prostration and respiratory depression.

Groups of mice given dietary erythromycin stearate at a dose of 0, 580, 1160, 2300, 2800 or 5000 mg/kg body weight per day for 14 days showed decreased feed consumption and lethargy, rough coat and hydration of cornea beginning at a dose of 1160 mg/kg body weight per day. The NOEL was 580 mg/kg body weight per day.

Rats were given erythromycin stearate in the diet at doses of 0, 360, 720, 1160, 1400 or 2250 mg/kg body weight per day for 14 days. Rats showed decreased body weight gain and feed consumption, lethargy, rough coat and

hyperaemic intestine at 1160, 1400 or 2250 mg/kg body weight per day. The NOEL was 720 mg/kg body weight per day.

Rats were given erythromycin stearate orally in the diet at doses of 0, 60, 120, 240, 480 or 1000 mg/kg body weight per day for 13 weeks. All rats receiving more than 60 mg/kg body weight per day were lethargic. At the highest dose, rats exhibited multinucleated syncytial hepatocytes. The NOEL was 60 mg/kg body weight per day.

There were increased mortalities in rats given oral erythromycin base or salts at a dose of 800 mg/kg body weight per day for 6 weeks.

Rats receiving an oral dose of 0, 0.12, 1.2 or 12 mg/kg body weight per day for 68 weeks showed no compound-related adverse effects. The NOEL for the study was 12 mg/kg body weight per day, the highest dose tested.

In four studies in dogs, erythromycin was administered either orally as a capsule or intravenously for up to 12 months at doses up to at least 100 mg/kg body weight per day. No adverse effects were reported in any of these studies.

Monkeys given erythromycin orally at a dose of 75 mg erythromycin base/kg body weight per day for 64 days exhibited no compound-related adverse effects.

Long-term (2-year) studies were carried out in mice and rats with dietary erythromycin stearate.

Mice receiving doses of 250 or 500 mg/kg body weight per day (female) or 270 or 545 mg/kg body weight per day (male) for 2 years showed no treatment-related carcinogenic effects. Inflammation of glandular stomach in male mice and lymphoid hyperplasia of urinary bladder in female mice were found with increased frequency at both doses.

Rats receiving doses of 180 or 370 mg/kg body weight per day (male) or 210 or 435 mg/kg body weight per day (female) for 2 years showed no treatment-related carcinogenic effects. Non-neoplastic effects observed included granulomas of the liver at 210 and 435 mg/kg body weight per day in females and at 370 mg/kg body weight per day in males and reticulum cell hyperplasia of the bone marrow at 210 and 435 mg/kg body weight per day in females. The LOEL was 210 mg/kg body weight per day based on the granulomas of the liver and reticulum cell hyperplasia of the bone marrow in female rats.

Assays covering an adequate range of genotoxic end-points were conducted with erythromycin stearate. Erythromycin stearate was not mutagenic in the *S. typhimurium* assay for strains TA98, TA100, TA1535 or TA1537, in the sister chromatid exchange test or in the chromosomal aberrations test (Chinese hamster ovary cells) in either the presence or absence of metabolic activation. In the mouse L5178Y lymphoma assay for mutagenicity, it was noted that the response was marginal, that it occurred at levels close to those resulting in precipitation of the compound and that the increase in mutant fraction was not dose related. The Committee concluded that, on the weight of evidence, erythromycin is unlikely to pose a genotoxic hazard.

Two reproductive toxicity studies were performed in rats. In one study, animals were given about 1 mg erythromycin thiocyanate/kg body weight per day in the diet for 100 days prior to mating. Further treatment was not given, and reproductive indices were examined over three generations. In a second study, animals received 125 mg erythromycin base/kg body weight per day over two generations. While no adverse effects were reported, both studies are of limited use due to the study design and limited availability of the original data.

Rats receiving oral doses of 3.3 mg erythromycin/kg body weight per day showed a decreased frequency of mitotic division in testes. In vitro, high concentrations of erythromycin impaired the motility and viability of sperm from humans (as well as ram, bull, rabbit and horse). The Committee concluded that, on the weight of the available evidence, there was no evidence of developmental or reproductive effects in rats.

Mice treated with 2000 mg erythromycin/kg body weight per day orally by gavage during gestation days 8–13 showed a significant decrease in maternal body weight at gestation day 19 and in fetal body weight, but no fetal malformations.

Epidemiological studies in the published literature identify a potential prenatal and postnatal effect following therapeutic administration of erythromycin to women. There was one report, with inadequate statistical power to reach adequate conclusions, of cardiovascular malformations in the fetuses from women receiving therapeutic treatment with erythromycin. Other studies have found no effect on fetal malformation in fetuses from treated women. The Committee determined that there was insufficient evidence to draw a conclusion at this time concerning effects on the fetus. Infantile hypertrophic pyloric stenosis has been identified as a possible effect in developing children following early postnatal exposure to erythromycin in breast milk. The Committee assumed a human therapeutic dose of 500 mg per person and 60 kg body weight, or 8 mg/kg body weight per day.

In humans, high doses above 2 g/day induced signs of gastrointestinal disturbance (nausea, vomiting, diarrhoea) in 5–30% of the patients. Hepatotoxicity was reported in patients after 10–16 days of treatment at a dose of 1 g erythromycin estolate/day and in children treated with 1.2 g/day. Epidemiological data in humans indicate that hypersensitivity reactions to erythromycin are rare and generally mild.

3.3 Microbiological data

Several studies evaluated the activity of erythromycin against anaerobic clinical isolates and bacterial species representative of those found in the human gastrointestinal tract. Erythromycin inhibited most of the *Eubacterium*, *Bifidobacterium* and *Lactobacillus* at 0.5 µg/ml or less. In *Clostridium* species, 64% and 84% of the strains were sensitive to 0.5 and 1.0 µg/ml, respectively. Erythromycin was less active against *Bacteroides fragilis* and other *Bacteroides* species. Antimicrobial susceptibility for 37 strains of *Bifidobacterium*, including *B. bifidum*, *B.*

longum and *B. infantis*, to erythromycin indicated that most of the bifidobacteria showed a MIC value below 0.19 µg/ml.

The lowest relevant concentration that completely inhibited the growth of 50% of a microbial culture (MIC_{50}) for erythromycin was 0.1 µg/ml for *Bifidobacterium* species.

