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SCIENTIFIC OPINION

Scientific Opinion on Chloramphenicol in food and feed[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Chloramphenicol is an antibiotic not authorised for use in food-producing animals in the European Union (EU). However, being produced by soil bacteria, it may occur in plants. The European Commission asked EFSA for a scientific opinion on the risks to human and animal health related to the presence of chloramphenicol in food and feed and whether a reference point for action (RPA) of 0.3 µg/kg is adequate to protect public and animal health. Data on occurrence of chloramphenicol in food extracted from the national residue monitoring plan results and from the Rapid Alert System for Food and Feed (RASFF) were too limited to carry out a reliable human dietary exposure assessment. Instead, human dietary exposure was calculated for a scenario in which chloramphenicol is present at 0.3 µg/kg in all foods of animal origin, foods containing enzyme preparations and foods which may be contaminated naturally. The mean chronic dietary exposure for this worst-case scenario would range from 11 to 17 and 2.2 to 4.0 ng/kg b.w. per day for toddlers and adults, respectively. The potential dietary exposure of livestock to chloramphenicol was estimated to be below 1 µg/kg b.w. per day. Chloramphenicol is implicated in the generation of aplastic anaemia in humans and causes reproductive/hepatotoxic effects in animals. Margins of exposure for these effects were calculated at 2.7×10^5 or greater and the CONTAM Panel concluded that it is unlikely that exposure to food contaminated with chloramphenicol at or below 0.3 µg/kg is a health concern for aplastic anaemia or reproductive/hepatotoxic effects. Chloramphenicol exhibits genotoxicity but, owing to the lack of data, the risk of carcinogenicity cannot be assessed. The CONTAM Panel concluded that, when applied to feed, the current RPA is also sufficiently protective for animal health and for public health, arising from residues in animal derived products.

KEY WORDS

chloramphenicol, food, feed, reference point for action, aplastic anaemia, natural occurrence, risk assessment

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SUMMARY

Chloramphenicol is a broad-spectrum antibiotic effective against Gram-positive and Gram-negative bacteria and, in the past, has been widely used to treat infections in both humans and animals. Chloramphenicol is not authorised for use in food-producing animals in the European Union (EU) but may be used in human medicine and in treatments for non-food-producing animals. Apart from its potential occurrence as a residue in food from illicit treatment of food-producing animals, chloramphenicol has also been used in feed and food enzyme products and may occur naturally in plants from its production by the soil bacterium *Streptomyces venezuelae*.

The EFSA Scientific Opinion entitled "*Guidance on methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin*" identified an approach for establishing RPAs for various categories of non-allowed pharmacologically active substances. However, the opinion also identified certain categories of non-allowed pharmacologically active substances that are considered to be outside the scope of the procedure, including substances causing blood dyscrasias (aplastic anaemia) such as chloramphenicol. As chloramphenicol is excluded from that opinion and taking into account its natural occurrence in the environment as a contaminant and its incidental use in fermentation processes or to protect the consumer from food and feed deterioration, the European Commission (EC) asked the European Food Safety Authority (EFSA) for a scientific opinion on the risks to human and animal health related to the presence of chloramphenicol in food and feed. The opinion should include an evaluation of the toxicity of chloramphenicol for humans, considering all relevant toxicological endpoints and identification of the toxicological relevance of chloramphenicol present in food, and an exposure assessment of the EU population to chloramphenicol, including the consumption patterns of specific (vulnerable) groups of the population. With regard to animals, the opinion should consider the exposure levels of chloramphenicol for the different farm animal species above which signs of toxicity can be observed or the level of transfer/carry-over of chloramphenicol from the feed to products of animal origin for human consumption which results in unacceptable levels of chloramphenicol. The EC also requested that an RPA of 0.3 µg/kg for chloramphenicol in food of animal origin be evaluated as to whether it is adequate to protect public health, and that the appropriateness of applying the RPA for food of animal origin to feed and food of non-animal origin for the protection of animal and public health be assessed.

Most of the sampling of food, and of related materials, for chloramphenicol testing in foods of animal origin is undertaken in the context of the national residue monitoring plans. Suitable screening methods measure chloramphenicol residues with sufficient sensitivity to satisfy the current regulatory requirements, at the minimum required performance limit (MRPL) of 0.3 µg/kg, and include immunoassay, biosensor and chromatographic techniques. Confirmatory methods, typically based on gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) techniques, have been developed for determination of chloramphenicol in a wide range of sample types and have decision limits (or limits of detection) in the range of < 0.01 to 0.15 µg/kg and detection capability (or limits of quantification) values in the range of 0.01 to 0.3 µg/kg.

Chloramphenicol has been found to occur in feed, such as straw, in a number of European Member States, and also in herbs, grass and soil samples. Studies have shown the natural formation of chloramphenicol by *Streptomyces venezuelae* in the soil, and its uptake into wheat stems and corn stalks and, at lower levels, into spikes and cobs. These studies demonstrate that plant materials can become contaminated as a result of the production of chloramphenicol by soil organisms.

Data on occurrence of chloramphenicol in food, reported by Member States from the national residue monitoring plans, have been extracted for the period 2002 to 2012; there were 306 targeted samples reported to be non-compliant for chloramphenicol. The animal species/food products in which chloramphenicol was reported were pigs, poultry, bovines, aquaculture, sheep/goats, rabbit, farmed game, honey and milk. Data were also extracted from the Rapid Alert System for Food and Feed

(RASFF) database for the years 2002 to 2013; there were 440 notification events reported for chloramphenicol, 402 for food and 38 for feed. The notifications related to a range of food products, particularly the categories of crustaceans and products thereof, honey and royal jelly, meat and meat products, milk and milk products and fish and fish products, and to feed. In addition, during 2013 there were 24 notification events relating to enzyme concentrates, enzyme preparations or foods containing enzyme preparations; 19 for food and 5 for feed. Three of these 19 notification events for food concerned enzyme-based food supplements.

The EFSA Panel on Contaminants in the Food Chain (CONTAM) concluded that these data, extracted from the EC's database relating to the national residue monitoring plan testing by Member States and the RASFF database, were too limited to carry out a reliable human dietary exposure assessment. Instead, the CONTAM Panel calculated the hypothetical human dietary exposure, considering as an occurrence value the RPA of 0.3 µg/kg, for a scenario where chloramphenicol is present in specific food groups (foods of animal origin, foods in which enzyme preparations, reported to be contaminated with chloramphenicol, may be used during food production, and grains and grain-based products in which chloramphenicol could occur naturally). The CONTAM Panel emphasises that this scenario represents a worst-case situation.

Applying the EFSA Comprehensive European Food Consumption Database to this exposure scenario would give mean chronic dietary exposure across the different European countries and dietary surveys of 11 to 17 ng/kg body weight (b.w.) per day for toddlers and of 2.2 to 4.0 ng/kg b.w. per day for adults.

The daily dietary exposure to chloramphenicol from enzyme-based food supplements at the concentrations reported in RASFF notifications ranged between 0.1 and 12 ng/kg b.w. per day.

The CONTAM Panel considered the exposure to chloramphenicol via feed enzymes for pigs and poultry and used a chloramphenicol concentration of 5.9 µg/kg compound feed. This level of chloramphenicol contamination in compound feed would result in a dietary exposure of ≤ 0.4 µg/kg b.w. per day for various categories of pigs and poultry. Based on available data on chloramphenicol levels in straw, the highest dietary exposure of cattle to chloramphenicol would be ≤ 0.5 µg/kg b.w. per day. Overall, potential dietary exposure of livestock to chloramphenicol from feed enzymes, straw or soil was estimated to be below 1 µg/kg b.w. per day.

Information on the effect of food processing on chloramphenicol is limited; some decrease in chloramphenicol has been reported due to processing, as well as the production of degradation products, but the toxic potential of these compounds is unclear. In the case of feed, no studies on the influence of feed processing (e.g. silage fermentation of grass, elevated temperatures and pressure in compound feed production) on chloramphenicol were identified.

In humans, chloramphenicol is highly bioavailable upon oral exposure and may easily cross both placental and mammary barriers. Under normal conditions, the drug is extensively biotransformed and rapidly eliminated, mainly as glucuronide derivatives. However, conditions known to depress the glucuronidation rate may allow the drug to enter reductive and/or oxidative pathways yielding toxic/reactive metabolites, which have been implicated in the generation of blood dyscrasias and possibly genotoxicity.

In ruminants, chloramphenicol is extensively metabolised in the rumen, resulting in poor absorption of the parent compound. In pigs, the available data indicate that chloramphenicol is widely bioavailable by the oral route and is distributed in all edible tissues. In avian species, chloramphenicol displays a limited oral bioavailability (35–45 %) and a remarkable first-pass effect. The parent drug and different metabolites have been detected in liver, muscle and eggs up to several days after termination of treatment. In horses, chloramphenicol is rapidly and extensively absorbed and widely distributed to tissues. In fish, metabolism of chloramphenicol is dependent on species and a variety of environmental

factors, such as water temperature and water flow. Cats exhibit a longer elimination half-life of the drug compared to other domestic animal species investigated.

Exposure of farm animals to radiolabelled chloramphenicol at doses formerly used therapeutically, typically around 50 mg/kg b.w., resulted in levels in meat, milk and eggs in the range of 1 to 100 mg/kg, expressed as chloramphenicol equivalents, during or shortly after the treatment. Linear extrapolation of these exposure levels to maximal intakes calculated for recent findings in feed enzymes, straw and soil indicate that levels in edible products would not exceed the current RPA of 0.3 µg/kg. Various metabolites were identified in carry-over studies with doses of chloramphenicol formerly used therapeutically but there is uncertainty about potential occurrence of residues of genotoxic metabolites in various animal species, with one study reporting their occurrence in broilers, whereas unpublished studies submitted to FAO/WHO could not confirm their presence in meat and organs of pigs, calves and broilers.

In mice, the oral median lethal dose (LD_{50}) was estimated to be 2 640 mg/kg b.w. and neurotoxic effects were observed after acute dosing at 1 250 mg/kg b.w. and higher. In dogs, neurotoxic effects were observed at 300 mg/kg b.w. given orally. Chloramphenicol causes toxicity in liver, small intestine, spleen and thymus of laboratory animals. Chloramphenicol also caused a concentration dependent inhibition of the activity of some cytochrome P450 (CYP)-enzymes in rat liver microsomal fractions. It also induced signs of haemolytic anaemia as well as an inhibitory action on the bone marrow. The most sensitive endpoint was liver toxicity, with effects found at the lowest tested dose of 25 mg/kg b.w. per day in rats. Consequently, a no observed adverse effect level (NOAEL) for repeated-dose toxicity could not be identified from these studies. Chloramphenicol caused dose-dependent mild reversible anaemia in laboratory animals at oral doses of 825 mg/kg b.w. per day or above, while severe non-reversible aplastic anaemia has not been observed. Chloramphenicol at doses of 25–112 mg/kg b.w. per day caused testes degeneration and effects on sperm quality in rats. Embryotoxicity and teratogenicity were found in laboratory animals orally exposed to chloramphenicol doses in the range of 500–2 000 mg/kg b.w. per day. Chloramphenicol is neurotoxic in certain species, shown by reduced learning ability in rats (50 mg/kg b.w. per day s.c.) and mice (25 to 200 mg/kg b.w. per day orally) and disturbed sleeping pattern in rats (400 mg/kg b.w. i.p.) and cats (165 mg/kg b.w. or higher orally).

While largely inactive in prokaryotic and lower eukaryotic genotoxicity test systems, chloramphenicol displays mutagenic and clastogenic activity *in vitro* in different types of mammalian cells, although it was negative in some tests. Moreover, several metabolites were shown to be much more active than chloramphenicol itself in inducing DNA-strand breaks in human cells. *In vivo*, chloramphenicol induced chromosomal aberrations in bone marrow in mice and rats and in blood cells of calves, following administration through different routes. Oral gavage studies showed clastogenic effects in newborn rats exposed transplacentally. The genotoxic activity of chloramphenicol is likely to depend on the metabolic competence of the exposed organism(s) in view of the higher toxic potencies of certain metabolites. No conclusion can be drawn regarding the potential carcinogenicity of chloramphenicol because of the lack of appropriate and well-documented long-term studies.

Although the mechanism for chloramphenicol-induced aplastic anaemia in humans has not been elucidated, nitroreduction to nitroso-chloramphenicol and the production of reactive oxygen species leading to DNA damage seem to be crucial factors in the induction of aplastic anaemia. Genetic predisposition, enhancing the ability of the bone marrow to reduce chloramphenicol into its myelotoxic derivative, also plays an important role.

The therapeutic use of chloramphenicol in humans has been reported to result in various adverse effects, with haematotoxicity being most frequent and severe. Reversible anaemia with or without leukopenia or thrombocytopenia, may be caused by an inhibitory effect of chloramphenicol on mitochondria. Aplastic anaemia caused by chloramphenicol is an idiosyncratic adverse reaction only observed in humans and for which no dose-response relationship has been established. While in case studies it has been clearly demonstrated that chloramphenicol exposure can cause aplastic anaemia, a

relationship could not be established in epidemiological studies. The CONTAM Panel noted that the design of such studies, in particular retrospective studies, appears not to be appropriate to detect such a relationship due to the low incidence of aplastic anaemia and the idiosyncratic nature of the disease. A positive association of chloramphenicol exposure with an increased risk of developing leukaemia was reported in one study but not observed in subsequent studies.

Despite the former widespread use of chloramphenicol as a veterinary drug, limited information is available concerning adverse effects in livestock, especially after oral treatment. Some effects were described in calves treated intramuscularly (i.m.) or intravenously (i.v.) with doses of 20–100 mg/kg b.w., including chromosome aberrations in lymphocytes from treated animals. In cats and dogs, prolonged treatment with high doses (more than 50 mg/kg b.w.) resulted in effects on the bone marrow/blood system.

The available animal and human data indicate that the derivation of a health-based guidance value for chloramphenicol is not appropriate. Three serious effects of chloramphenicol, i.e. aplastic anaemia in humans and reproductive and liver toxicity in animals, were envisaged as providing a basis for reference points for the risk characterisation. Clinical case studies addressing aplastic anaemia show that doses in a range from 4 to 410 mg chloramphenicol/kg b.w. per day administered over periods spanning from several days to months are associated with the development of aplastic anaemia. The lowest dose of 4 mg/kg b.w. chloramphenicol per day was selected as a reference point from the case studies on systemic exposure from which an exposure could be estimated. At a dose level of 25 mg/kg b.w. per day, reproductive and liver toxicity were observed in rats; this effect dose was selected as a reference point to assess the risk of possible reproductive/hepatotoxic effects of exposure to chloramphenicol. Owing to the lack of appropriate data, the CONTAM Panel cannot assess the risk of carcinogenicity.