A decision-tree for evaluating the potential effect of veterinary drug residues on human intestinal microflora was developed by the Committee at its fifty-second meeting (Annex 1, reference *140*). The present Committee used the decision-tree to answer the following questions in its assessment of erythromycin:

1. *Does the ingested residue have antimicrobial properties?*

Yes, erythromycin is a macrolide antibiotic commonly used in poultry, livestock and human clinical practice to treat infections due to Gram-positive bacteria, such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and enterococci. In veterinary medicine, it is used for the treatment of mastitis in cows, chronic respiratory diseases due to mycoplasmas in poultry and infectious diseases due to erythromycin-sensitive bacteria. Anaerobes are relatively sensitive to erythromycin. Erythromycin is not active against Gram-negative bacilli.

2. *Does the drug residue enter the lower bowel?*

Yes. Erythromycin is poorly absorbed from the gastrointestinal tract in humans. Following oral administration of erythromycin, 50% of the dose was absorbed in humans. In addition, 20 h after oral administration of 10 mg of radiolabelled erythromycin to rats, approximately 37–43% of the administered radioactivity was recovered in the intestinal tract and faeces. Therefore, oral doses of erythromycin are available to the gastrointestinal tract microflora.

3. *Is the ingested residue transformed irreversibly to inactive metabolites by chemical transformation, by metabolism mediated by the host or intestinal microflora in the bowel and/or by binding to intestinal contents?*

No. There is no specific information on the ability of intestinal microflora to metabolize erythromycin. However, the metabolism of erythromycin by cytochrome P450-mediated *N*-demethylation reactions occurs in the liver of various species of rodents, ruminants and humans. Des-*N*-methyl-erythromycin is the major metabolite present in the bile and intestinal contents of rats after oral administration of 10 mg of erythromycin. The antimicrobial activity of des-*N*-methyl-erythromycin is presumably low, and the only form of erythromycin known to be active is the free base.

4. *Do data on the effects of the drug on the colonic microflora provide a basis to conclude that the ADI derived from toxicological data is sufficiently low to protect the intestinal flora?*

No. A number of in vitro and in vivo studies have demonstrated the potential for adverse effects of erythromycin on the intestinal microflora. Studies of toxicity have not identified adverse findings at low oral doses, and thus the toxicological

ADI would not be expected to provide adequate protection for the intestinal microflora.

5. *Do clinical data from the therapeutic use of the class of drugs in humans or data from in vitro or in vivo model systems indicate that effects could occur in the gastrointestinal tract?*

Yes. Gastrointestinal effects are the most commonly reported adverse reactions to therapeutic use of erythromycin in humans. The effects include abdominal pain, nausea, vomiting and diarrhoea. In humans, doses of 2 g/day or more can induce signs of gastrointestinal disturbance.

6. *Determine the most sensitive adverse effect(s) of the drug on the human intestinal microflora.*

The available data indicate that oral exposure to erythromycin is associated with disruption of the colonization barrier, rather than emergence of resistance. There are no studies available on the emergence of resistance to erythromycin in human intestinal microflora. It was concluded that disruption of the colonization barrier is the most appropriate end-point for the determination of a microbiological ADI.

7. *If disruption of the colonization barrier is the concern, determine the MIC of the drug against 100 strains of predominant intestinal flora and take the geometric mean MIC of the most sensitive genus or genera to derive an ADI using the formula discussed at the forty-seventh meeting of the Committee (Annex 1, reference 125). Other model systems may be used to establish a no-observed-effect concentration (NOEC) to derive an ADI.*

Using all relevant data acquired in studies conducted in vitro and in vivo, the Committee considered that MIC studies for erythromycin against a range of bacterial species representative of those typically found in the human gastrointestinal tract were the most appropriate to use in determining a microbiological ADI.

An evaluation of the MIC_{50} values for relevant gastrointestinal microflora provides a figure of 0.1 µg/ml for *Bifidobacterium*. This value can be used to calculate a microbiological ADI, as follows:

$$\text{Upper limit of ADI} = \frac{MIC_{50} \times MCC}{FA \times SF \times BW}$$

where:

MIC_{50} = minimum inhibitory concentration for 50% of strains of the most sensitive relevant organism. The MIC_{50} for the most sensitive relevant genus of the gut flora was 0.1 µg/ml (0.1 µg/g) for *Bifidobacterium*.

MCC = mass of colonic contents: a value of 220 g, determined by the Committee at its forty-seventh meeting (Annex 1, reference *125*), was used in the calculation.

FA = available fraction of the dose; the microbiologically active residue is erythromycin. Erythromycin is poorly absorbed in humans. In addition, in rats, approximately 37–43% of an oral dose of erythromycin was recovered in the intestinal tract and faeces. Therefore, a conservative estimate of the fraction of the dose available to the gastrointestinal microflora is 50%. Thus, the value in the equation is 0.5.

SF = safety factor: the magnitude of the safety factor depends on the quality and quantity of the microbiological data available. A value of 1 is appropriate when relevant microbiological data on disruption of the colonization barrier are available, as is the case in the current assessment. Thus, the safety factor should be 1.

BW = body weight: a value of 60 kg has been adopted for an adult.

Hence,

$$\text{Upper limit of ADI} = \frac{0.1\ \mu g/g \times 220\ g}{0.5 \times 1 \times 60\ kg}$$

$$= 0.7\ \mu g/kg\ \text{body weight}$$

4. EVALUATION

The Committee considered that microbiological effects were more relevant than toxicological effects for the establishment of an ADI for erythromycin. Due to the deficiencies and uncertainties in the toxicological database, the Committee was unable to establish a toxicological ADI with any confidence. However, the microbiological data did permit calculation of a robust microbiological ADI. To help compare the microbiological ADI with potentially relevant toxicological end-points, the Committee adopted a margin of exposure approach. This involved obtaining the ratio of the respective LOEL to the upper bound of the microbiological ADI.

The most relevant animal study for the evaluation of the safety of residues of erythromycin is the 2-year study in rats given erythromycin stearate in the diet. A NOEL was not established in this study, and the LOEL for non-tumorigenic effects was 210 mg/kg body weight per day in female rats based on granulomas of the liver and reticulum cell hyperplasia of the bone marrow. The upper bound of the microbiological ADI, 0.7 µg/kg body weight, provides a 300 000-fold margin of exposure over the LOEL in rats. The Committee concluded that, although based on a LOEL, this margin of exposure is such that residues of erythromycin are unlikely to pose a risk of systemic toxicity to humans.