In accordance with the exposure scenario in which specific food groups (foods of animal origin, foods in which enzyme preparations, reported to be contaminated with chloramphenicol, may be used during food production and grains and grain-based products in which chloramphenicol could occur naturally) are considered to be contaminated with chloramphenicol at the RPA value of 0.3 µg/kg, the median chronic dietary exposure across European countries and dietary surveys for the average consumer results in a margin of exposure (MOE) for aplastic anaemia of approximately 2.7×10^5 for toddlers and 1.3×10^6 for adults and an MOE for reproductive/hepatotoxic effects of approximately 1.7×10^6 for toddlers and 8.1×10^6 for adults. Considering these large MOEs, and the relatively low frequency of occurrence (1 in 20 000 to 40 000) of aplastic anaemia following systemic treatment of patients with chloramphenicol (4 to 410 mg/kg b.w.), it is unlikely that exposure to food contaminated with chloramphenicol at or below 0.3 µg/kg is a health concern with respect to the risk of developing aplastic anaemia, or reproductive/hepatotoxic effects.

In the case of enzyme-based food supplements, considered to be contaminated with chloramphenicol at the highest observed level of 1 800 µg/kg, MOEs of 3.3×10^5 for aplastic anaemia and 2.1×10^6 for reproductive/hepatotoxic effects were calculated. Exposure to such an enzyme-based food supplement is unlikely to represent a health concern with respect to aplastic anaemia or reproductive/hepatotoxic effects.

Potential dietary exposure of livestock to chloramphenicol from feed enzymes, straw or soil was estimated to be below 1 µg/kg b.w. per day. Some adverse effects were described in farm animals but for dosages in the mg/kg b.w. range. It is unlikely that exposures around 1 µg/kg b.w. per day would result in adverse effects.

The CONTAM Panel evaluated whether an RPA of 0.3 µg/kg for chloramphenicol in food of animal origin is adequate to protect public health and concluded that the current RPA is adequate to protect against potential adverse health effects of chloramphenicol with respect to aplastic anaemia or reproductive/hepatotoxic effects. The CONTAM Panel also concluded that it is appropriate to apply

the RPA for food of animal origin to food of non-animal origin and feed for the protection of animal and public health.

The CONTAM Panel recommends that information be generated on the stereoselectivity of the production routes used for chemical synthesis systems used to produce chloramphenicol and the extent to which the potential presence of different enantiomers in the chloramphenicol preparation used may have influenced the observed adverse effects. There is a need for information on the carcinogenicity of chloramphenicol and on the mechanisms underlying the genotoxic effects of chloramphenicol. Further studies are required on the presence of chloramphenicol in soil and on the possible uptake of chloramphenicol by cereals and vegetables, including the formation of plant metabolites. The potential formation of reactive intermediates of chloramphenicol, which could result in residues in foods of animal origin, should be studied. Additional data are needed on the occurrence of toxic metabolites and the formation of bound residues in edible tissues of food-producing animals.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Chloramphenicol is an antimicrobial that was originally derived from the bacterium *Streptomyces venezuelae*, a species of soil-dwelling Gram-positive bacterium of the genus *Streptomyces*. It was introduced into clinical practice in 1949. It was the first antibiotic to be manufactured synthetically on a large scale. It is cheap and easy to produce.

Both the European Medicines Agency[4] and the WHO/FAO Joint Expert Committee on Food Additives (JECFA)[5] have concluded that it was not possible to derive an acceptable daily intake (ADI) for chloramphenicol in the human diet. The International Agency for Research on Cancer (IARC) has classified chloramphenicol in Group 2A[6] (likely carcinogenic to humans).

In human medicine, it has long been a first-line agent for treatment of infections. In developed nations, resistance and safety concerns have largely reduced its use to topical treatment although it is still being used for life threatening conditions in humans when other antibiotics are less effective. In low-income countries, chloramphenicol is still widely used because it is inexpensive and readily available. Safety concerns related to chloramphenicol relate to bone marrow toxicity (bone marrow suppression and aplastic anaemia), leukaemia and grey baby syndrome.

In veterinary medicine in the European Union, chloramphenicol was included[7] in Regulation (EEC) No 2377/90[8] in Annex III "*List of pharmacologically active substances used in veterinary medicinal products for which provisional maximum residue limits have been fixed*" for use in "*all food producing animals*" with a provisional maximum residue limit (expiring in July 1994) of 10 µg/kg for the target tissues muscle, liver, kidney and fat. Its use in food producing animals in the European Union came to an end in 1994 by the reclassification of chloramphenicol to the list of prohibited substances[9]. Formulations containing chloramphenicol currently authorised within the European Union are restricted to use in non-food producing animals.

Findings of chloramphenicol

From 2001 onwards, a wide presence of chloramphenicol was detected in fishery products mostly originating in South-East Asia. Fishery products (shrimp, crayfish, crab…) were most affected with levels of chloramphenicol mostly below 10 µg/kg, but with exceptional levels up to almost 300 µg/kg. Less strict legislation related to veterinary medicinal products (VMPs) in South-East Asia (absence of provisions related to general prohibition on the off-label use of VMPs and on the use of non-approved VMPs) in combination with wide availability of pharmacologically active substances as chemicals has been reported as a possible cause of this episode. Other tainted food commodities were fish (levels below 5 µg/kg), honey, pollen and propolis (levels mostly below 10 µg/kg, exceptions up to 5000 µg/kg), milk powders (levels mostly below 1 µg/kg) and casings (levels mostly below 2 µg/kg).

[4] Chloramphenicol Summary Report − Committee for Veterinary Medicinal Products − available online at http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500012060.pdf

[5] 12th JECFA, 1968; 32nd JECFA 1987; 42nd JECFA, 1994.

[6] IARC vol. 50: 169, 1990.

[7] Commission Regulation (EEC) No 675/92 of 18 March 1992 amending Annexes I and III of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (OJ L 73, 19.3.1992, p. 8).

[8] Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (OJ No L 224, 18.8.1990, p.1), repealed by Regulation (EC) No 470/2009 of the European Parliament and of the Council laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council (OJ L 152, 16.6.2009, p. 11) and Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (OJ L 15, 20.1.2010, p. 1).

[9] Commission Regulation (EC) No 1430/94 amending annexes I, II, III and IV of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (OJ L 156, 23.6.1994, p. 6).

As risk management tool, a reference point for action (RPA) was set[10] specifying the actions to be undertaken when analytical tests carried on imported consignments of products of animal origin confirmed the presence of chloramphenicol at or above 0.3 µg/kg. All tested food containing residues at or above the RPA was considered non-compliant and removed from the food chain (destruction, re-dispatch, recall). The decision further contains provisions related to confirmed findings below the RPA indicating a recurrent pattern. Several safeguard measures[11] imposing obligatory testing on imports were adopted in view of consumer protection. When import checks demonstrated that all consignments were compliant as regards residues of chloramphenicol, these safeguard measures were lifted or no longer prolonged.

The Commission and the Member States agreed[12] to apply this approach including possible enforcement actions, with the necessary changes, to food of animals origin produced within the Union. As a consequence of this agreement, Member States perform follow-up investigations to determine the cause of the residues and to prevent repetition and impose enforcement measures (recall, movement restrictions...) in accordance with Directive 96/23/EC when confronted with cases of residues of chloramphenicol in food of animal origin of intra-Union origin. As chloramphenicol is a prohibited substance for inclusion in veterinary medicinal products for food producing animals, the expected outcome of such findings is an illegal use/abuse of a veterinary medicinal product destined for companion animals in food producing animals. However, on several occasions[13] these follow-up investigations were unable to disclose the origin of the residues.

In December 2012, follow-up investigations launched following simultaneous confirmed findings of chloramphenicol at or below 0.3 µg/kg in several pig farms in Sweden were unable to reveal illegal use or abuse. Further enquiries revealed that straw supplied to the animals contained confirmed levels of chloramphenicol[14]. The affected farms were blocked. The straw was removed and animals were fed animal feed containing no chloramphenicol. The farms remained blocked until monitoring of the animals (urine) was no longer able to demonstrate the presence of residues of chloramphenicol, at which time the restrictive measures were lifted. Findings of chloramphenicol in plant materials with levels ranging from 1 to 50 µg/kg (with exceptions up to 450 µg/kg) have been reported[15].

In summer 2013, investigations following findings of chloramphenicol in feed enzymes lead to enzyme producers in Asia. The investigations revealed levels of chloramphenicol mostly below 55 µg/kg (with exceptions up to 47.000 µg/kg) in enzymes destined for feed production. Levels up to 1900 µg/kg were detected in enzymes destined for food production. In this incident, an the same action level (0.3 µg/kg) applicable to products of animal origin was used as well to determine compliance in all stages of the feed (feed enzymes, premixes, compound feed) and food chain (food enzymes, food). The range of detected residues points towards the possible intentional addition during the fermentation process (to suppress development of unwanted bacteria) or to the final product (for stabilisation / protection reasons).

[10] Commission Decision 2005/34/EC laying down harmonised standards for the testing for certain residues in products of animal origin imported from third countries.

[11] e.g. Commission Decision 2008/630/EC on emergency measures applicable to crustaceous imported from Bangladesh and intended for human consumption (OJ L 205, 1.8.2008, p.49); Commission Decision 2002/994/EC concerning certain protective measures with regard to the products of animal origin imported from China (OJ L 348, 21.12.2002, p.154); Commission Decision 2010/381/EU on emergency measures applicable to consignments of aquaculture products imported from India and intended for human consumption (OJ L 174, 9.7.2010, p.51).

[12] SANCO -E.2(04)D/521927 available online at: http://ec.europa.eu/food/committees/regulatory/scfcah/controls_imports/summary35_en.pdf

[13] See "Questionnaires submitted by the Member States on the actions taken in case of non-compliant results" in the annual reports on the implementation of national residue monitoring plans in the Member States (Council Directive 96/23/EC) for the years 2008 to 2011 available at: http://ec.europa.eu/food/food/chemicalsafety/residues/monitoring_en.htm

[14] If possible reference to be provided by Sweden (Ingrid Nordlander).

[15] Berendsen et al. Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. Anal Bioanal Chem (2010) 397:1955-1963.

In the Scientific Opinion "*Guidance on methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin*"[16], the CONTAM Panel proposed several criteria where the European Commission might consider it appropriate to consult EFSA for a substance-specific risk assessment. One of proposed criteria was in case of residues of substances causing blood dyscrasias (such as aplastic anaemia).

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

As chloramphenicol is excluded from this opinion and taking into account its natural occurrence in environment as contaminant and its incidental use in fermentation processes or to protect food and feed from deterioration, the Commission requests EFSA in accordance with Article 29 of Regulation (EC) No 178/2002 for a scientific opinion on the risks to human and animal health related to the presence of chloramphenicol in food and feed.

In particular this opinion should comprise the:

a) evaluation of the toxicity of chloramphenicol for humans, considering all relevant toxicological endpoints and identification of the toxicological relevance of chloramphenicol present in food;

b) exposure of the EU population to chloramphenicol, including the consumption patterns of specific (vulnerable) groups of the population;

c) exposure levels of chloramphenicol for the different farm animal species (difference in sensitivity between animal species) above which

- signs of toxicity can be observed (animal health/impact on animal health) or
- the level of transfer/carry-over of chloramphenicol from the feed to the products of animal origin for human consumption results in unacceptable levels of chloramphenicol;

d) evaluation whether a reference point for action of 0.3 µg/kg for chloramphenicol in food of animal origin is adequate to protect public health;

e) assessment of the appropriateness to apply the reference point for action for food of animal origin to feed and food of non-animal origin for the protection of animal and public health

[16] EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2013). Guidance on methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin. EFSA Journal 2013;11(4):3195, 24 pp. doi:10.2903/j.efsa.2013.3195

ASSESSMENT

1. Introduction

Chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$ (2,2-dichloro-N-[1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl] acetamide), is a broad-spectrum antibiotic with bacteriostatic action. Chloramphenicol is effective against Gram-positive and Gram-negative bacteria. Chloramphenicol was discovered in 1949 and has, in the past, been widely used to treat infections in both humans and animals. In human medicine, chloramphenicol was initially used in the treatment of typhoid and subsequently in the treatment of bacterial meningitis, central nervous system (CNS) infections and as a topical treatment for bacterial conjunctivitis.

Originally, chloramphenicol was obtained from the bacterium *Streptomyces venezuelae*. It may be produced by chemical synthesis followed by a step to isolate stereoisomers; a fermentation process also has been described (IARC, 1990) that does not require separation of stereoisomers (NTP, 2011). In this opinion, only the stereoisomer with antibacterial activity, which is the one produced by bacteria (the RR-p-chloramphenicol isomer) is considered.

Bone marrow toxicity is the most serious adverse effect associated with chloramphenicol treatment, occurring either as bone marrow suppression or aplastic anaemia. Bone marrow suppression is a direct toxic effect of chloramphenicol and is usually reversible, whereas aplastic anaemia is idiosyncratic, being rare, unpredictable and unrelated to the dose, and is generally fatal. Owing to the safety and bacterial resistance concerns, chloramphenicol is no longer used as a primary antibacterial in human medicine in developed countries, with the exception of its use to treat bacterial meningitis and as a topical treatment for bacterial conjunctivitis. However, because chloramphenicol is a very effective antibacterial and may be easily and cheaply manufactured, it is still used widely in some developing countries in treatments for both humans and animals.

In veterinary medicine, chloramphenicol is not authorised for use in food-producing animals in the European Union (EU) following an evaluation by the Committee for Medicinal Products for Veterinary Use (CVMP) (CVMP, 1994). Chloramphenicol is still used for treatment of infections in non-food-producing animals.

The EFSA scientific opinion entitled "*Guidance on methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin*" (EFSA CONTAM Panel, 2013) identified an approach based on both analytical and toxicological considerations for establishing RPAs for various categories of non-allowed pharmacologically active substances. However, the opinion also identified certain categories of non-allowed pharmacologically active substances for which toxicological screening values based on the procedure described might not be sufficiently health protective and such substances are considered to be outside the scope of the procedure. Such substances include those causing blood dyscrasias (such as aplastic anaemia) or allergy or which are high-potency carcinogens. For such substances, including chloramphenicol, a specific risk assessment is required.

1.1. Previous assessments

Chloramphenicol has been the subject of several previous assessments by international, European and national organisations.

1.1.1. International and European agencies

Chloramphenicol was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 12th, 32nd, 42nd and 62nd meetings (FAO/WHO, 1969, 1988, 1995, 2004a). In its most recent evaluation, JECFA concluded from epidemiological data that treatment with chloramphenicol is associated with the induction of aplastic anaemia, which may be fatal. However, no dose–response

relationship or a threshold dose for the induction of aplastic anaemia was identified in humans. As is the case with other idiosyncratic immune system-mediated adverse reactions, no animal model could be developed for chloramphenicol. Based on the evidence that chloramphenicol is genotoxic *in vivo*, JECFA considered it prudent to assume that chloramphenicol could cause some effects, such as cancer, through a non-thresholded genotoxic mechanism. JECFA concluded that it was not appropriate to establish an acceptable daily intake (ADI) for chloramphenicol. JECFA also evaluated the possibility that foods are occasionally contaminated from environmental sources and concluded that this source of contamination cannot be ruled out (FAO/WHO, 2004a). Since no ADI was established, and because there was insufficient information on which to choose a suitable marker residue, JECFA was unable to assign maximum residue limits (MRLs) for chloramphenicol (FAO/WHO, 2004b).