Infantile hypertrophic pyloric stenosis has been identified as a possible effect in developing children following early postnatal exposure to erythromycin. The Committee assumed a human therapeutic dose of 500 mg per person and 60 kg body weight, or 8 mg/kg body weight per day. While a potential might exist for a postnatal effect in infants exposed through the breast milk from mothers receiving therapeutic erythromycin, the upper bound of the microbiological ADI, 0.7 µg/kg

body weight, provides a greater than 10 000-fold margin of exposure over the dose associated with postnatal effects. The Committee concluded that residues of erythromycin are unlikely to pose a risk to breast-fed infants.

Therefore, the Committee established an ADI of 0–0.7 µg/kg body weight on the basis of the MIC_{50} of 0.1 µg/g for *Bifidobacterium.*

A toxicological monograph was prepared.

5. REFERENCES

Abramov, L.A., Yust, I.C., Fierstater, E.M. & Vardinon, N.E. (1978) Acute respiratory distress caused by erythromycin hypersensitivity. *Arch. Intern. Med.*, **138**, 1156–1158.

AMA (1986) Macrolides and lincosamides. In: *Drug evaluations*, 6th ed. Philadelphia, PA, W.B. Saunders Co. for the American Medical Association, pp. 1395–1407.

Anderson, R., Fernandes, A.C. & Eftychis, H.E. (1984) Studies on the effects of ingestion of a single 500 mg oral dose of erythromycin stearate on leucocyte motility and transformation and on release in vitro of prostaglandin E2 by stimulated leucocytes. *J. Antimicrob. Chemother.*, **14**, 41–50.

Anderson, R.C., Harris, P.N. & Chen, K.K. (1952) The toxicity and distribution of ilotycin. *J. Am. Pharm. Assoc. Am. Pharm. Assoc. (Baltim.)*, **49**, 555–558.

Anderson, R.C., Harris, P.N. & Chen, K.K. (1955) Further toxicological studies with ilotycin (erythromycin, Lilly). *J. Am. Pharm. Assoc. Am. Pharm. Assoc. (Baltim.)*, **44**, 199–204.

Anderson, R.C., Lee, C.C., Worth, H.M. & Harris, P.N. (1959) Pharmacology and toxicology of propionyl erythromycin ester lauryl sulfate. *J. Am. Pharm. Assoc. Am. Pharm. Assoc. (Baltim.)*, **48**, 623–628.

Andremont, A. & Tancrede, C. (1981) Reduction of the aerobic Gram negative bacterial flora of the gastro-intestinal tract and prevention of traveller's diarrhea using oral erythromycin. *Ann. Microbiol. (Paris)*, **132B**, 419–427.

Andremont, A., Raibaud, P. & Tancrede, C. (1983) Effect of erythromycin on microbial antagonisms: a study in gnotobiotic mice associated with a human fecal flora. *J. Infect. Dis.*, **148**, 579–587.

Andremont, A., Gerbaud, G. & Courvalin, P. (1986) Plasmid-mediated high-level resistance to erythromycin in *Escherichia coli. Antimicrob. Agents Chemother.*, **29**, 515–518.

Anonymous (undated a) *Repeated dose toxicity studies in monkeys.* Submitted to WHO by Ceva Sante Animale Company, Libourne, France.

Anonymous (undated b) *Multigeneration reproduction study in rats.* Submitted to WHO by Ceva Sante Animale Company, Libourne, France.

ASHP (1991) Erythromycins. In: McEvoy, G.K., ed. *AHFS drug information.* Bethesda, MD, American Society of Hospital Pharmacists, pp. 194–205.

Baggot, J.D. & Gingerich, D.A. (1976) Pharmacokinetic interpretation of erythromycin and tylosin activity in serum after intravenous administration of a single dose to cows. *Res. Vet. Sci.*, **21**, 318–323.

Barlam, T. & Neu, C.H. (1984) In vitro comparison of the activity of RU 28965, a new macrolide, with that of erythromycin against aerobic and anaerobic bacteria. *Antimicrob. Agents Chemother.*, **25**, 529–531.

Bartkus, J.M., Juni, B.A., Ehresmann, K., Miller, C.A., Sanden, G.N., Casseday, P.K., Saubolle, M., Lee, B., Long, J., Harrison, A.R. & Besser, J.M. (2003) Identification of a mutation associated with erythromycin resistance in *Bordetella pertussis*: implications for surveillance of antimicrobial resistance. *J. Clin. Microbiol.*, **41**, 1167–1172.

Berndtson, W.E. & Foote, R.H. (1976) Survival and fertility of antibiotic-treated bovine spermatozoa. *J. Dairy Sci.*, **59**, 2130–2133.

Bessard [initials not provided] (1987) *Cours de pharmacologie: Macrolides.* Association des enseignants de pharmacologie des Facultés des Médecine, pp. 475–480.

Bligny, S. (1988a) *Erythromycin injectable. Pharmacokinetic study, residue study.* Unpublished Report No. PK 5251/E-00. Submitted to WHO by Ceva Sante Animale Company, Libourne, France.

Bligny, S. (1988b) *Erythromycin soluble. Pharmacokenetic study, residue study.* Unpublished Report No. PK 8400/E-00. Submitted to WHO by Ceva Sante Animale Company, Libourne, France.

Boggiano, B.G. & Gleeson, M. (1976) Gastric acid inactivation of erythromycin stearate in solid dosage forms. *J. Pharm. Sci.*, **65**, 497–502.

Braun, P. (1969) Hepatotoxicity of erythromycin. *J. Infect. Dis.*, **119**, 300–306.

Burrows, G.E. (1980) Pharmacotherapeutics of macrolides, lincomycins, and spectinomycin. *J. Am. Vet. Med. Assoc.*, **176**, 1072–1077.

Champney, W.S., Chittum, H.S. & Tober, C.L. (2003) A 50S ribosomal subunit precursor particle is a substrate for the ErmC methyltransferase in *Staphylococcus aureus* cells. *Curr. Microbiol.*, **46**, 453–460.

Cooper, W.O., Ray, W.A. & Griffin, M.R. (2003) Prenatal prescription of macrolide antibiotics and infantile hypertrophic pyloric stenosis. *Obstet. Gynecol.*, **100**, 101–106.

Courvalin, P., Ounissi, H. & Arthur, M. (1985) Multiplicity of macrolide–lincosamide–streptogramin antibiotic resistance determinants. *Antimicrob. Chemother.*, **16**, 91–100.