The International Agency for Research on Cancer (IARC) evaluated chloramphenicol in 1975, 1987 and most recently in 1990. During the evaluation in 1990, IARC concluded that there is limited evidence for carcinogenicity of chloramphenicol in humans and inadequate evidence in experimental animals. The overall evaluation was that chloramphenicol is probably carcinogenic to humans (Group 2A) (IARC, 1990).

The CVMP of the European Agency for the Evaluation of Medicinal Products (EMEA; now the European Medicines Agency (EMA)) evaluated chloramphenicol in 1994 and concluded that no ADI could be established for chloramphenicol due to the inability to identify a threshold level for the induction of aplastic anaemia in humans, the genotoxicity in a number of *in vitro* and *in vivo* tests, the lack of an adequate carcinogenicity study, the lack of a no observed effect level (NOEL) for fetotoxicity and the lack of an adequate reproductive toxicity study. The CVMP concluded that no MRLs for chloramphenicol could be elaborated because no ADI could be established, no information about residues of toxicological concern was available and there was insufficient information to confirm a "marker" residue that would reflect total residues (CVMP, 1994).

1.1.2. National agencies

The National Institute for Public Health and Environment (RIVM; Rijksinstituut voor Volksgezondheid en Milieu) evaluated the risk of chloramphenicol occurrence in shrimps in 2001. Chloramphenicol was detected in shrimps at concentrations between 1 and 10 µg/kg. Based on a mean shrimp consumption of 8.4 g per week and a chloramphenicol concentration of 10 µg/kg, the exposure was estimated to be 0.17 ng/kg b.w. per day for a 70 kg b.w. person. From a two-year study in C57BL/6N mice receiving chloramphenicol via drinking water (Sanguineti et al., 1983), an additional lifetime cancer risk of 1 in 10^6 was estimated to be associated with an oral intake in the range of 1–5 µg/kg b.w. per day. It was concluded that the consumption of shrimps at the observed chloramphenicol concentrations is a negligible risk to public health (RIVM, 2001).

In 2004, l'Agence française de sécurité sanitaire des aliments (AFSSA; now ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail)) evaluated the risk of chloramphenicol occurrence in cheese. The source of contamination was the yeast used for cheese making. Based on a maximum occurrence value of 0.2 µg/kg and a mean and 95th percentile cheese consumption, a mean and a highest exposure was estimated to be 0.15 and 0.49 ng/kg b.w. per day, respectively, for children (2–14 years). AFSSA concluded that the highest exposure is 2 000 fold lower than the intake of 1 000 ng/kg b.w. per day, which was according to the RIVM (2001) associated with an additional cancer risk of 1:10^6. It underlined, however, that this was a theoretical approach (AFSSA, 2004).

In 2002, the German Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV), now Federal Institute for Risk Assessment (BfR), evaluated the risk of low chloramphenicol levels in food, such as muesli for consumers. The chloramphenicol levels in muesli, which were contaminated by honey were 0.6 and 12.6 µg/kg[17]. In its risk assessments, the BgVV, in principle,

[17] http://www.bfr.bund.de/cm/343/chloramphenicol_in_muesli.pdf

followed the CVMP evaluation in 1994. However, based on epidemiological studies which showed that the application of chloramphenicol as eye drops did not identify side effects in the form of aplastic anaemia, the BgVV reinforced the conclusion of Woodward (1991) that "*there are no data to implicate the presence of residues of chloramphenicol in foods consumed by humans as a cause of aplastic anaemia*". Moreover, the BgVV considered it unlikely, that microgram doses may reach target organs to trigger toxic effects. In summary, the BgVV concluded that chloramphenicol concentrations in food at the low µg/kg range constitute no quantifiable risk to the health of the consumer[18].

The Subcommittee on the Classification of Reproduction Toxic Compounds of the Health Council of the Netherlands evaluated the effects of chloramphenicol on reproduction, development and lactation. The committee noted that available human data on the developmental effects of chloramphenicol were insufficient to draw conclusions but, based on the prenatal and postnatal developmental effects in laboratory animals (increased embryo lethality and fetal lethality, delayed development, malformations, effects on neurobehaviour of offspring, effects on mitochondrial function and morphology), the committee concluded that it is "*a presumed human reproductive toxicant*". Owing to the lack of appropriate human and animal data, no conclusion was drawn for effects on fertility. In the absence of data on the toxicity of chloramphenicol in human milk, the committee was not able to calculate a safe level for chloramphenicol in human milk (Health Council of the Netherlands, 2012).

The Netherlands Food and Consumer Product Safety Authority (NVWA; Nederlandse Voedsel- en Warenautoriteit) evaluated the risks for human and animal health in relation to the occurrence of chloramphenicol in straw given to veal calves. Based on the highest concentration of chloramphenicol detected in straw (8.7 µg/kg) and a consumption of 100 g of straw per day, it was estimated that veal calves are exposed to a maximum of 1 µg per day. Pharmacokinetic studies have shown that chloramphenicol does not accumulate in edible tissue of calves and is excreted via the urine, primarily as metabolites. However, no chloramphenicol was detected in the urine samples tested. Based on the estimated low exposure of the veal calves and the absence of chloramphenicol in urine samples, the NVWA concluded that the presence of chloramphenicol in straw did not result in an increased risk to public or animal health. Since chloramphenicol is classified as probably carcinogenic to humans (Group 2A) by IARC, the exposure should be limited to 0.15 µg per day according to the Threshold of Toxicological Concern (TTC) approach. Therefore, the NVWA concluded that no increased risk to public health should be expected when consuming, per day, less than 500 g of meat containing chloramphenicol at a concentration of less than 0.3 µg/kg (NVWA, 2012).

1.2. Chemical characteristics

Chloramphenicol (2,2-dichloro-N-[1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide; Chemical Abstracts Service (CAS) No 56-75-7) is a white to greyish-white or yellowish-white fine crystalline powder or consists of fine crystals, needles or elongated plates with the molecular formula $C_{11}H_{12}Cl_2N_2O_5$ and a molecular weight of 323.13 g/mol (Figure 1). It has a bitter taste.

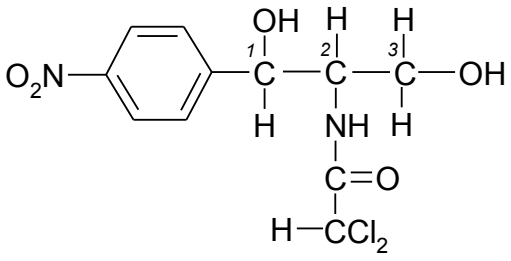

Figure 1: Chemical structure of p-chloramphenicol

[18] http://www.bfr.bund.de/cm/343/gesundheitliche_bewertung_von_chloramphenicol_cap_in_lebensmitteln.pdf

The main properties of chloramphenicol are summarised as follows (HSDB, 2011). The melting point is 150.5–151.5 °C. Chloramphenicol is very soluble in methanol, ethanol, butanol, ethyl acetate, acetone and chloroform, fairly soluble in ether and insoluble in benzene, petroleum ether and vegetable oils. Solubility in water is 2.5 g/L at 25 °C. Aqueous solutions are neutral, and neutral and acid solutions are stable on heating. The octanol/water partition coefficient (log K_{ow}) is 1.14 and the vapour pressure is 1.7×10^{-12} mmHg at 25 °C (EPI Suite, estimated[19]). Henry's law constant is estimated as 2.3×10^{-18} atm-m^3/mol at 25 °C (EPI Suite, estimated).

Four stereoisomers are possible, of which only the alphaR,betaR (1R,2R or D-threo) form is active (see Section 3.4). It seems likely that chemical synthesis of the drug would lead to a mixture of all four stereoisomers, in contrast to the production by bacteria. There is, however, no information to what extent commercial preparations contained the different isomers.

In clinical practice, chloramphenicol is most commonly used in three applications: either as a crystalline powder for oral administration, or palmitate ester as a suspension for oral administration, or as a succinate ester for parenteral administration. As both esters are inactive, they require hydrolysis to chloramphenicol for anti-bacterial activity. While the palmitate ester is hydrolysed in the small intestine prior to absorption, the succinate ester acts as a prodrug which is converted to chloramphenicol while it is circulating in the body (Ambrose, 1984).

1.3. Therapeutic use of chloramphenicol in humans

Despite its well-known side effects, chloramphenicol is a broad-spectrum antibiotic still used in the treatment of serious infections, such as meningitis and brain abscesses, typhoid fever and severe *Haemophilus influenzae* infections. The usual dosages and route of administration are the following: 25 mg/kg b.w. per day intravenously (i.v.) in four divided doses in neonates, 37.5–50 mg/kg b.w. per day i.v. in four divided doses in infants over seven days, and 250–500 mg orally every six hours. In all cases dosages must be adjusted to result in blood peak drug levels not higher than 25 µg/mL to avoid adverse effects (Smyth and Pallett, 1988). Eye drops (0.5 % active principle) or ocular ointments (1 % active principle) are available for the treatment of ocular infections. Capsulated crystalline chloramphenicol and chloramphenicol palmitate are available for oral administration, while chloramphenicol sodium succinate is the formulation of choice for parenteral use.

2. Legislation

According[20] to Article 3 of Regulation (EC) No 470/2009 of the European Parliament and of the Council[21] any pharmacologically active substance intended for use in the Union in veterinary medicinal products (VMPs) which are to be administered to food-producing animals shall be subject to an opinion of the EMA on the MRL, formulated by the CVMP. The opinion consists of a scientific risk assessment and risk management recommendations. Pharmacologically active substances, for which the opinion concludes that no MRL is needed or that a (provisional) MRL should be established, are subsequently classified in Table 1 "allowed substances" of Regulation (EU) 37/2010[22]. All use of other pharmacologically active substances in VMPs is not allowed. A specific group of the non-allowed substances is the group of "prohibited substances", listed in Table 2 of Regulation (EU) 37/2010. This group of "prohibited substances" includes, *inter alia*, chloramphenicol. For these prohibited substances no MRL could be recommended because available data are not sufficient to

[19] http://www.epa.gov/opptintr/exposure/pubs/episuite.htm

[20] In this scientific opinion, where reference is made to European legislation (regulations, directives, decisions), the reference should be understood as relating to the most current amendment, unless otherwise stated.

[21] Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council Text with EEA relevance. OJ L 152, 16.6.2009, p. 11–22.

[22] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1–72.

allow a safe limit to be identified or because a final conclusion concerning human health with regard to residues of a substance could not be established, given the lack of scientific information.

Article 18 of Regulation (EC) No 470/2009 stipulates that, for substances which are not classified as "allowed substances" in accordance with that Regulation, an RPA may be established in order to ensure the functioning of controls for food of animal origin. Food of animal origin containing residues of such substances at or above the RPA is considered not to comply with Union legislation. Until now, RPAs have only been based on analytically driven minimum required performance limits (MRPLs), and no consideration has been given to the toxicological profile of non-allowed substances. The MRPLs for chloramphenicol and a few other prohibited substances are specified in Annex II of Commission Decision 2002/657/EC[23]. For chloramphenicol, an MRPL value of 0.3 µg/kg is specified for meat, eggs, milk, urine, aquaculture products and honey. Under the terms of Commission Decision 2005/34/EC[24], these MRPLs are currently to be used as RPAs, irrespective of the matrix tested, for the purpose of control of residues when analytical tests are being carried out in the framework of import control. However, this decision regulated only imports from third countries and did not apply to food produced within the Union. As a number of products of animal origin originating from Member States were found to contain chloramphenicol and other prohibited substances below and above the MRPLs, the European Commission and the Member States agreed to apply the approach laid down in Decision 2005/34/EC, with the necessary changes, also to food of animal origin produced within the Union. This implies, in particular, that the MRPLs set according to Commission Decision 2002/657/EC shall also be used as RPAs. This approach, moreover, means that any detection of substances whose use is not authorised in the Union, regardless of the level found, shall be followed by an investigation into the source of the substance in question and appropriate enforcement measures applied, in particular aiming at the prevention of recurrence in the case of documented illegal use (SANCO-E.2(04)D/521927)[25].

Maximum limits for chloramphenicol in feed or food of non-animal origin, are not specified in the European Union.

It should be emphasised that chloramphenicol is still authorised according to national legislation in particular Member States for the treatment of animals not intended for food production and also as a human drug (e.g. eye ointment).

3. Methods of analysis

3.1. Sampling and storage

Most of the sampling of food, and of related materials, for chloramphenicol testing in foods of animal origin is undertaken in the context of the national residue monitoring plans as specified in Council Directive 96/23/EC[26], with residue testing undertaken in accordance with Commission Decision 2002/657/EC. For details of the protocols and procedures specified for such sampling and testing, see Section 4.2.1.

Commission Decision 2002/657/EC states that samples shall be obtained, handled and processed in such a way that there is a maximum chance of detecting the substance. Sample handling procedures shall prevent the possibility of accidental contamination or loss of analytes. In the case of chloramphenicol, the substance may be relatively rapidly metabolised *in vitro* in tissue samples, particularly liver, due to oxidation catalysed by the cytochrome P450 (CYP) system followed by phase

[23] Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. OJ L 221, 17.8.2002, p. 8–36.
[24] Commission Decision 2005/34/EC laying down harmonised standards for the testing for certain residues in products of animal origin imported from third countries. OJ L 16, 20.1.2005, p. 61–63.
[25] http://ec.europa.eu/food/fs/rc/scfcah/biological/rap16_en.pdf
[26] Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. OJ L 125, 23.5.1996, p. 10–32.

II glucuronic acid conjugation. To prevent a decrease in chloramphenicol levels, tissue samples may be frozen, immediately after sampling, and a CYP inhibitor, such as piperonyl butoxide, added during sample homogenisation prior to residue extraction (Parker and Shaw, 1988; Sanders et al., 1991; Cooper et al., 1998).

3.2. Determination of chloramphenicol

3.2.1. Extraction and sample clean-up

Extraction of chloramphenicol from sample matrices is most often carried out using solvent extraction, commonly with ethyl acetate but also using aqueous/solvent mixtures such as dilute salt solution and acetonitrile. Typically, the solvent extract is subjected to defatting by washing with hexane and further clean-up is achieved using solid phase extraction (SPE). A wide range of SPE materials are used, including reversed phase (such as C_{18} and polymeric sorbents), combination of reversed phase with normal phase (such as silica or magnesium silicate) and cation exchange. Depending on both the sample type and/or whether the method is a screening or confirmatory method, fewer or more sample extract purification steps may be required. For example, in the case of urine samples the defatting step may be omitted, while for some screening methods simple dilution of a honey sample may be sufficient.