Czeizel, A.E., Rockenbauer, M., Sorensen, H.T. & Olsen, J. (1999) A population-based case–control teratogenic study of oral erythromycin treatment during pregnancy. *Reprod. Toxicol.*, **13**, 531–536.

Davis, T.E. (1995) Interstitial cell adenoma of the rat testis: why is it such a problem in toxicology studies using Sprague-Dawley rats? *Toxicol. Pathol.*, **23**, 83–86.

Davydova, N., Streltsov, V., Wilce, M., Liljas, A. & Garber, M. (2002) L22 ribosomal protein and effect of its mutation on ribosome resistance to erythromycin. *J. Mol. Biol.*, **322**, 635–644.

Delgado, S., Florez, A.B. & Mayo, B. (2005) Antibiotic susceptibility of *Lactobacillus and Bifidobacterium* species from the human gastrointestinal tract. *Curr. Microbiol.*, **50**, 202–207.

Dewdney, J.M., Maes, L., Rynaud, J.P., Blanc, F., Scheid, J.P., Jackson, T., Lens, S. & Verschueren, C. (1991) Risk assessment of antibiotic residues of β-lactams and macrolides in food products with regard to their immuno-allergic potential. *Food Chem. Toxicol.*, **29**, 477–483.

Dieter, M., French, J., Boorman, G. & Luster, M. (1987) Metabolic characterization of mouse bone marrow cells responsive to estrogenic inhibition: Hexose monophosphate shunt enzyme activity in enriched populations of mature cells and progenitor cells. *J. Leukoc. Biol.*, **41**, 212–219.

DiSanto, A.R. & Chodos, D.J. (1981) Influence of study design in assessing food effects on absorption of erythromycin base and erythromycin stearate. *Antimicrob. Agents Chemother.*, **20**, 190–196.

Edelstein, P.H. (2004) Pneumococcal resistance to macrolides, lincosamides, ketolides, and streptogramin B agents: molecular mechanisms and resistance phenotypes. *Clin. Infect. Dis.*, **38** (Suppl. 4), S322–S327.

Fail, P.A., Martin, P. & Sokal, D. (2000) Comparative effects of quinacrine and erythromycin in adult female rats: a nonsurgical sterilization study. *Fertil. Steril.*, **73**, 387–394.

Farrell, D.J., Douthwaite, S., Morrissey, I., Bakker, S., Poehlsgaard, J., Jakobsen, L. & Felmingham, D. (2003) Macrolide resistance by ribosomal mutation in clinical isolates of *Streptococcus pneumoniae* from the PROTEKT 1999–2000 study. *Antimicrob. Agents Chemother.*, **47**, 1777–1783.

Fiese, E.F. & Steffen, S.H. (1990) Comparison of the acid stability of azithromycin and erythromycin A. *J. Antimicrob. Chemother.*, **25**, 39–47.

Finegold, S.M. (1977) Antimicrobial agent susceptibility of anaerobic bacteria. In: *Anaerobic bacteria in human disease.* New York, Academic Press, pp. 513–533.

Fraschini, F., Scaglione, F., Ferrara, F., Marelli, O., Braga, P.C. & Teodori, F. (1986) Evaluation of the immunostimulating activity of erythromycin in man. *Chemotherapy*, **32**, 286–290.

Frost, D.V., Main, B.T., Perdue, H.S., Stein, R.J., Blockus, L.E., Otto, G.F. & Hamlin, K.E. (1965) *Erythromycin thiocyanate: results of chronic toxicity study in rats (RG-LT-5) at 68 weeks.* Submitted to WHO by Amdal Company, Agricultural Division, Abott Laboratories, North Chicago, IL.

Fujita, K. (2006) Cytochrome P450 and anticancer drugs. *Curr. Drug Metab.*, **7**, 23–37.

Griffith, R.S. & Black, H.R. (1970) Erythromycin. *Med. Clin. North Am.*, **54**, 1199–1215.

Haanpera, M., Huovinen, P. & Jalava, J. (2005) Detection and quantification of macrolide resistance mutations at positions 2058 and 2059 of the 23S rRNA gene by pyrosequencing. *Antimicrob. Agents Chemother.*, **49**, 457–460.

Hargreaves, C.A., Rogers, S., Hills, F., Rahman, F., Howell, R.J. & Homa, S.T. (1998) Effects of co-trimoxazole, erythromycin, amoxycillin, tetracycline and chloroquine on sperm function in vitro. *Hum. Reprod.*, **13**, 1878–1886.

Heusghem, C. & Lechat, P. (1973) *Les effets indésirables des médicaments*. Paris, Masson, pp. 456, 706, 778–779.

Hirakata, Y., Kaku, M., Mizukane, R., Ishida, K., Furuya, N., Matsumoto, T., Tateda, K. & Yamaguchi, K. (1992) Potential effects of erythromycin on host defense systems and virulence of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, **36**, 1922–1927.

Huber, W.G. (1977) Streptomycin, chloramphenicol, and other antibacterial agents — Erythromycin. Chemotherapy of microbial, fungal, and viral diseases. In: Meyer-Jones, L., Booth, N.H. & McDonald, L.E., eds. *Veterinary pharmacology and therapeutics*. Ames, IA, Iowa State University Press, pp. 953–954.

Hughes, B.R. & Cunliffe, W.J. (1987) Erythromycin and gastrointestinal motility. *Lancet*, **2** (8571), 1340.

Kallen, B.A. & Otterblad Olausson, P. (2003) Maternal drug use in early pregnancy and infant cardiovascular defect. *Reprod. Toxicol.*, **17**, 255–261.

Kallen, B.A., Otterblad Olausson, P. & Danielsson, B.R. (2005) Is erythromycin therapy teratogenic in humans? *Reprod. Toxicol.*, **20**, 209–214.

Kaltiala, E.H., Rasanen, O., Herva, E. & Karki, N.T. (1967) Subchronic toxicity of some erythromycin compounds in rats. *Acta Pharmacol. Toxicol.*, **25**, 435–446.

Keller, H. & Follath, F. (1988) Erythromycin. In: Dukes, M.N.G., ed. *Meyler's side effects of drugs*, 11th ed. Amsterdam, Elsevier, pp. 557–559.

Kroboth, P.D., Brown, A., Lyon, J.A., Kroboth, F.J. & Juhl, R.P. (1982) Pharmacokinetics of single-dose erythromycin in normal and alcoholic liver disease subjects. *Antimicrob. Agents Chemother.*, **21**, 135–140.

Leclerc, R. (2002) Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. *Antimicrob. Resist.*, **34**, 482–492.