Other approaches have been applied to extraction/clean-up of chloramphenicol from samples such as matrix solid phase dispersion (MSPD), supercritical fluid extraction (SFE), immunoaffinity chromatography (IAC) and molecularly imprinted polymers (MIPs). MSPD and SFE provide alternative systems to classical solvent extraction for chloramphenicol extraction and clean-up. MSPD involves intimate mixing of sample and a sorbent, such as C_{18} or a MIP, packing into a column and washing and elution of chloramphenicol from the MSPD column. SFE involves use of solvents under supercritical conditions to wash and elute chloramphenicol from the sample dispersed on an inert material with subsequent trapping of the analyte on a sorbent. IAC and MIPs involve use of chloramphenicol-specific antibodies immobilised on a support material, in the case of IAC, or a chloramphenicol-specific imprinted polymer, in the case of MIPs, packed into a column to trap the analyte from the sample extract and allow for washing steps prior to elution of the analyte from the column.

Examples of the application of these extraction and sample clean-up approaches are described in the following sections on screening and confirmatory methods.

3.2.2. Screening methods

Screening methods are designed to identify the possible presence of chloramphenicol in test samples using relatively simple, rapid and inexpensive techniques. The purpose of such screening methods is to remove from further investigation those samples that do not contain measurable quantities of chloramphenicol residues and concentrate confirmatory methods on the relatively small number of samples that the results of the screening method suggest may contain chloramphenicol residues. Apart from their relative simplicity, screening methods should measure chloramphenicol residues with sufficient sensitivity to satisfy the current regulatory requirements, at the MRPL of 0.3 µg/kg (Commission Decision 2002/657/EC), and should be designed to avoid false compliant results. A wide range of screening methods have been described for determination of chloramphenicol in liquid and solid samples (Samsonova et al., 2012; Zaidi, 2013). These screening methods may be grouped into the categories of microbial inhibition tests, immunoassays (including radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), immunofiltration card and dipstick assays, biosensors, chip-based assays), and assays based on direct measurement of chloramphenicol by electrochemistry, and chromatographic (gas chromatography (GC) and high-performance liquid chromatography (HPLC)) techniques. Certain methods, particularly microbial inhibition tests, some immunofiltration card and dipstick assays, methods based on capillary electrophoresis and some chromatographic techniques, do not have sufficient sensitivity and, therefore, are not considered in detail in this opinion.

Microbial inhibition tests, such as the EC four-plate test (Lynas et al., 1998; Tajik et al., 2010) and the one-plate method (Koenen-Dierick et al., 1995) for chloramphenicol in tissue samples and some commercially available tests for chloramphenicol in milk samples (Althaus et al., 2003), are not sufficiently sensitive to determine chloramphenicol residues in test samples at the MRPL; the sensitivity of these methods for chloramphenicol was reported to be 300 µg/kg, 30 000 g/kg and 12 000 µg/kg, respectively. The most sensitive microbial inhibition test method was reported by Shakila et al. (2007) with a sensitivity of 1 µg/kg for shrimp tissue, but this involved an extraction procedure using a 100-g sample.

Immunoassays have been very widely applied as screening methods for chloramphenicol. Initially, radioimmunoassays were applied in the 1980s to a range of sample types and had limits of detection (LODs) of 0.2–5 µg/kg (Arnold et al., 1984; Arnold and Somogyi, 1985; Boertz et al., 1985; Agthe and Scherk, 1986; Beck et al., 1987; Freebairn et al., 1988). Subsequently, ELISAs, both in-house and commercial kit methods, have been developed with LODs ranging from 0.1 to 10 µg/kg. Of these, some of the more sensitive ELISAs are commercial kits for which LODs of ≤ 0.3 µg/kg in seafood, porcine muscle and kidney, eggs, honey and milk are reported (Impens et al., 2003; Posyniak et al., 2003; Scortichini et al., 2005; Shen and Jiang, 2005). All of these methods require, as a minimum sample pre-treatment, solvent extraction and clean-up steps such as SPE and/or defatting with hexane to achieve LODs close to 0.1 µg/kg.

The sensitivity of the ELISA for chloramphenicol was significantly improved by using a biotin–streptavidin system (Wang et al., 2010) or fluoroimmunoassay (Li et al., 2006), providing LODs much lower than the MRPL for chloramphenicol of 0.3 µg/kg.

An immunofiltration/dipstick method for detection of chloramphenicol in milk was reported by Nouws et al. (1988), with an LOD of 0.1 µg/kg, but this required relatively extensive sample pre-treatment including deproteinisation, solvent extraction and SPE clean-up.

A number of biosensor-based assays have been developed for chloramphenicol using various systems for measuring the response due to analyte-antibody interaction. Such biosensors include (a) an amperometric system using a coated glassy carbon electrode and chloramphenicol labelled with hydrazine, which was applied to chloramphenicol determination in beef, chicken and pork with an LOD of 0.045 µg/kg (Kim et al., 2010); and (b) an impedimetric system using a modified gold electrode that is label-free (change in resistance due to antibody/antigen binding on the electrode surface is measured), which was applied to chloramphenicol determination in shrimp with an LOD of 0.0016 µg/kg (Chullasat et al., 2011).

The most commonly used biosensor technique validated and applied to analysis of chloramphenicol in food samples is surface plasmon resonance (SPR). A number of applications of SPR biosensor technology have been reported for chloramphenicol and chloramphenicol glucuronide in milk (Gaudin and Maris, 2001), and in honey, poultry and pork tissues and prawns (Ashwin et al., 2005; Ferguson et al., 2005); reported LOD and/or decision limit (CCα)[27] values were in the range of 0.005–0.1 µg/kg. Typical sample pre-treatment steps required for SPR analysis range from no pre-treatment for milk samples, to dilution with buffer for honey samples, to solvent extraction (with or without SPE clean-up) for tissue samples. Further rapid and enhanced sensitivity (LOD < 0.05 µg/kg) for chloramphenicol determination in honey samples was reported by Yuan et al. (2008, 2009) through use of large gold nanoparticles (40 nm) for signal enhancement and use of chloramphenicol-carbamate-PEG-NH_2 as the covalently bound chloramphenicol derivative, which allows for fast association/dissociation of the chloramphenicol antibody and does not require surface regeneration of the sensor chip.

[27] CCα is the decision limit at and above which it can be concluded with an error probability of α that a sample is non-compliant.

Microarrays allow for simultaneous detection of a number of substances, normally in miniaturised formats. Peng and Bang-Ce (2006) described a microarray on a glass slide for chloramphenicol, clenbuterol and tylosin using analyte-specific antibodies and a secondary antibody with a fluorescent dye. Chloramphenicol, together with the other analytes, was determined in milk, cheese, chicken and pork with an LOD of 0.03 µg/kg.

Thongchai et al. (2010) described a chemiluminescent technique, with pre-concentration of chloramphenicol from honey using a MIP, all contained in a microfluidic system, to determine chloramphenicol at a limit of quantification (LOQ) of 0.008 µg/L.

Gas chromatography with electron capture detection (GC–ECD) methods have been described for chloramphenicol in porcine tissues (muscle, liver, kidney) and urine with LOD/LOQ values of 0.2/0.3 µg/kg for muscle, 2.0/3.0 µg/kg for kidney and liver and 0.4/0.6 µg/kg for urine (Gude et al., 1995). More recently, GC–ECD methods for poultry muscle/liver tissues with an LOQ of 0.05/0.1 µg/kg (Zhang et al., 2006), for porcine, bovine, poultry, game and fish muscle with CCα/detection capability (CCβ)[28] values of 0.07/0.12 µg/kg (Cerkvenik-Flajs, 2006) and for goat's milk with LOD/LOQ values of 0.03/0.10 µg/kg (da Silva et al., 2010) have been published. All of these methods used solvent extraction (with salt/acetonitrile, basic ethyl acetate and acetonitrile, ethyl acetate or water), defatting with hexane, clean-up by IAC or SPE and formation of silyl derivatives of chloramphenicol prior to GC.

High-performance liquid chromatography (HPLC) with ultraviolet (UV) and diode-array detector (DAD) methods for chloramphenicol, with sensitivity lower than 0.3 µg/kg, include an IAC-based method for milk samples with a reported LOD of 0.02 µg/L (van de Water et al., 1989) and a method for fish samples based on solvent extraction coupled with IAC clean-up and detection at 278 nm with an LOQ of 0.25 µg/kg (Zhang et al., 2013). For animal feed, Viñas et al. (2006) described a method involving ethyl acetate extraction, SPE clean-up and determination of chloramphenicol by HPLC–DAD with an LOD of 0.7 µg/kg. A method for feed water, and for milk and honey samples, using a single-step two-phase extraction system and determination of chloramphenicol by HPLC–UV reported LOD/LOQ values of 0.3/1.0 µg/L (Han et al., 2011).

3.2.3. Confirmatory methods

Commission Decision 2002/657/EC specifies the criteria required for confirmatory methods, that is methods providing unequivocal identification and quantification of the analyte. The confirmatory method must provide information on the chemical structure of the analyte and, in the case of a non-allowed pharmacologically active substance, such as chloramphenicol, chromatographic techniques combined with mass spectrometry (MS) are suitable. For the chromatographic separation, the ratio of the chromatographic retention time of the analyte to that of the internal standard, i.e. the relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of ± 0.5 % for GC and ± 2.5 % for liquid chromatography (LC). The mass spectrometric detection may be carried out by recording full mass spectra, for example in an ion trap, or by selected ion monitoring (SIM) and selected reaction monitoring (SRM), for example in a triple quadrupole MS (Samanidou and Nisyriou, 2008). Specifications for the type of diagnostic ions that are acceptable, their relative intensities (for full scan spectra recorded in single MS, a minimum of four ions shall be present with a relative intensity of ≥ 10 % of the base peak) and correspondence with those of the calibration standard (maximum permitted tolerances of 20–50 %, depending on the relative intensity to the base peak) are laid down. The molecular ion shall be included if it is present in the reference spectrum with a relative intensity of ≥ 10 %. In the case of mass spectrometric techniques other than full-scan, the system of identification points (IPs) is used for interpretation of the results. For non-allowed pharmacologically active substances, such as chloramphenicol, a minimum of four IPs are required. This requirement may be satisfied by application of low-resolution MS (GC–MS, LC–MS), measuring four mass fragments, or by the very widely used techniques of liquid chromatography–tandem MS

[28] CCβ is the detection capability, meaning the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β.

(LC–MS/MS) and gas chromatography–tandem MS (GC–MS/MS), measuring one precursor ion and two transition products. If the analyses are performed with high-resolution MS, only two mass fragments are needed to earn four IPs. In any case, the relative ion intensities have to lie within the maximum permitted tolerances, which are the same as those described above for analyses in full scan mode.

A number of GC–MS methods, using negative chemical ionisation MS (NCI–MS), have been described for chloramphenicol analysis. Two methods are described for chloramphenicol determination in milk samples, both using acetonitrile extraction, and either defatting with hexane and silica gel SPE clean-up (LOD/LOQ of 0.025/0.037 µg/L (Fürst et al., 1988)) or dual SPE clean-up (CCα/CCβ values of 0.083/0.14 µg/kg (Sniegocki et al., 2007)). The former method was applied, also, to the determination of chloramphenicol in meat and egg samples. A method developed for poultry muscle and liver used ethyl acetate extraction, defatting by freezing and washing with hexane and SPE clean-up with an LOD of 0.1 µg/kg (Shen et al., 2009). Two methods are described for determination of chloramphenicol in urine, using ethyl acetate extraction, SPE clean-up and fractionation by HPLC, one applied to bovine urine, muscle and egg samples with an LOD of 0.1 µg/kg (van der Heeft et al., 1991) and the other applied to urine (CCα/CCβ values of 0.05/0.3 µg/kg) and shrimp (CCα/CCβ values of 0.05/0.1 µg/kg) (van Rossum et al., 2003). A method for shellfish samples used extraction with acetonitrile/4 % salt solution (1:1 v/v), defatting with hexane and dual SPE clean-up, reporting CCα/CCβ values of 0.07/0.09 µg/kg (Polzer et al., 2006). SFE, together with *in situ* derivatisation, was used to determine chloramphenicol in shrimp with an LOD of < 0.02 µg/kg (Liu et al., 2010). Sanchez-Brunete et al. (2005) described a method for honey using dissolution of the sample in water and SPE clean-up to obtain LOD/LOQ values of 0.05/0.2 µg/kg, with determination by electron impact ionisation (EI)–MS. For feed water, Rejtharová and Rejthar (2009) report a method using MIP with CCα/CCβ values of 0.005/0.007 µg/L.

LC–MS/MS has become the most widely used methodology for confirmatory analysis of chloramphenicol in a broad range of sample types.

An overview of LC–MS/MS methods for determination of chloramphenicol in liquid milk and milk powders shows a variety of approaches to extraction and clean-up. Simple extraction with acetonitrile or acetonitrile/salt solution may be used (Rønning et al., 2006; Cronly et al., 2010a; Wang et al., 2011; Freitas et al., 2013) with, in some cases, further clean-up by C_{18} SPE (Sniegocki et al., 2007), or extraction with a 10 % trichloroacetic acid solution and clean-up by SPE on a reversed phase polymeric sorbent (Guy et al., 2004). Other published methods use ethyl acetate extraction with clean-up either by liquid/liquid partitioning with hexane (Rodziewicz and Zawadzka, 2008) or by MSPD (Rezende et al., 2012). Centrifugation, to remove fat, has been used followed by extraction and clean-up using SPE on C_{18} and on neutral aluminium oxide sorbents (Sorensen et al., 2003) or using a MIP sorbent (Mohamed et al., 2007). Direct SPE on reversed phase polymeric sorbent has also been used (Chen et al., 2011). The CCα values reported for these methods range from 0.007 to 0.13 µg/kg and the CCβ values range from 0.01 to 0.21 µg/kg.

Methods for honey and associated products (such as propolis and bee pollen) typically involve extraction of honey diluted in water with ethyl acetate (Bononi and Tateo, 2008; Taka et al., 2012; Douny et al., 2013), acetonitrile or acetonitrile/salt solution (Rønning et al., 2006; Cronly et al., 2010a), or methanol/1 % metaphosphoric acid (Fujita et al., 2008). Some methods describe direct extraction and clean-up of chloramphenicol from honey with C_{18} SPE (Bogusz et al., 2004) or use of C_{18} SPE for further clean-up of an ethyl acetate extract (Ortelli et al., 2004) or of a dichloromethane/acetone extract (Forti et al., 2005) of the honey samples. Other methods involve use of supported liquid–liquid extraction (LLE) on diatomaceous earth cartridges (Kaufmann and Butcher, 2005; Vivekanandan et al., 2005), MIP (Shi et al., 2010) and IAC (Mackie et al., 2013) techniques. The CCα values reported for these methods range from 0.007 to 0.08 µg/kg and the CCβ values range from 0.013 to 0.12 µg/kg.