Lee, C.C., Anderson, R.C. & Chen, K.K. (1953) Tissue distribution of erythromycin in rats. *Antibiot. Chemother.*, **3**, 920–924.

Lee, C.C., Anderson, R.C. & Chen, K.K. (1956a) Distribution and excretion of radioactivity in rats receiving *N*-methyl-C^{14}-erythromycin. *J. Pharmacol. Exp. Ther.*, **117**, 265–273.

Lee, C.C., Anderson, R.C. & Chen, K.K. (1956b) The excretory products of *N*-methyl-C^{14}-erythromycin in rats. *J. Pharmacol. Exp. Ther.*, **117**, 274–280.

Lim, K.S., Huh, C.S. & Baek, Y.J. (1993) Antimicrobial susceptibility of bifidobacteria. *J. Dairy Sci.*, **76**, 2168–2174.

Luster, M., Boorman, G., Korach, K., Dieter, M. & Hong, L. (1984) Mechanism of estrogen-induced myelotoxicity: Evidence for thymic regulation. *Int. J. Immunopharmacol.*, **6**, 287–294.

McCormack, W., George, H., Donner, A., Kodgis, L.F., Alpert, S., Lowe, E.W. & Kass, E.H. (1977) Hepatotoxicity of erythromycin estolate during pregnancy. *Antimicrob. Agents Chemother.*, **12**, 630–635.

Midtvedt, T. (1981) Erythromycin. In: *Side effects of drugs annual.* Amsterdam, Excerpta Medica, pp. 270–271.

Moriguchi, M., Fujita, M. & Koeda, T. (1972) Teratological studies on SF-837 in mice. *Jpn. J. Antibiot.*, **25**, 193–199.

Nold, J.B. (2001) Diseases and neoplasms of the aging rat. In: *47th annual pathology of laboratory animals (POLA) course.* Washington, DC, Armed Forces Institute of Pathology.

Nouws, J.F.M. & Ziv, G. (1979) Distribution and residues of macrolide antibiotics in normal dairy cows. *Arch. Lebensmittelhyg.*, **30**, 202–208.

NTP (1988) *NTP toxicology and carcinogenesis studies of erythromycin stearate (CAS No. 643-22-1) in F344/N rats and B6C3F1 mice (feed studies).* Research Triangle Park, NC, United States Department of Health and Human Services, National Institutes of Health, National Toxicology Program, pp. 1–167 (Technical Report Series 338).

O'Connors, M., Gregory, S.T. & Dahlberg, A.E. (2004) Multiple defects in translation associated with altered ribosomal protein L. *Nucleic Acids Res.*, **32**, 5750–5756.

Oliver, L.E., Iser, J.H., Stening, G.F. & Smallwood, R.A. (1972) Abdominal pain and erythromycin estolate. *Lancet*, **2** (7784), 980.

Payot, S., Avrain, L., Magras, C., Praud, K., Cloeckaert, A. & Chalus-Dancla, E. (2004) Relative contribution of target gene mutation and efflux to fluoroquinolone and erythromycin resistance, in French poultry and pig isolates of *Campylobacter coli*. *Int. J. Antimicrob. Agents*, **23**, 468–472.

Periti, P., Mazzei, T., Mini, E. & Novelli, A. (1989) Clinical pharmacokinetic properties of the macrolide antibiotics — Effects of age and various pathophysiological states. *Clin. Pharmacokinet.*, **16**, 193–214.

Pineau, T., Galtier, P., Bonfils, C., Derancourt, J. & Maurel, P. (1990) Purification of a sheep liver cytochrome P-450 from the P450IIIA gene subfamily. *Biochem. Pharmacol.*, **39**, 901–909.

Pradalier, A., Dry, J. & Luce, H. (1980) Réflexion sur l'allergie médicamenteuse. *Concours méd. (Paris)*, **102**, 5993–6011.

Raab, W. (1977) Investigations into acute unwanted effects of erythromycin, lincomycin and clindamycin. *J. Clin. Pharmacol. Biopharmacol.*, **15**, 90–97.

Ras, G.J. & Anderson, R. (1986) An in-vitro study of oral therapeutic doses of co-trimoxazole and erythromycin stearate on abnormal polymorphonuclear leucocyte migration. *J. Antimicrob. Chemother.*, **17**, 185–193.

Reynolds, J.E.F., ed. (1989) Erythromycin. In: *Martindale: The extra pharmacopoeia*, 29th ed. London, Pharmaceutical Press, pp. 222–230.

Roberts, M.C., Sutcliffe, J., Courvalin, P., Jensen, L.B., Rood, J. & Seppala, H. (1999) Nomenclature for macrolide and macrolide–lincosamide–streptogramin B resistance determinants. *Antimicrob. Agents Chemother.*, **43**, 2823–2830.

Schlegel, P.N., Chang, T.S.K. & Marshall, F.F. (1991) Antibiotics: potential hazards to male fertility. *Fertil. Steril.*, **55**, 235–242.

Shanson, D.C., Tidbury, P., McNabb, W.R. & Tadayon, M. (1984) The pharmacokinetics and tolerance of oral erythromycin stearate compared with erythromycin ethylsuccinate: implications for preventing endocarditis. *J. Antimicrob. Chemother.*, **14**, 157–163.

Shulman, J.A. & Sellers, T.F. (1971) Chemotherapy of bacterial infections. VII: Other important antibiotics. In: DiPalma, J.R., ed. *Drill's pharmacology in medicine*, 4th ed. New York, McGraw-Hill, pp. 1729–1731.

Sutter, V.L. & Finegold, S.M. (1976) Susceptibility of anaerobic bacteria to 23 antimicrobial agents. *Antimicrob. Agents Chemother.*, **10**, 736–752.

Sweetman, S.C., ed. (2002) Erythromycin. In: *Martindale: the complete drug reference*, 33rd ed. London, Pharmaceutical Press, pp. 201–204.

Tait-Kamradt, A., Davies, T., Cronan, M., Jacobs, M.R., Appelbaum, P.C. & Sutcliffe, J. (2000) Mutations in 23S rRNA and ribosomal protein L4 account for resistance in pneumococcal strains selected in vitro by macrolide passage. *Antimicrob. Agents Chemother.*, **44**, 2118–2125.

Tancrede, C., Andremont, A. & Guerlin, N. (1980) Effet de l'érythromycine sur les populations d'entérobactéries du tube digestif chez l'homme. *Nouv. Presse Med.*, **9**, 2742–2743.