For animal tissues, fish and shellfish and egg samples, extraction with ethyl acetate (Bogusz et al., 2004), with basic (2 % ammonium hydroxide) ethyl acetate (Zhang et al., 2008) or with acetonitrile (Rønning et al., 2006) is used, together with liquid/liquid partitioning with hexane (Hammack et al., 2003; Vinci et al., 2005; Yibar et al., 2011; Douny et al., 2013) or with petroleum ether and isooctane (Tyagi et al., 2008) to remove fat. Further clean-up of the extract is carried out using SPE on silica (Mottier et al., 2003), on a reversed phase polymeric sorbent (Gikas et al., 2004), on C_{18} (Gantverg et al., 2003), on a cation exchange sorbent (Xia et al., 2013) or on graphene (Wu et al., 2012). Other methods developed for the determination of chloramphenicol in tissue samples include hexane/chloroform (1:1, v/v) washing to remove fat, followed by MIP extraction and clean-up (Rejthar et al., 2012), ethyl acetate extraction followed by MSPD (Rezende et al., 2012), and homogenisation in buffer followed by IAC (Mackie et al., 2013). A method for determination of chloramphenicol in kidney tissue involved extraction with sodium acetate solution followed by deconjugation of the chloramphenicol glucuronide metabolite with β-glucuronidase, prior to supported LLE on a diatomaceous earth column (Kaufmann and Butcher, 2005). Lu et al. (2012) described an on-line MSPD procedure for extraction and clean-up of soft-shelled turtle tissues. The CCα values reported for these methods range from 0.01 to 0.15 µg/kg and the CCβ values range from 0.02 to 0.26 µg/kg. Cronly et al. (2010b) describe an LC–MS/MS method for prohibited medicinal additives, including chloramphenicol, in pig and poultry compound feed, validated at a level of 100 µg/kg.

In addition, there are a number of published methods using a single quadrupole MS detector (Ramos et al., 2003; Takino et al., 2003; van de Riet et al., 2003; Penney et al., 2005; Mărghitaş et al., 2010; Ozcan and Aycan, 2013), covering the analysis of samples such as milk, honey, eggs, fish and shellfish and muscle, liver and kidney tissues, with reported LOD and/or LOQ values generally below the MRPL of 0.3 µg/kg. A number of methods based on use of an ion-trap detector have been applied for the determination of chloramphenicol in milk (Gallo et al., 2005), in feed water (Ardsoongnearn et al., 2014) and in animal feed (Moragues et al., 2012); the method for feed water, using SPE on a reversed phase polymeric sorbent, reports LOD/LOQ values of 0.01/0.025 µg/L while the method for feed, using dual extraction with ethyl acetate and C_{18} SPE clean-up, reports CCα/CCβ values of 6 and 8 µg/kg. Another method used high-resolution MS (HRMS) for determination of chloramphenicol in pork, poultry and fish products with an LOQ value of 0.1 µg/kg (Xu et al., 2011).

3.3. Analytical quality assurance: performance criteria, reference materials and proficiency testing

The performance criteria for methods used to test for chloramphenicol are those laid down in Commission Decision 2002/657/EC for screening and confirmatory methods to be used for Group A substances. Methods must have satisfactory performance for the characteristics of specificity, trueness, ruggedness, and stability of the analyte in standard solutions and in test matrices. The methods must be validated for recovery, repeatability, within-laboratory reproducibility, calibration curves, CCα and CCβ according to procedures specified in the Decision or equivalent procedures.

The Joint Research Centre–Institute for Reference Materials and Measurements (JRC–IRMM) has produced a number of reference materials for chloramphenicol in meat. The original certified reference material (CRM) was BCR-445, a porcine muscle sample produced in 1997 with a certified chloramphenicol content of 8.9 ± 0.9 µg/kg. The concentration of chloramphenicol in this CRM was relatively high and, therefore, largely unsuitable for method validation and method performance control for testing at the MRPL of 0.3 µg/kg. In 2010, the JRC–IRMM replaced BCR-445 with a new CRM, ERM-BB130 which is an incurred porcine muscle material with a certified chloramphenicol mass fraction of 0.230 ± 0.021 µg/kg. A study on the suitability of ERM-BB130 for use as a quality control tool for screening methods for chloramphenicol was undertaken by Zeleny et al. (2010). While differences among the assays were observed, in terms of bias, repeatability or goodness of fit, ERM-BB130 was found to be suitable as a quality control sample for the screening assays.

Several proficiency tests and interlaboratory studies have been reported for chloramphenicol in various food products. In 2001, the European Union Reference Laboratory (ANSES—EU RL) prepared three

samples of incurred porcine muscle and a blank sample for distribution to 14 laboratories for analysis by GC– or LC–MS methods. Three of the laboratories reported false positive results, none reported false negative results and the Z-scores for the incurred samples were satisfactory (≤ 2) for all but one participant; the assigned chloramphenicol contents in the incurred samples were 2.1, 4.9 and 6.5 µg/kg (Hurtaud-Pessel et al., 2002). In 2006, incurred samples of shrimp, crayfish and prawns were prepared containing chloramphenicol and distributed to 20 official control laboratories in Germany; the results obtained were very good, with reproducibility standard deviation for five samples ranging from 17 to 24 % and the median concentrations lying between 0.43 and 0.51 µg/kg chloramphenicol (Polzer et al., 2006). In the United Kingdom (UK), the Food Analysis Performance Assessment Scheme (FAPAS) provides samples of honey, milk, prawns and animal tissue (bovine kidney) containing chloramphenicol for testing[29].

3.4. Enantiomeric analyses

A method based on chiral liquid chromatography in combination with tandem mass spectrometric detection was developed to discriminate between the four (RR, SS, RS, SR) para-stereoisomers of chloramphenicol (Berendsen et al., 2011a, b), of which, in principle, only the RR-p-chloramphenicol isomer has antimicrobial properties (Maxwell and Nickel, 1954; Hahn et al., 1954). However, it remains to be determined whether other stereoisomers than the RR-p-isomer can be expected since, contrary to the production by bacteria, it is unclear whether the chemical synthesis results in the selective production of only the RR-p-isomer. The method has been applied to urine samples using clean-up by SPE and liquid–liquid extraction and separation of isomers on a chiral α1-acid glycoprotein (AGP) LC column with detection by negative electrospray ionisation (ESI) MS/MS; CCα/CCβ values were 0.005/0.13 µg/L (Berendsen et al., 2011a).

4. Occurrence of chloramphenicol

Linked to the previous use of chloramphenicol as a veterinary medicine, most controls on the presence of chloramphenicol are focused on food of animal origin. However, more recently chloramphenicol has also been detected in plant material and in food and feed enzymes. Because the current MRPL of 0.3 µg/kg was established in November 2001 (see Section 2), the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) considered occurrence data for samples that were collected since 2002. It should be noted that some of the studies described in Section 4.1, for studies reported in the scientific literature, may also be included in the databases described in Section 4.2, relating to samples taken in national residue monitoring plans.

4.1. Previously reported occurrence data

4.1.1. Occurrence in plants, feed and food of non-animal origin

Recent data show that chloramphenicol may be present in feed and food of non-animal origin, possibly due to natural occurrence. Hanekamp et al. (2003) reported the presence of chloramphenicol in a sample of Spanish white wine at a level of 2.7 µg/L. Berendsen et al. (2010) showed the presence of chloramphenicol in various herbs (*Thalictrum*, *Artemisia*, *Thermopsis* species) collected in 2006 from Mongolia, but also herbs bought in the Netherlands (Parusahaan Jamu herbs) or the USA (*Artemisia frigida*). In general, levels were in the range of 0.3–50 µg/kg, but three Mongolian samples contained substantially higher levels of 160, 175 and 450 µg/kg. Whether these herbs are also sold for direct consumption or whether such high levels can be expected in other herbs commercially sold is unclear. A second set of Mongolian samples collected in 2009 also showed the presence of chloramphenicol but at much lower levels (0.2–3.8 µg/kg). These samples included several grass samples, both leaves and roots as well as soil samples. Stolker et al. (2012) reported the presence of chloramphenicol in 12 of 21 samples of straw collected in the Netherlands with levels in a range of 0.1–11 µg/kg. According to Berendsen et al. (2013), another 37 of 104 straw samples, primarily from the Netherlands, tested positive, with 7 above 0.3 µg/kg, the highest level of which was 6.8 µg/kg.

[29] http://fapas.com/proficiency-testing-schemes/fapas/

Nordkvist (2013) examined 209 samples of Swedish straw and detected chloramphenicol in 117 (56 %) of the samples but only in 26 was the level higher than 2 µg/kg. The highest amounts, about 20 and 32 µg/kg, were found in straw from the south (Skåne) and the east (the Baltic island Gotland) of Sweden, respectively.

To further examine the origin of the chloramphenicol in herbs and plants, Berendsen et al. (2013) performed studies demonstrating its natural formation by *Streptomyces venezuelae* in the soil but also its degradation by other soil organisms, thus explaining the previously observed presence in soil but at low levels. When wheat and maize were cultivated under experimental conditions and provided once a week with water containing chloramphenicol (7.5 or 75 mg in 100 mL, added weekly for 10 consecutive weeks), the antibiotic was detected in wheat stems and maize stalks at levels ranging from several µg/kg to several hundreds of µg/kg. Levels in the spikes and cobs were 30 and 15 times lower, respectively. Overall, the amount of chloramphenicol that was detected in the plants varied between 0.001 and 0.19 % of the applied dose. This study offers an explanation for the contamination of plant materials with chloramphenicol.

4.1.2. Occurrence in food of animal origin

No comprehensive reviews on the occurrence of chloramphenicol in food of animal origin were identified in the scientific literature. The information presented below provides examples of the occurrence of chloramphenicol in food of animal origin. In general, the available information does not allow for identification of the origin of the chloramphenicol found.

4.1.2.1. Honey and royal jelly

Several studies on the occurrence of chloramphenicol in honey have been conducted. The Food Standards Agency (FSA, 2002) analysed 16 honey samples collected at retail level in the UK in January and February 2002 (reporting limit: 0.3 µg/kg). Chloramphenicol was reported in 10 samples with concentrations ranging from 0.9 to 7.2 µg/kg.

In Belgium, locally produced and imported honeys were analysed in 2002 by ELISA (LOD = 0.1 µg/kg) and confirmation was performed by LC–MS (LOQ = 0.1 µg/kg). Chloramphenicol was not detected in the locally produced honeys (n = 93) but, for the imported honey, 40 out of 85 samples contained chloramphenicol. Of these 40 positive samples, 31 were from China and the other nine samples were of unknown origin (Reybroeck, 2003).

Raw honey samples from Argentina (n = 25), Australia (n = 35), Cuba (n = 64), China (n = 32) and Thailand (n = 20) were analysed by LC–MS/MS (both CCα and CCβ were quoted as < 0.1 µg/kg; year of sample collection not indicated). None of the Australian honeys contained chloramphenicol, while 97 % of the Chinese samples contained chloramphenicol with an average chloramphenicol concentration of 4.8 µg/kg (range: 0.1–75 µg/kg). Intermediate results were observed for the samples from Argentina (8 % positive samples, mean concentration 0.1 µg/kg), Cuba (6.3 % positive samples, mean concentration 0.3 µg/kg) and Thailand (15 % positive samples, mean concentration 1.4 µg/kg) (Verzegnassi et al., 2003).

Honey samples collected in Switzerland, including honey originating from Asian countries, were analysed by LC–MS/MS (LOD/LOQ = 0.2/0.5 µg/kg; year of sample collection not reported). Chloramphenicol was detected in 13 of 75 samples (17 %; maximum concentration: 6.0 µg/kg) (Ortelli et al., 2004).

In India, 17 honey samples originating from different geographical regions were analysed using an LC–MS/MS method (LOD = 0.05 µg/kg; year of sample collection not reported). Three samples contained more than 2 µg/kg and six samples contained between 0.3 and 1.7 µg/kg (Vivekanandan et al., 2005).

Between 2003 and 2004, 35 royal jelly samples imported into Italy were analysed by LC–MS/MS (LOD/LOQ = 0.15/0.3 µg/kg). Chloramphenicol was quantified in 83 % of the samples with a mean concentration of 6.1 µg/kg and the highest chloramphenicol concentration detected was 28 µg/kg (Calvarese et al., 2006).

Sheridan et al. (2008) analysed 126 honey samples, collected between 2005 and 2007, originating from 25 countries, by LC–MS/MS (LOD/LOQ = 0.2/0.6 µg/kg). Chloramphenicol was detected in 9 % of the samples and the highest concentration detected was 91 µg/kg. The samples containing chloramphenicol originated from China, Russia, Georgia and Moldova.

In Italy, Baggio et al. (2009) reported on the analysis of chloramphenicol in honey samples (n = 505) collected between 2003 and 2007. A first screening was done using ELISA (CCβ = 0.1 µg/kg) and positive results were confirmed by LC–MS/MS (CCα/CCβ = 0.11/0.12 µg/kg). Chloramphenicol was detected in eight samples and the highest concentration was 20 µg/kg.

Bonvehi and Gutiérrez (2009) reported on 567 Basque honey samples (year of collection not reported) analysed by a commercial radioimmunoassay kit method (reported CCα = 0.3 µg/kg) and no positive samples were detected.

Chloramphenicol was detected in three of 12 honey samples analysed with HPLC–DAD (LOD/LOQ = 0.87/2.92 µg/kg). The samples were collected in July 2009. The positive samples originated from India (4.4 µg/kg), Australia (3.6 µg/kg) and Switzerland (3.7 µg/kg) (Johnson and Jadon, 2010).

In Romania, 12 honey samples (year of collection not reported) collected from beekeepers in Romania were analysed by LC–MS (LOD/LOQ = 0.13/0.27 µg/kg) and chloramphenicol was detected in one sample (1.4 µg/kg) (Mărghitaş et al., 2010). In addition, Simion et al. (2011) analysed 82 honey samples, collected from beekeepers, using ELISA (LOD/LOQ/CCα/CCβ not reported). The samples were collected between 2007 and 2010. Chloramphenicol was detected in three samples, but the concentrations were below the RPA (range: 0.06–0.212 µg/kg).

4.1.2.2. Milk

In 2002, 27 samples of raw milk were collected in Slovenia and analysed by a GC–ECD method (CCα/CCβ = 0.18/0.21 µg/kg) and/or an immunoassay (CCα = 0.2–0.25 µg/kg). Confirmation was via LC–MS (LOD/LOQ = 0.1/0.2 µg/kg). Chloramphenicol was detected in two samples (reported concentrations: 0.5 µg/kg and < 0.2 µg/kg) (Dolajš et al., 2007).

Bilandžić et al. (2011b) reported the results for raw milk samples collected in the framework of the National Residue Monitoring plan in the Republic of Croatia between 2008 and 2010. Analysis was carried out using ELISA (LOD/LOQ/CCβ = < 0.01/< 0.01/0.23 µg/kg). In 2008, 299 samples were analysed for chloramphenicol and a mean concentration of 0.012 µg/L (maximum concentration: 0.118 µg/L) was reported. For 2009 and 2010, 356 and 146 samples were analysed with mean concentrations of 0.006 and 0.005 µg/L (maximum concentrations: 0.092 and 0.026 µg/L), respectively. In addition, in 2011, the same authors collected 119 samples of raw milk in Croatia and reported a mean concentration of 0.005 µg/L (maximum concentration: 0.05 µg/L) (Bilandžić et al., 2011a). The CONTAM Panel consider that these data should not be used as a reliable measure for the occurrence of chloramphenicol in milk since all values reported were below the CCβ of 0.23 µg/kg.