Tsubaki, M. & Ichikawa, Y. (1985) Isolation and characterization of two constitutive forms of microsomal cytochrome P-450 from a single bovine liver. *Biochim. Biophys. Acta*, **830**, 244–247.

Villa, P., Begue, J.M. & Guillouzo, A. (1984) Effects of erythromycin derivatives on cultured rat hepatocytes. *Biochem. Pharmacol.*, **33**, 4098–4101.

Wilson, J.T. & van Boxtel, C.J. (1978) Pharmacokinetics of erythromycin in man. *Antibiot. Chemother.*, **25**, 181–203.

Wong, K.Y., Boose, G.M. & Issitt, C.H. (1981) Erythromycin-induced hemolytic anemia. *J. Pediatr.*, **98**, 647–649.

Wrighton, S.A., Schuetz, E.G., Watkins, P.B., Maurel, P., Barwick, J., Ailey, B.S., Hartle, H.T., Young, B. & Guzelian, P. (1985) Demonstration in multiple species of inducible hepatic cytochromes P-450 and their mRNAs related to the glucocorticoid-inducible cytochrome P-450 of the rat. *Mol. Pharmacol.*, **28**, 312–321.

Yazid, A.M., Ali, A.M., Shuhaimi, M., Kalaivaani, V., Rokiah, M.Y. & Reezal, A. (2000) Antimicrobial susceptibility of bifidobacteria. *Lett. Appl. Microbiol.*, **31**, 57–62.

Zhang, Y., Zhang, Q. & Xu, K.Z. (1997) Tissue and body fluid distribution of antibacterial agents in pregnant and lactating women. *Zhonghua Fu Chan Ke Za Zhi*, **32**, 288–292.

Ziv, G. (1980a) Practical pharmacokinetic aspects of mastitis therapy — 3: Intramammary treatment. *Vet. Med. Small Anim. Clin.*, **75** (4), 657–670.

Ziv, G. (1980b) Practical pharmacokinetic aspects of mastitis therapy — 1: Parenteral treatment. *Vet. Med. Small Anim. Clin.*, **75** (2), 277–290.

TYLOSIN

A toxicological monograph on tylosin has not been prepared. No data were submitted, and a brief review of the toxicological information available in the scientific peer-reviewed literature did not allow an evaluation of the compound. The interested reader can refer to the summary of discussions on tylosin at the sixty-sixth meeting of the Committee in the report *Evaluation of Certain Veterinary Drug Residues in Food*, WHO Technical Report Series 939.

ANNEX 1

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 of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). 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 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives, Vol. II. Food colours*, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants* (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants*. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. *Specifications for identity and purity and toxicological evaluation of food colours*. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
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 of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases*. FAO Nutrition Meetings Report Series, No. 40A, 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 of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.

14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. *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). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. *Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. *Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents* (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. *Specifications for the identity and purity of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. *Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants* (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
27. *Toxicological evaluation of some enzymes, modified starches, and certain other substances.* FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
28. *Specifications for the identity and purity of some enzymes and certain other substances.* FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
29. *A review of the technological efficacy of some antioxidants and synergists.* FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
30. *Evaluation of certain food additives and the contaminants mercury, lead, and cadmium* (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO

Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.

31. *Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate.* FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers.* FAO Food and Nutrition Paper, No. 4, 1978.
35. *Evaluation of certain food additives* (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
37. *Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives.* FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
38. *Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances* (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
39. *Toxicological evaluation of some food colours, thickening agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
40. *Specifications for the identity and purity of certain food additives.* FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.
41. *Evaluation of certain food additives* (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 10, 1976.
43. *Specifications for the identity and purity of some food additives.* FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. *Summary of toxicological data of certain food additives.* WHO Food Additives Series, No. 12, 1977.
46. *Specifications for identity and purity of some food additives, including antioxidants, food colours, thickeners, and others.* FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. *Summary of toxicological data of certain food additives and contaminants.* WHO Food Additives Series, No. 13, 1978.
49. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 7, 1978.
50. *Evaluation of certain food additives* (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.

51. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 14, 1980.
52. *Specifications for identity and purity of food colours, flavouring agents, and other food additives.* FAO Food and Nutrition Paper, No. 12, 1979.
53. *Evaluation of certain food additives* (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 15, 1980.
55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives).* FAO Food and Nutrition Paper, No. 17, 1980.
56. *Evaluation of certain food additives* (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 16, 1981.
58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives).* FAO Food and Nutrition Paper, No. 19, 1981.
59. *Evaluation of certain food additives and contaminants* (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 17, 1982.
61. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 25, 1982.
62. *Evaluation of certain food additives and contaminants* (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
63. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 18, 1983.
64. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 28, 1983.
65. *Guide to specifications, general notices, general methods, identification tests, test solutions, and other reference materials.* FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. *Evaluation of certain food additives and contaminants* (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.
67. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 19, 1984.
68. *Specifications for the identity and purity of food colours.* FAO Food and Nutrition Paper, No. 31/1, 1984.
69. *Specifications for the identity and purity of food additives.* FAO Food and Nutrition Paper, No. 31/2, 1984.
70. *Evaluation of certain food additives and contaminants* (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 34, 1986.
72. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 20. Cambridge University Press, 1987.
73. *Evaluation of certain food additives and contaminants* (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 21. Cambridge University Press, 1987.
75. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 37, 1986.

76. *Principles for the safety assessment of food additives and contaminants in food.* WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.
77. *Evaluation of certain food additives and contaminants* (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987 and corrigendum.
78. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 22. Cambridge University Press, 1988.
79. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 38, 1988.
80. *Evaluation of certain veterinary drug residues in food* (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.
81. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 23. Cambridge University Press, 1988.
82. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41, 1988.
83. *Evaluation of certain food additives and contaminants* (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
85. *Evaluation of certain veterinary drug residues in food* (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 25, 1990.
87. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/2, 1990.
88. *Evaluation of certain food additives and contaminants* (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 26, 1990.
90. *Specifications for identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 49, 1990.
91. *Evaluation of certain veterinary drug residues in food* (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 27, 1991.
93. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/3, 1991.
94. *Evaluation of certain food additives and contaminants* (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 28, 1991.
96. *Compendium of food additive specifications* (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990. Rome, Food and Agricultural Organization of the United Nations, 1992 (2 volumes).
97. *Evaluation of certain veterinary drug residues in food* (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. *Toxicological evaluation of certain veterinary residues in food.* WHO Food Additives Series, No. 29, 1991.

99. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/4, 1991.
100. *Guide to specifications—General notices, general analytical techniques, identification tests, test solutions, and other reference materials.* FAO Food and Nutrition Paper, No. 5, Ref. 2, 1991.
101. *Evaluation of certain food additives and naturally occurring toxicants* (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 828, 1992.
102. *Toxicological evaluation of certain food additives and naturally occurring toxicants.* WHO Food Additive Series, No. 30, 1993.
103. *Compendium of food additive specifications: addendum 1.* FAO Food and Nutrition Paper, No. 52, 1992.
104. *Evaluation of certain veterinary drug residues in food* (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 31, 1993.
106. *Residues of some veterinary drugs in animals and food.* FAO Food and Nutrition Paper, No. 41/5, 1993.
107. *Evaluation of certain food additives and contaminants* (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 32, 1993.
109. *Compendium of food additive specifications: addendum 2.* FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. *Evaluation of certain veterinary drug residues in food* (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 33, 1994.
112. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/6, 1994.
113. *Evaluation of certain veterinary drug residues in food* (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 34, 1995.
115. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/7, 1995.
116. *Evaluation of certain food additives and contaminants* (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
117. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 35, 1996.
118. *Compendium of food additive specifications: addendum 3.* FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. *Evaluation of certain veterinary drug residues in food* (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 36, 1996.
121. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/8, 1996.
122. *Evaluation of certain food additives and contaminants* (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.

123. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 37, 1996.
124. *Compendium of food additive specifications: addendum 4.* FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.
125. *Evaluation of certain veterinary drug residues in food* (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 38, 1996.
127. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/9, 1997.
128. *Evaluation of certain veterinary drug residues in food* (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 39, 1997.
130. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/10, 1998.
131. *Evaluation of certain food additives and contaminants* (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
132. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 40, 1998.
133. *Compendium of food additive specifications: addendum 5.* FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
134. *Evaluation of certain veterinary drug residues in food* (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.
135. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 41, 1998.
136. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/11, 1999.
137. *Evaluation of certain food additives* (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.
138. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 42, 1999.
139. *Compendium of food additive specifications: addendum 6.* FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.
140. *Evaluation of certain veterinary drug residues in food* (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.
141. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 43, 2000.
142. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/12, 2000.
143. *Evaluation of certain food additives and contaminants* (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.
144. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 44, 2000.
145. *Compendium of food additive specifications: addendum 7.* FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.
146. *Evaluation of certain veterinary drug residues in food* (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.
147. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 45, 2000.

148. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/13, 2000.
149. *Evaluation of certain food additives and contaminants* (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 901, 2001.
150. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 46, 2001.
151. *Compendium of food additive specifications: addendum 8.* FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.
152. *Evaluation of certain mycotoxins in food* (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 906, 2002.
153. *Safety evaluation of certain mycotoxins in food.* WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper 74, 2001.
154. *Evaluation of certain food additives and contaminants* (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, 2002.
155. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 48, 2002.
156. *Compendium of food additive specifications: addendum 9.* FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.
157. *Evaluation of certain veterinary drug residues in food* (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.
158. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 49, 2002.
159. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/14, 2002.
160. *Evaluation of certain food additives and contaminants* (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.
161. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 50, 2003.
162. *Compendium of food additive specifications: addendum 10.* FAO Food and Nutrition Paper No. 52, Add. 10, 2002.
163. *Evaluation of certain veterinary drug residues in food* (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.
164. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 51, 2003.
165. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/15, 2003.
166. *Evaluation of certain food additives and contaminants* (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.
167. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 52, 2004.
168. *Compendium of food additive specifications: addendum 11.* FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.
169. *Evaluation of certain veterinary drug residues in food* (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.
170. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/16, 2004.
171. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 53, 2005.
172. *Compendium of food additive specifications: addendum 12.* FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.

173. *Evaluation of certain food additives* (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.
174. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 54, 2005.
175. *Compendium of food additive specifications: addendum 13.* FAO Food and Nutrition Paper, No. 52, Add. 13, 2005.
176. *Evaluation of certain food contaminants* (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005.
177. *Safety evaluation of certain contaminants in food.* WHO Food Additives Series, No. 55/FAO Food and Nutrition Paper, No. 82, 2005.
178. *Evaluation of certain food additives* (Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 934, 2005.
179. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 56, 2006 (in press).
180. *Compendium of food additive specifications: addendum 14.* FAO Food and Nutrition Paper, No. 52, Add. 14 (in preparation).
181. *Evaluation of certain veterinary drug residues in food* (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 939, 2006 (in preparation).
182. *Residues of some veterinary drugs in animals and foods.* FAO JECFA Monographs, No. 2, 2006 (in preparation).
183. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 57, 2006.

ANNEX 2

ABBREVIATIONS USED IN THE MONOGRAPHS

ADI	acceptable daily intake
ATCC	American Type Culture Collection
CFU	colony-forming unit(s)
DNA	deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GLP	Good Laboratory Practice
HFA	human flora-associated
Ig	immunoglobulin
IPCS	International Programme on Chemical Safety
IU	International Units
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
LOEL	lowest-observed-effect level
MIC	minimum inhibitory concentration
MIC_{50}	minimum inhibitory concentration for 50% of strains of the most sensitive relevant organism
Mr	relative molecular mass
MRL	maximum residue limit
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
PELS	propionyl erythromycin ester lauryl sulfate
RNA	ribonucleic acid
S9	9000 × *g* supernatant of rat liver
TRS	Technical Report Series
USA	United States of America
WHO	World Health Organization

ANNEX 3
JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES, ROME, 22–28 FEBRUARY 2006

Members

Professor A. Anadón, Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain

Dr D. Arnold, Consultant, Berlin, Germany *(Vice-Chairman)*

Professor A. Boobis, Experimental Medicine and Toxicology, Division of Medicine, Faculty of Medicine, Imperial College, London, England

Dr R. Ellis, Consultant, Myrtle Beach, SC, USA

Dr A. Fernández Suárez, Instituto Nacional de Tecnología Agropecuaria, Instituto de Tecnología de los Alimentos, Buenos Aires, Argentina [1]

Dr K. Greenlees, Toxicologist, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA

Dr J. MacNeil, Center for Veterinary Drug Residues, Canadian Food Inspection Agency, Saskatoon Laboratory, Saskatoon, Saskatchewan, Canada

Dr J.G. McLean, Professor Emeritus, Camberwell, Victoria, Australia *(Chairman)*

Dr J. Palermo-Neto, Department of Pathology, Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil

Dr J.L. Rojas Martínez, Laboratorio Nacional de Servicios Veterinarios, Ministerio de Agricultura y Ganadería, Barreal de Heredia, Heredia, Costa Rica

Dr P. Sanders, Directeur, Agence française de sécurité sanitaire des aliments, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les disinfectants, Fougères, France

Professor G.E. Swan, Dean, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

Professor J. Wongtavatchai, Faculty of Veterinary Science, Department of Medicine, Chulalongkorn University, Bangkok, Thailand[2]

---

[1] Dr A. Fernández Suárez was unable to attend the meeting but prepared the first draft of one of the residue monographs.

[2] Professor J. Wongtavatchai was unable to attend the meeting but prepared the first draft of one of the residue monographs.

Secretariat

Dr A. Bruno, Food Standards Officer, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Staff Member)*

Dr C. Cerniglia, Director, Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, AR, USA *(WHO Temporary Adviser)*

Dr M. de Lourdes Costarrica, Senior Officer, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Staff Member)*

Dr L.G. Friedlander, Leader, Residue Chemistry Team, Division of Human Food Safety, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA *(FAO Consultant)*

Dr N. Iseki, Senior Standards Officer, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Staff Member)*

Dr Sang-Hee Jeong, Senior Researcher, Toxicology and Chemistry Division, National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, Anyang City, Republic of Korea *(WHO Temporary Adviser)*

Dr J. Lewicki, Division of Pharmacology and Toxicology, Department of Preclinical Sciences, Warsaw Agricultural University, Warsaw, Poland *(FAO Consultant)*

Professor S. McEwen, Professor and Interim Chair, Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada *(WHO Temporary Adviser)*

Dr S. Ozawa, Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan *(WHO Temporary Adviser)*[3]

Dr P. Reeves, Australian Pesticides and Veterinary Medicines Authority, Kingston, ACT, Australia *(FAO Consultant)*

Professor L. Ritter, Executive Director, Canadian Network of Toxicology Centres, Professor, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada *(WHO Temporary Adviser)*

Dr G. Roberts, Consultant Toxicologist, Greenway, ACT, Australia *(WHO Temporary Adviser)*

Ms M. Sheffer, Orleans, Ontario, Canada *(WHO Editor)*

Dr S. Sundlof, Director, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA *(WHO Temporary Adviser)*

[3] Dr S. Ozawa was invited but unable to attend the meeting.

Dr A. Tritscher, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland *(WHO Joint Secretary)*

Dr A. Wennberg, FAO Joint Secretary to JECFA, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Joint Secretary)*

ANNEX 4

RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA AND FURTHER INFORMATION REQUIRED

Colistin (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–7 µg/kg body weight, on the basis of the MIC_{50} of 1 µg/g of colistin base for *E. coli.*

Residue definition: Sum of colistin A and colistin B

Recommended maximum residue limits (MRLs)

Species	Fat[a] (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)	Milk[b] (µg/kg)	Eggs (µg/kg)
Cattle	150	200	150	150	50	
Sheep	150	200	150	150	50	
Goat	150	200	150	150		
Pig	150	200	150	150		
Chicken	150	200	150	150		300
Turkey	150	200	150	150		
Rabbit	150	200	150	150		

[a] The MRL includes skin + fat where appropriate.

Erythromycin (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–0.7 µg/kg body weight, on the basis of the MIC_{50} of 0.1 µg/g for *Bifidobacterium.*

Residue definition: Erythromycin A

Recommended maximum residue limits (MRLs)

Species	Fat[a] (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)	Eggs (µg/kg)
Chicken	100	100	100	100	50
Turkey	100	100	100	100	

[a] The MRL includes skin + fat where appropriate.

Flumequine (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–30 µg/kg body weight at its sixty-second meeting (WHO TRS No. 925, 2004).

Residue definition: Flumequine

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)
Black tiger shrimp (*Penaeus monodon*)	500[a]
Shrimp	500[a,b]

[a] The MRL is temporary. The following information is requested by the end of 2008: (1) Information on the approved dose for the treatment of diseases in shrimp and the results of residue depletion studies conducted at the recommended dose.
[b] The assignment of the temporary MRL applies to all freshwater and marine shrimp.

Melengestrol acetate (production aid)

Acceptable daily intake: The Committee established an ADI of 0–0.03 µg/kg body weight at its fifty-fourth meeting (WHO TRS No. 900, 2001).

Residue definition: Melengestrol acetate

Recommended maximum residue limits (MRLs)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	18	2	10	1

Ractopamine hydrochloride (production aid)

Acceptable daily intake: The Committee established an ADI of 0–1 µg/kg body weight at its sixty-second meeting (WHO TRS No. 925, 2004).

Residue definition: Ractopamine

The Committee maintained the MRLs recommended at its sixty-second meeting (WHO TRS No. 925, 2004):

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	10	90	40	10
Pig	10	90	40	10

To reconsider the MRLs for cattle, the Committee requested a residue depletion study with unlabelled drug in feed at the maximum recommended dose. This study should provide sufficient additional data to determine if the relationship used in establishing the MRLs for cattle liver and kidney requires adjustment and provide median values for residues in these tissues.

Trichlorfon (Metrifonate) (insecticide)

Acceptable daily intake: The Committee confirmed the ADI of 0–2 µg/kg body weight established at its sixtieth meeting (WHO TRS No. 918, 2003).

Residues: The MRLs that were recommended by the sixtieth meeting of the Committee were not reconsidered and were maintained.

Triclabendazole (anthelminthic)

Acceptable daily intake: The Committee established an ADI of 0–30 µg/kg body weight at its fortieth meeting (WHO TRS No. 832, 1993).

Residue definition: Keto-triclabendazole

Recommended maximum residue limits (MRLs)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Muscle (µg/kg)
Cattle	100	100	200	150
Sheep	100	100	200	150
Goat	100	100	200	150

This volume contains monographs prepared at the sixty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Rome, Italy, from 22 to 28 February 2006.

The toxicological monographs in this volume summarize data on the veterinary drug residues that were evaluated toxicologically by the Committee: the antimicrobial agents colistin and erythromycin. For the third antimicrobial agent, tylosin, a toxicological monograph has not been prepared. No data were submitted, and a brief review of the toxicological information available in the scientific peer-reviewed literature did not allow an evaluation of the compound.

This volume and others in the WHO Food Additives Series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories, and universities.