In Former Yugoslav Republic of Macedonia (FYROM), 497 raw milk samples collected between 2008 and 2011 were analysed by ELISA (LOD/LOQ/CCβ = < 0.01/0.014/0.18 µg/kg). The authors reported a mean concentration of 0.019 µg/kg (maximum concentration: 0.074 µg/kg) (Dimitrieska-Stojkovic et al., 2011). The CONTAM Panel consider that these data should not be used as a reliable measure for the occurrence of chloramphenicol in milk since all values reported were below the CCβ of 0.18 µg/kg.

Azzouz et al. (2011) analysed cow's milk (n = 13; raw, whole, semi-skimmed and skimmed), goat's milk (n = 2; whole and semi-skimmed) and powdered milk (n = 2) collected in Spain and Morocco (year of sample collection not reported). GC–MS (LOD = 0.0002 µg/kg) was used for the analysis but no chloramphenicol was detected in any of the samples.

4.1.2.3. Fish and other seafood

Chloramphenicol was measured in 19 fish and shrimp samples collected in China. Chloramphenicol was detected by GC–ECD (LOD/LOQ = 0.04/0.1 µg/kg, year of sample collection not reported) in nine samples with the highest concentration being 242 µg/kg (Ding et al., 2005).

Shen et al. (2006) analysed chloramphenicol in 20 shrimp samples collected from local food markets in China (year of sample collection not reported). Time-resolved fluoroimmunoassay (TR-FIA; LOD/LOQ = 0.05/0.1 µg/kg) and GC–ECD (LOD/LOQ not reported) were used for the analysis. Chloramphenicol was detected in six samples using both methods in the range 0.2–13.8 µg/kg (analysis with TR-FIA) and 0.1–11.3 µg/kg (analysis with GC–ECD). In addition, Li et al. (2006) used a method based on TR-FIA (LOD/LOQ = 0.04/0.15 µg/kg) to analyse aquaculture tissue samples (n = 35, year of sample collection not reported) from local food markets in China and detected chloramphenicol in four samples (concentrations not specified).

The Department of Health, Government of South Australia, analysed 17 samples of imported crab meat using LC–MS/MS (LOD = 0.1 µg/kg, sampling period not reported). Chloramphenicol was detected in six samples at concentrations between 0.1 and 0.3 µg/kg. A mean middle bound (MB) concentration of 0.094 µg/kg was reported (Eckert, 2006).

In the framework of a Canadian total diet study, 12 composite samples of marine, freshwater and canned fish and shrimps were collected between 2002 and 2004. Chloramphenicol was analysed using LC–MS (LOD = 0.1 µg/kg) and was not detected in any of the samples (Tittlemier et al., 2007).

Seven fish samples were collected in 2010 in Croatia and analysed for their chloramphenicol content with ELISA (CCβ = 0.28 µg/kg) and confirmed by LC–MS/MS (CCα/CCβ = 0.17/0.19 µg/kg). The mean chloramphenicol concentration was 0.011 µg/kg (maximum concentration: 0.019 µg/kg) (Bilandžić et al., 2011c). The CONTAM Panel consider that these data should not be used as a reliable measure for the occurrence of chloramphenicol in fish since all values reported were below the CCβ of 0.28 µg/kg.

Samples of fish and other seafood (n = 21) collected by the Brazilian Federal Inspection Services were analysed using LC–MS/MS (CCα/CCβ = 0.04/0.06 µg/kg, year of sample collection not reported), but no detectable amounts of chloramphenicol were found in any of the samples (Barreto et al., 2012).

4.1.2.4. Meat and meat products

Samples of poultry (n = 33), bovine (n = 109) and pig (n = 46) meat and of meat products (n = 21) were collected in 2010 in Croatia and analysed for their chloramphenicol content with ELISA (CCβ = 0.28 µg/kg) and confirmed by LC–MS/MS (CCα/CCβ = 0.17/0.19 µg/kg). Mean concentrations of 0.016, 0.011, 0.016 and 0.004 µg/kg were reported for bovine, pig, poultry meats and for meat products, respectively. The highest concentration of 0.2 µg/kg was detected in a sample of bovine meat (Bilandžić et al., 2011c). The CONTAM Panel consider that these data should not be used as a reliable measure for the occurrence of chloramphenicol in meat and meat products since all values reported were below the CCβ of 0.28 µg/kg.

4.2. Current occurrence results

4.2.1. Data sources

Data on occurrence of chloramphenicol in food and feed are not currently collected by EFSA. The only data on chloramphenicol present in the EFSA Chemical Occurrence database had been

voluntarily submitted during the year 2012 by the Czech Republic, and contained 460 entries on animals and animal products. All of these data were left censored (values below the LOD or LOQ). The data provider confirmed that these same data were also submitted to the EC's database on residues of veterinary medicines, relating to the National Residue Monitoring Plan (see below). For this reason, they were not further analysed for the purposes of this opinion.

4.2.1.1. National Residue Monitoring Plans

Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products requires that Member States should draft a national residue monitoring plan for the groups of substances detailed in Annex I. These plans must comply with the sampling rules in Annex IV of the Directive. Chloramphenicol is in the Group A6 of prohibited substances, as listed in Table 2 of Commission Regulation (EU) No 37/2010[30], for which MRLs cannot be established. These substances are not allowed to be administered to food-producing animals.

The minimum number of each species of animal to be controlled each year for all kinds of residues and substances is specified as a proportion of the animals of each species slaughtered in the previous year. In the case of Group A substances, substances having anabolic effect and unauthorised substances, a proportion of the total samples taken are to be from live animals or related materials (feed, drinking water, urine, faeces, etc.) on farms and the remainder of the samples are to be taken at the slaughterhouse. Each subgroup of Group A, such as Group A6, which includes chloramphenicol, must be checked each year using a minimum of 5 % of the total number of samples to be collected for Group A. Sampling under the national residue monitoring plan should be targeted; samples should be taken on-farm and at slaughterhouse level with the aim of detecting illegal treatment.

Member States submit data on the occurrence of non-compliant results determined in the residue monitoring, including for chloramphenicol, to the European Commission's database on residues of veterinary medicines[31]. Data on occurrence of chloramphenicol in food have been extracted from the EC's database on residues of veterinary medicines. This database contains the annual sampling plan and the results from 2004 onwards[32] provided by all Member States. The results are reported as aggregate data with the following level of detail:

- animal category and animal products: bovines, pigs, sheep and goats, horses, poultry, aquaculture, milk, eggs, rabbit, farmed game, wild game and honey;
- production volume;
- sampling strategy: targeted, suspect, import and others;
- number of samples analysed for each substance group as defined in Annex I of Council Directive 96/23/EC and for each animal category or animal product;
- number of non-compliant results within each substance group or subgroup and within each animal category or animal product;
- place of sampling: farm or slaughterhouse.

However, there is no indication of the sample matrix tested (muscle, blood, urine, kidney, fat, etc.) and no concentration for the chemical residue or contaminant detected in the sample is provided. In addition, the number of samples analysed for the individual substances are reported by the Member States only if there is at least one non-compliant sample for the substance in question. Where all samples are compliant, the number of samples analysed is not reported. Furthermore, where controls

[30] Formerly Annex IV of Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ L 224,18.8.1990 p. 1–8.
[31] https://webgate.ec.europa.eu/residues/index.cfm
[32] The results for the year 2013 currently present in the EC's database are provisional and will be complete and available at the end of 2014.

are carried out at farm and slaughterhouse, the total number of samples recorded may refer either to samples taken at farm or at slaughterhouse depending on where the non-compliant samples were found, and this may be on a substance group basis rather than on the individual substance basis. Where non-compliant samples were found at both farm and slaughterhouse, the number of samples represents the sum of samples taken at both sampling points.

Data on chloramphenicol reported by Member States during 2002 and 2003 have been extracted from the Commission staff working papers on the implementation of national residue monitoring plans in the Member States in 2002 and 2003. Unfortunately, data presented in these papers are not consistent with the reports for the following years. The number of samples analysed for each food category represents, in most cases, the total of samples for all prohibited substances. Only for the food categories of bovine, pigs, poultry and sheep and goats does the number of samples represent those analysed only for the Group A6 substances, which includes chloramphenicol.

4.2.1.2. Rapid Alert System for Food and Feed

In addition, the CONTAM Panel considered the Rapid Alert System for Food and Feed (RASFF) database for information on occurrence of chloramphenicol in food and feed. Searches in the RASFF database were performed for the hazard category "veterinary residues — chloramphenicol" that had been notified between 1 January 2002 and 31 December 2013.

Notifications are provided by Member States when non-compliant samples for a contaminant are found, and quantified values are also provided. However, information on the total number of samples analysed, the number of compliant samples and the concentrations and the type of analysis undertaken, was rarely provided.

4.2.2. Distribution of samples across food categories and feed

4.2.2.1. National Residue Monitoring Plans

In the period 2002–2012, 768 734 targeted samples were analysed for Group A6 prohibited substances, including chloramphenicol, by the European Member States. The number of targeted samples analysed for Group A6 prohibited substances through the years were 70 412 for 2002, 90 887 for 2003, 65 999 for 2004, 61 119 for 2005, 68 975 for 2006, 68 450 for 2007, 57 671 for 2008, 66 971 for 2009, 70 828 for 2010, 73 258 for 2011 and 74 164 for 2012. For chloramphenicol, the results in the residue database are:

- There were 306 targeted samples reported to be non-compliant for chloramphenicol distributed across the years, as shown in Table 1.
- The animal species in which chloramphenicol was mostly reported were pigs, poultry and bovines with 91, 74 and 68 non-compliant cases, respectively. Other categories for which non-compliant samples were reported include aquaculture, sheep/goats, rabbit, farmed game, honey and milk (Table 1).

Table 1: Distribution of non-compliant samples (targeted sampling) for chloramphenicol across years reported in the European Commission's database on residues of veterinary medicines (total number of samples analysed for chloramphenicol not reported)

Category	Year											Total
	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	
Bovines	9	6	12	8	9	6	9	2	2	3	2	68
Poultry	2	4	18	11	11	7	5	9	3	3	1	74
Aquaculture	1	1	2	3	1	3	1			1		13
Sheep/goats		1	2	5	3	1	1	1			1	15
Rabbit				3					2	2	1	8
Pigs	13	6	7	4	13	15	6	10	6	1	10	91
Farmed game	1						1					2
Honey		1			1	1				1		4
Milk		2	5	2	4	9	1	3	3	1	1	31

4.2.2.2. Rapid Alert System for Food and Feed

The findings in the RASFF database for chloramphenicol are shown below:

- There were 440 notification events[33] reported and distributed across the years as seen in Table 2.
- There were 402 notification events reported for food and 38 for feed products (Table 2).
- The notifications for food covered the following product categories: cephalopods and products thereof, confectionery, crustaceans and products thereof, dietetic foods, enzyme-based food supplements, fortified foods, farmed crustaceans and products thereof[34], farmed fish and products thereof (other than crustaceans and molluscs)[34], fish and fish products, food additives and flavourings, honey and royal jelly, meat and meat products (other than poultry), milk and milk products, other food products/mixed, poultry meat and poultry meat products, prepared dishes and snacks, wild-caught crustaceans and products thereof[34], wild-caught fish and products thereof (other than crustaceans and molluscs)[34].
- The notifications for feed affected the following product categories: animal nutrition[34] compound feeds, feed additives, feed for food-producing animals[34], feed materials, feed premixtures and milk and milk products.
- There were 24 notification events reported for enzyme concentrates, enzyme preparations or target food containing enzyme preparations; 19 for food and five for feed, all of them during the year 2013.
  - One notification event (reference number 2013.1222) is reported as a food additive and flavouring and concerns a bread production intermediate based on an enzyme preparation containing cellulase. The concentration of chloramphenicol (1 900 µg/kg) was reported as analysed in the enzyme preparation and calculated as 0.38 µg/kg in the bread production intermediate. Based on the quantity of the intermediate product added to baked goods, it was foreseen that the target foods would contain traces of chloramphenicol significantly below the LOD of the officially recognised analytical methods.
  - Three of the 24 notification events concern enzyme-based food supplements containing enzyme concentrates/enzyme preparations. One of the events (reference number 2013.1544), concerns a supplement in which the origin of the chloramphenicol was the

[33] The total number of notification events is not the sum of the total number of notifications, because one notification event may include more than one notification. Notification events include alerts, border rejections, information, information for attention, information for follow-up and news.
[34] This product category is no longer used in the RASSF database.

cellulose enzyme concentrate. The concentration of chloramphenicol is reported as 7 600 µg/kg in the enzyme concentrate and as 1 800 µg/kg in the final product. Another event (reference number 2013.1503) concerns a supplement containing a mixture of different enzyme concentrates i.e. pectinase, glucoamylase, protease, β-glucanase, lipase, galactosidase, xylanase, cellulase, amylase and invertase. The concentration of chloramphenicol was only reported in the final product and was 18 µg/kg. The last event (reference number 2013.1685) is reported as "other food products/mixed" in the notification event, but it refers to an enzyme-based food supplement. The origin of chloramphenicol was the enzyme preparation that was used for the manufacturing of the supplement. The type of enzymes present in the supplement were: protease, amylase, amyloglucosidase, lipase, cellulase, lactase and pectinase. The concentration of chloramphenicol was reported as 9.4 µg/kg in the enzyme preparation.

- Two notification events relate to enzyme preparations containing pectinase. In the first (reference number 2013.1207), the pectinase enzyme concentrate had chloramphenicol concentration values in the range 2 100–31 400 µg/kg. Based on these figures, the content of chloramphenicol in the enzyme preparation was calculated to be between 500 and 5 000 µg/kg. Taking into consideration the highest concentration value of pectinase in the enzyme concentrate and its content in the enzyme preparation, it was calculated that the concentration of chloramphenicol would be below 0.3 µg/kg in the target foods, namely wine and juices. In the second event (reference number 2013.1284) the concentration of chloramphenicol was reported as 92 µg/kg in the enzyme preparation.
- One notification event (reference number 2013.1272) relates to a pectinesterase enzyme preparation used in juices. The concentration of chloramphenicol in the two samples of the enzyme concentrate analysed was 519 and 180 µg/kg. For the latter sample, the concentration of chloramphenicol in the enzyme preparation was calculated to be 10.8 µg/kg.
- Three notification events relate to enzyme preparations containing amylase. The target foods for this type of enzyme are bread and fine bakery ware products. In the first event (reference number 2013.1163), the range of chloramphenicol concentrations was 23–150 µg/kg in an enzyme preparation used as a pre-baking mix. No chloramphenicol was detected from analysis of the target foods. In the second event (reference number 2013.1195), the chloramphenicol concentrations in the two samples analysed were 8.7 and 45 µg/kg in an enzyme preparation used in bakery products. Calculations based on the recommended inclusion levels of the enzyme preparation in the target food indicated that chloramphenicol would be significantly below the LOD in the target foods. In the third event (reference number 2013.1364), the concentration of chloramphenicol in an amylase enzyme concentrate was reported as 4.2 µg/kg and 0.69 µg/kg in the baking premixture. The calculated concentration of chloramphenicol in the target food was below the LOD.
- One notification event (reference number 2013.1620) relates to a lactase enzyme preparation. The chloramphenicol concentration in the lactase concentrate for the two samples analysed was 47 and 160 µg/kg. The target foods for this type of enzyme are lactose-free milk and dairy products.
- One notification event (reference number 2013.1212) relates to an enzyme preparation containing glucanase. The chloramphenicol concentration was reported as 409 µg/kg in the glucanase enzyme concentrate and 230 µg/kg in the enzyme preparation. The target food of the enzyme preparation are fruit juices, wines, bread and fine bakery wares.
- Two notification events relate to the papain enzyme concentrate. In the first (reference number 2013.1418), the chloramphenicol concentration in refined papain was reported as 21 µg/kg. In the second (reference number 2013.1425), the chloramphenicol concentration in crude papain was reported as 0.5 µg/kg. The target foods for which these enzyme concentrates are used are beef, bakery ware and beer.

- Four notification events relate to enzyme preparations containing xylanase. In the first event (reference number 2013.1150), the chloramphenicol concentration range was 55–860 µg/kg in the xylanase enzyme concentrate and, when applying a dilution factor of 0.004, this results in a concentration of 0.98 µg/kg in the enzyme preparation used as a processing aid in bread and biscuits. According to the recommended use levels of the enzyme preparation (5 to 50 mg/kg of bread), the calculated concentration of chloramphenicol in the target foods ranged from 0.00017 to 0.01582 µg/kg. In the second event (reference number 2013.1154), the concentration of chloramphenicol was reported as 4 500 µg/kg in the xylanase enzyme concentrate, amounting to a concentration range of 90–430 µg/kg in the enzyme preparation used in the bakery industry. In the third event (reference number 2013.1312), the concentration of chloramphenicol was 7.48 µg/kg in the enzyme preparation. In the last event (reference number 2013.1537) the chloramphenicol concentration was 7 µg/kg in xylanase enzyme concentrate.
- One notification event (reference number 2013.1432) relates to a bread product intermediate based on enzyme preparations without specifying their identity. The concentration of chloramphenicol was reported as 0.73 µg/kg in the bread product intermediate.
- There are five notification events for feed relating to enzyme preparations. The first event (reference number 2013.1017) concerns an enzyme preparation containing a mixture of xylanase, hemicellulase and protease. The chloramphenicol concentration range for the xylanase enzyme concentrate was 1.35–672.07 µg/kg and for the hemicellulase was 2.97–319.97 µg/kg. Protease enzyme concentrate gave two negative results and one value below 0.3 µg/kg (0.16 µg/kg). The chloramphenicol concentration range in the enzyme preparation was 0.13–9.07 µg/kg. The second event (reference number 2013.1148) is related to the first one, but was recorded as a separate notification because of the different origin of the raw material. It concerns an enzyme preparation containing a mixture of cellulase, lipase and xylanase. The concentration of chloramphenicol was 735 and > 1 000 µg/kg in the two samples of cellulase enzyme concentrate analysed and 0.37 µg/kg in the xylanase enzyme concentrate. Chloramphenicol was not detected in the lipase enzyme concentrate. The third event (reference number 2013.1292) relates to a xylanase enzyme concentrate and enzyme preparation. The chloramphenicol concentration range in the xylanase enzyme concentrate was 27–47 000 µg/kg. A concentration of 6 µg/kg was reported for one sample of the xylanase enzyme preparation. The fourth event (reference number 2013.1134) relates to an enzyme preparation containing β-glucanase and xylanase. The concentration of chloramphenicol was 59 µg/kg in the enzyme preparation. The last event (reference number 2013.1077) relates to a xylanase enzyme concentrate. The concentration of chloramphenicol in the two samples analysed was 8 and 380 µg/kg and the calculated concentration of chloramphenicol in the compound feed was below the LOD.

Table 2: Distribution of the Rapid Alert System for Food and Feed notifications for chloramphenicol for 2002–2013

Category	Year											
	2002	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Cephalopods and products thereof										1		
Confectionery		2										
Crustaceans and products thereof	92	6	11	1	2	5	3	2	2	7	1	5
Dietetic foods, food supplements, fortified foods						2	1					3[(a)]
Farmed crustaceans and products thereof	3	1										
Farmed fish and products thereof (other than crustaceans and molluscs)	1	1										
Fish and fish products	10	1	1	1	3			1	2	2		
Food additives and flavourings												1[(b)]
Honey and royal jelly	34	17	7	25	7	1	3					
Meat and meat products (other than poultry)	9	12	1		2	3	2	3	1	3	9	
Milk and milk products	19	10	6	1	1	1						
Other food products/mixed	1											16[(c)]
Poultry meat and poultry meat products	4	2	4		1	1					1	
Prepared dishes and snacks			1									
Wild-caught crustaceans and products thereof	7	12										
Wild-caught fish and products thereof (other than crustaceans and molluscs)		1										
Feed	21	3	3				3			2	1	5[(d)]
Total	**201**	**68**	**34**	**28**	**16**	**13**	**12**	**6**	**5**	**15**	**12**	**30**

(a): Two notification events relating to the category "dietetic foods, food supplements, fortified foods" refer to an enzyme used as a raw material for the production of the products notified. Information on the concentration of chloramphenicol is only available for one of them.
(b): The notification event concerns an enzyme preparation.
(c): All notification events relating to the food category "other food products/mixed" refer to enzyme preparations. For one of them, the target food is an enzyme-based food supplement.
(d): All notification events for feed relate to enzyme preparations.

4.3. Food and feed processing

4.3.1.1. Food processing

The effect of cooking and cold storage on chloramphenicol residues in bovine meat was studied by O'Brien et al. (1981). A microbiological assay was used for the analysis (LOD not reported). Steaks were grilled for 10, 20 or 30 minutes. A maximum temperature between 30 and 56 °C was attained in steaks that were grilled for 10 minutes and a reduction of the annular zone diameter of between 0 and 7.1 % was observed. For grilling times of 20 and 30 minutes, maximum temperatures of 58–75 °C and 77–82 °C were attained and reductions of the annular zone diameter of 14.2–50 % and 27.3–61 %, respectively, were observed. Roasts were cooked for two hours at 190 °C. The maximum temperature in the centre of the roast was 51–87 °C, and 87–101 °C at the outer layer of the roast. The reductions of the annular zone diameter at the outer layer, at mid-depth and at the centre of the roast were 55.2–75 %, 42.8–100 % and 37.4–74 %, respectively. The influence of cold storage on chloramphenicol concentrations was studied by storing muscle tissue at 4 and –20 °C. Storage at 4 °C resulted in reductions of the annular zone diameter of 85.4–100 % after two weeks, and 100 % after four weeks. At –20 °C, no reductions of the annular zone diameter were observed after 2, 4, 6 and 77 weeks, while reductions of 0–32 % and 73.3–100 % were reported after 12 and 24 weeks of storage, respectively.

Costa et al. (1993) studied the stability of chloramphenicol residues in rabbit muscle during storage and cooking. Sample extracts were analysed using HPLC–UV (LOD = 1 µg/kg) and confirmation was performed by GC–MS. Storage of rabbit meat for 30 days at –20 °C had no influence on the chloramphenicol concentration (650 µg/kg vs. 710 µg/kg, the concentration before storage). The chloramphenicol concentration was reduced to 60 µg/kg (91.6 % reduction) when the meat was stored at –20 °C for 30 days and afterwards boiled for one hour. No chloramphenicol was detected in the boiling water. After roasting for 30 minutes at 100 °C, no effect on the chloramphenicol concentration was observed (640 µg/kg), while at 160 °C the concentration was decreased to 270 µg/kg (59 % reduction). Following 30 minutes roasting at 220 °C, no chloramphenicol was detected (< 1 µg/kg).

Shakila et al. (2006) studied the effect of heat treatments on chloramphenicol concentrations in shrimps using a microbial assay with *Photobacterium leiognathi*, which has a minimum detection level of 1 µg/mL. Blended shrimps were spiked at a concentration of 5 000–10^5 µg/kg and heated in test tubes. Boiling shrimps at 100 °C for 10, 20 and 30 minutes resulted in chloramphenicol reductions of 6, 12 and 29 %, respectively. Pressure-cooking at 121 °C for 10 and 15 minutes resulted in reductions of 9 and 16 %, respectively. The CONTAM Panel noted the high chloramphenicol concentrations that were used and the long heating conditions which are not representative for the preparation of shrimps for human consumption. Therefore, the Panel considered these data not suitable to indicate the effect of cooking on chloramphenicol levels in shrimps.

Besides the temperature and duration of the heat treatment, the matrix also has an influence on the heat stability of chloramphenicol. Franje et al. (2010) studied the stability of chloramphenicol at 100 °C during different heating times in water, salted water, soybean sauce and blended chicken meat. The samples were analysed by capillary electrophoresis with UV–DAD detection (LOQ = 2.5 µg/mL) and samples were spiked at final concentrations of 50 and 100 µg/mL. The heat stability of chloramphenicol in the different matrices was ranked as follows: water ≥ salt water > soybean sauce > meat. The influence of the duration of the heat treatment on chloramphenicol reduction was also investigated in this study. It was shown that microwave heating of chloramphenicol in water for 5 minutes has a similar effect as 30 minutes' boiling. GC–EI–MS analysis was used to structurally identify different degradation products after heating chloramphenicol in water and meat and four structures were proposed by the authors. The structures of the proposed compounds differ from the metabolites that are known to be involved in the toxic actions of chloramphenicol (see Section 7.1.1).

The influence of emulsifying, curing and heating on chloramphenicol concentrations in pork meat was studied by Epstein et al. (1988). Chloramphenicol was quantified using GC–ECD (LOD/LOQ not

reported). Emulsifying and curing under chilled conditions reduced chloramphenicol from an initial concentration of 48.4 µg/kg to 21.9 and 10.6 µg/kg, respectively. When the emulsified and cured meat was further processed in casings at 68 °C or canned and heated at 122 °C, chloramphenicol was not detected (< 5 µg/kg).

In addition to the studies on heat treatments, the stability of chloramphenicol under cooling conditions was studied. Storage of spiked chicken muscle (14.7 ± 0.58 µg/kg; mean ± standard deviation) at 4 °C for five days caused no change in chloramphenicol concentration (14.2 ± 0.38 µg/kg), and similar results were observed for spiked milk stored at 4 °C for 11 days (7.1 ± 0.41 µg/kg on day 0 vs. 7.6 ± 0.21 µg/kg on day 11). In addition, storage at –18 °C for 30, 60 and 90 days did not result in a reduction of chloramphenicol in chicken muscle (14.8 ± 0.26 µg/kg on day 90). Storage of milk at –18 °C resulted in chloramphenicol concentrations of 5.4 ± 0.13 after one and six months of storage, compared with 7.1 ± 0.41 µg/kg on day 0. Analysis was performed by HPLC–DAD (LOD = 2 (meat) and 0.4 µg/kg (milk)) (Ramos et al., 2003).

Cheng et al. (2012) studied the influence of the processing steps preheating, filtration, vacuum concentration and pasteurisation on the chloramphenicol concentration in spiked honey (7 µg/kg). A total reduction of 14 % occurred, of which 9.9 % was caused by vacuum concentration. In addition, several macroporous adsorption resins were tested for their ability to adsorb chloramphenicol from honey and adsorption rates up to 86 % were observed. A commercial ELISA kit was used for the analysis (LOD = 0.05 µg/kg).

Overall, only limited information about the effect of food processing on chloramphenicol is available; some decrease in chloramphenicol has been reported as well as the production of degradation products, but the toxic potential of these compounds is unclear.

4.3.1.2. Feed processing

The natural occurrence of chloramphenicol in animal feed and the presence of chloramphenicol in feed enzymes added to compound feed have only recently been discovered, and no studies on the influence of feed processing (e.g. silage fermentation of grass, elevated temperatures and pressure in compound feed production) on chloramphenicol were identified.

5. Food and feed consumption

5.1. Food consumption

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was constructed in 2010 from existing detailed national information on food consumption. Competent authorities in the European countries provided EFSA with data from the most recent national dietary surveys in their countries at the level of consumption by the individual consumer. These included food consumption data concerning infants (two surveys from two countries), toddlers (eight surveys from eight countries), other children (16 surveys from 14 countries), adolescents (14 surveys from 12 countries), adults (21 surveys from 20 countries), the elderly (nine surveys from nine countries) and the very elderly (eight surveys from eight countries) for a total of 32 different dietary surveys carried out in 22 different countries. Surveys on children were mainly obtained through the Article 36 project "Individual food consumption data and exposure assessment studies for children" (acronym EXPOCHI) (Huybrechts et al., 2011).

Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, consumption data were collected using different methodologies and thus they are not suitable for direct country-to-country comparison.

5.1.1. EFSA's Comprehensive European Food Consumption Database

As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011a), dietary surveys with only one day per subject were not considered for the calculation of chronic

dietary exposure, as they are not adequate to assess repeated exposure. Similarly, subjects who participated for only one day in dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic dietary exposure assessment. Therefore, for chronic dietary exposure assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries. These included infants from 2 surveys (2 countries), toddlers from 7 surveys (7 countries), other children from 15 surveys (13 countries), adolescents from 12 surveys (10 countries), adults from 15 surveys (14 countries), the elderly from 7 surveys (7 countries) and the very elderly from 6 surveys (6 countries) (Appendix A, Table A1).

Within the dietary studies, subjects were classified in different age classes as defined below:

- Infants: < 12 months old
- Toddlers: > 12 months to < 36 months old
- Other children: ≥ 36 months to < 10 years old
- Adolescents: ≥ 10 years to < 18 years old
- Adults: > 18 years to < 65 years old
- Elderly: ≥ 65 years to < 75 years old
- Very elderly: ≥ 75 years old

Consumption records were coded according to the FoodEx classification system, which was developed by the DATA Unit in 2009 (EFSA, 2011a).

The dietary surveys considered for the chronic dietary exposure assessment and the numbers of subjects in the different age classes are presented in Appendix A, Table A1. Further details on how the Comprehensive Database is used are found in the Guidance of EFSA (2011b).

5.2. Feed consumption

The CONTAM Panel considered the consumption of compound feed because of the occurrence of chloramphenicol in feed enzymes (see Section 4.2.2).

Approximately 150 million tonnes of compound feeds are produced annually in the EU (FEFAC, 2012). Feed enzymes are used in nearly all poultry and pig compound feeds, but rarely in feed for ruminants (complementary feed, silage). Therefore, only poultry and pigs were considered for the exposure to chloramphenicol via compound feed. The CONTAM Panel considered all feed consumed by pigs and poultry to be compound feed. Table 3 shows feed intakes proposed by EFSA FEEDAP Panel (2012).

Table 3: Live weights and feed intakes of pigs and poultry (EFSA FEEDAP Panel, 2012)

Animal	Live weight (kg)	Feed intake (kg per day)
Piglets	20	1
Pigs for fattening	100	3
Sows	200	6
Chickens for fattening	2	0.12
Laying hens	2	0.12
Turkeys for fattening	12	0.4
Ducks	3	0.14

6. Dietary exposure assessment in humans and animals

6.1. Dietary exposure assessment of chloramphenicol in humans

6.1.1. Previously reported human dietary exposure assessments

Bilandžić et al. (2011b) estimated the dietary exposure to chloramphenicol from raw milk using occurrence data collected between 2008 and 2010 (see Section 4.1). Based on an average milk consumption of 300 mL per day for adults and mean chloramphenicol concentrations, an average exposure of 0.4 ng/kg b.w. per day from raw milk was estimated. For occurrence data collected in 2011, a mean daily exposure to chloramphenicol from raw milk of 0.28 ng/kg b.w. per day was estimated (Bilandžić et al., 2011a). Because all reported concentrations of chloramphenicol in milk were below the CCβ value for the method, the CONTAM Panel do not consider that these data may be used to provide a reliable estimate for the exposure to chloramphenicol from milk.

A similar estimation was done by Dimitrieska-Stojkovic et al. (2011) using occurrence data in raw milk collected between 2008 and 2011 in FYROM (see Section 4.1). Based on a daily average milk consumption of 200 mL for adults, an average dietary exposure of 0.74 ng/kg b.w. per day from raw milk was estimated. Because all reported concentrations of chloramphenicol in milk were below the CCβ value for the method, the CONTAM Panel do not consider that these data may be used to provide a reliable estimate for the exposure to chloramphenicol from milk.

The Department of Health, Government of South Australia, estimated dietary exposure to chloramphenicol from the consumption of imported crab meat. It was estimated that an average consumer of crab has an intake of 3.4 ng per day and a high consumer 9 ng per day, using the mean concentration of 0.094 µg/kg in crab meat (Eckert, 2006).

In 2003, JECFA estimated the chloramphenicol dietary exposure from shrimps. Based on a median concentration of 0.5 µg/kg in shrimps and a high consumption level of seafood of 3.9 g/kg b.w. per day, exposure was estimated to be 2 ng/kg b.w. per day. JECFA noted that other products of animal origin could also occasionally contain chloramphenicol (FAO/WHO, 2004a).

In addition, several national agencies evaluated the dietary exposure from specific foods in which chloramphenicol had been detected (see Section 1.1).

6.1.2. Dietary exposure to chloramphenicol for different scenarios

Only limited chloramphenicol occurrence data in food were available for this opinion (see Section 4.2). Therefore, the CONTAM Panel concluded that these data are too limited to carry out a reliable human dietary exposure assessment. Instead, the CONTAM Panel calculated the hypothetical human chronic dietary exposure using the RPA value of 0.3 µg/kg for four scenarios;

- scenario 1, in which all foods of animal origin are contaminated with chloramphenicol (meat and meat products, fish and other seafood, milk and dairy products and honey);
- scenario 2, which includes foods in which enzyme preparations, reported to be contaminated with chloramphenicol, may be used during food production (beef, bread and rolls, fine bakery wares, wine and wine-like drinks, fruit juices and mixed fruit juices);
- scenario 3, which includes grains and grain-based products in which chloramphenicol could occur naturally;
- scenario 4, the combination of scenarios 1, 2 and 3.

The CONTAM Panel emphasises that these scenarios represent the worst-case situations in which all food covered by each scenario are contaminated with chloramphenicol at the RPA, a highly unlikely situation.

For calculating the chronic dietary exposure to chloramphenicol, food consumption and body weight, data at the individual level were accessed in the Comprehensive Database. Exposure was calculated by multiplying the occurrence concentration of 0.3 µg/kg ($\bar{o}_f$) for each food or food group (f belonging to F sets of food groups) with their respective consumption amount ($c_{f,d,i}$) per kg b.w. (bw_i) separately for each individual (i belonging to I set of individuals) in the database, calculating the sum of exposure for each survey day (d belonging to D_i set of days surveyed for an individual i) and then deriving the daily average for the survey period (the operation $|D_i|$ represents the number of days in the survey of each individual).

The method used can be described according to the following equation calculating the individual exposure:

$$\bar{e}_i = \frac{\sum_{d \in D_i} \sum_{f \in F} \bar{o}_f \cdot c_{f,d,i}}{|D_i| \cdot bw_i}$$

Mean and 95th percentile chronic dietary exposure were calculated for the total population separately for each survey and age class using consumption data at individual level from the Comprehensive database (see Section 5.1.1) and for all four scenarios. Chronic dietary exposure estimates were calculated for 26 different dietary surveys carried out in 17 different European countries. Not all countries provided consumption information for all age groups, or in some cases the same country provided more than one consumption survey. In accordance with the specification of the EFSA Guidance on the use of the Comprehensive Database (EFSA, 2011b), 95th percentile estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust and therefore they should not be considered in the risk characterisation. For each age group, Table 4 provides the minimum, median and maximum of the mean and 95th percentile chronic dietary exposure values across European countries and dietary surveys. The mean chronic dietary exposure to chloramphenicol would range for scenario 1 from 1.1 to 23 ng/kg b.w. per day, for scenario 2 from 0.4 to 7.0 ng/kg b.w. per day, for scenario 3 from 0.5 to 3.2 ng/kg b.w. per day, and for scenario 4 from 2.2 to 24 ng/kg b.w. per day. The 95th percentile chronic dietary exposure to chloramphenicol would range for scenario 1 from 2.4 to 31 ng/kg b.w. per day, for scenario 2 from 1.7 to 12 ng/kg b.w. per day, for scenario 3 from 1.1 to 5.9 ng/kg b.w. per day, and for scenario 4 from 4.4 to 35 ng/kg b.w. per day.

Table 4: Summary statistics for the chronic dietary exposure assessment (ng/kg body weight per day) of chloramphenicol estimated by age class for different scenarios. The minimum, median and maximum of the mean and 95th percentile exposure values across European countries and dietary surveys are shown.

Age class	Number of surveys	Scenario 1[a]			Scenario 2[b]			Scenario 3[c]			Scenario 4[d]		
		Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Mean chronic dietary exposure (average consumer)													
Infants	2	5.6	–[e]	23	0.4	–[e]	1.7	0.5	–[e]	1.0	7.6	–[e]	24
Toddlers	7	7.1	9.8	13	1.3	3.1	7.0	1.8	2.5	3.2	11	15	17
Other children	15	2.4	6.6	11	1.9	2.9	5.1	1.5	2.2	3.2	4.7	10	16
Adolescents	12	1.4	2.7	3.9	1.4	1.6	2.5	1.0	1.3	1.8	3.4	4.4	5.9
Adults	15	1.1	1.9	2.8	0.9	1.2	1.9	0.6	0.9	1.1	2.2	3.1	4.0
Elderly	7	1.1	1.4	2.3	0.5	1.3	1.6	0.6	0.9	1.0	2.4	2.9	3.6
Very elderly	6	1.2	1.5	2.3	0.9	1.3	1.6	0.8	0.9	1.1	2.4	2.9	3.8

Table 4: Summary statistics for the chronic dietary exposure assessment (ng/kg body weight per day) of chloramphenicol estimated by age class for different scenarios. The minimum, median and maximum of the mean and 95th percentile exposure values across European countries and dietary surveys are shown (continued).

Age class	Number of surveys	Scenario 1[a]			Scenario 2[b]			Scenario 3[c]			Scenario 4[d]		
		Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
		95th percentile chronic dietary exposure (high consumer)[f]											
Infants	1	_ [g]	_ [g]	_ [g]	_ [g]	_ [g]	_ [g]	_ [g]	_ [g]	_ [g]	_ [g]	_ [g]	_ [g]
Toddlers	4	16	17	31	5.9	8.3	12	3.5	4.2	5.3	20	23	35
Other children	15	5.4	12	20	3.7	6.1	11	2.6	3.9	5.9	8.8	16	25
Adolescents	12	3.3	5.1	7.9	2.9	3.6	6.7	1.8	2.5	3.6	6.5	8.4	11
Adults	15	2.5	3.8	5.1	1.7	2.6	5.1	1.1	1.8	2.1	4.4	5.4	7.2
Elderly	7	2.4	2.7	4.7	1.7	2.8	4.0	1.1	1.7	1.8	4.7	4.8	6.6
Very elderly	5	2.4	2.8	3.3	2.1	2.7	3.6	1.6	1.7	1.9	4.4	4.8	5.5

Min: minimum; Max: maximum.
Note: In order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to 2 figures.
(a): Scenario 1: all foods of animal origin are contaminated with chloramphenicol (meat and meat products, fish and other seafood, milk and dairy products and honey).
(b): Scenario 2 includes foods in which enzyme preparations, reported to be contaminated with chloramphenicol, may be used during food production (beef, bread and rolls, fine bakery wares, wine and wine-like drinks, fruit juices and mixed fruit juices);
(c): Scenario 3 includes grains and grain-based products in which chloramphenicol could occur naturally.
(d): Scenario 4 is the combination of scenarios 1, 2 and 3.
(e): Not calculated; estimates available only from two dietary surveys.
(f): The 95th percentile estimates obtained from dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011b) and therefore were not included in this table.
(g): Estimates available from only one dietary survey: 22 ng/kg body weight (b.w.) per day (scenario 1), 6.1 ng/kg b.w. per day (scenario 2), 3.7 ng/kg b.w. per day (scenario 3) and 24 ng/kg b.w. per day (scenario 4).

6.1.2.1. Contribution of different food groups to chloramphenicol dietary exposure

Taking into consideration the limited occurrence data available, the CONTAM Panel considered only certain food groups for the calculation of chronic dietary exposure in the four scenarios. These include all food products for which non-compliant samples were reported in the EC database on residues of veterinary medicines (Section 4.2.2, Table 1), in addition to foods where, according to the notifications in the RASFF database, enzymes are added and grain and grain-based products where chloramphenicol could occur naturally.

The contribution (%) of the individual food groups to chronic dietary exposure to chloramphenicol varied between the dietary surveys. This could be explained by the specific food consumption patterns in the individual European countries and even in the different regions of one country. It should be borne in mind that in two dietary surveys, foods (e.g. bread, fine bakery products) were disaggregated to ingredients (e.g. flour) and therefore these studies did not qualify for calculation of the contribution of food groups to the chronic dietary exposure. The contribution to chloramphenicol chronic dietary exposure for the seven individual food groups was assessed separately for each survey and age group. For the worst-case scenario (scenario 4), a summary of the median values, calculated from the average contribution of each food group across the dietary surveys, and the range of the lowest and highest average contribution is shown in Table 5.

Milk and dairy products made the largest contribution to the chronic dietary exposure to chloramphenicol in all age classes. Their contribution was higher in toddlers (59 %) and other children (54 %).

The next food group that contributed to the chronic dietary exposure of chloramphenicol in all age groups was grain and grain-based products, either because of treatment with enzymes or because of the natural occurrence of chloramphenicol.

Table 5: Contribution (%) of the different food groups to chronic dietary exposure to chloramphenicol in scenario 4[(a)]. Median values across dietary surveys and the range of the average contribution are presented.

Food group	Median contribution across dietary surveys (lowest–highest average contribution) (%)						
	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Meat and meat products	– [(c)] (0.4–4.7)	6.1 (4.9–9.8)	9.4 (5.3–17)	14 (9.4–21)	16 (10–26)	14 (12–22)	14 (11–22)
Fish and other seafood	– [(c)] (0.2–0.3)	1.1 (0.5–5.2)	1.3 (0.7–5.7)	2.7 (0.8–6.5)	3.1 (1.0–8.4)	4.2 (0.7–6.6)	3.7 (0.7–5.3)
Milk and dairy products	– [(c)] (69–95)	59 (51–73)	54 (34–61)	39 (23–56)	36 (23–49)	27 (24–52)	32 (26–44)
Honey	– [(c)] (0)	0 (0–0.3)	0.1 (0–0.4)	0.1 (0–0.2)	0.1 (0–0.3)	0.3 (0–0.7)	0.3 (0–0.7)
Grains and grain-based products[(b)]	– [(c)] (2.3–14)	17 (11–25)	22 (9.8–36)	28 (22–38)	31 (16–40)	32 (18–36)	34 (22–37)
Fruit and vegetable juices	– [(c)] (1.6–13)	13 (5.1–26)	12 (0–22)	11 (0–38)	6.8 (0–27)	5.8 (1.4–19)	4.8 (1.4–18)
Wine and wine-like drinks	– [(c)] (0)	0 (0)	0 (0–0.3)	0.3 (0–0.6)	5.9 (0.8–11)	13 (1.8–16)	11 (4.5–16)

Note: In order to avoid the impression of too high precision, the numbers for all contributions are rounded to 2 figures.

(a): Scenario 4 in which specific food groups (foods of animal origin, foods in which enzyme preparations, reported to be contaminated with chloramphenicol, may be used during food production and grains and grain-based products in which chloramphenicol could occur naturally) are considered to contain chloramphenicol at the concentration level of 0.3 µg/kg.

(b): FI/1 survey excluded from calculation of the contribution of "grains and grain-based products".

(c): Median value not calculated as only two dietary surveys were available.

6.1.2.2. Dietary exposure from enzyme-based food supplements

Overall, the Comprehensive Database contains limited information on the consumption of food supplements. Only some of the surveys registered and, consequently, reported the consumption of supplements but there were still no food consumption data available on enzyme-based food supplements for which notifications were included in the RASFF database. Based on this, the CONTAM Panel decided to calculate dietary exposure to chloramphenicol from enzyme-based food supplements taking into consideration the recommended dosage, the size of the single serving and the concentration of chloramphenicol as found in the notification events.

The daily dietary exposure to chloramphenicol from the enzyme-based food supplement reported in RASFF under notification number 2013.1544 has been estimated by multiplying the concentration of chloramphenicol as analysed in the supplement (1 800 µg/kg) with the weight of a single serving (0.45 g) and the number of suggested servings per day (one), divided by 70 kg b.w. (default adult body weight according to EFSA SC, 2012). This resulted in a value for exposure to chloramphenicol of 12 ng/kg b.w. per day. The source of chloramphenicol contamination for this supplement was reported to be the enzyme cellulase, which was further processed in the production of two different supplements available in different formats from the same company. Unfortunately, the concentration of chloramphenicol was available for only one of the supplements affected, so a calculation of the daily exposure was not possible for the second type of supplement.

For the enzyme-based food supplement notified in RASFF under notification number 2013.1503, the concentration of chloramphenicol as analysed was 18 µg/kg, the recommended serving per day was one and the weight of a single serving was estimated to be 0.4 g. Following a similar calculation to the previous case, the estimated daily dietary exposure to chloramphenicol was 0.1 ng/kg b.w. per day.

Finally, for the last enzyme-based food supplement reported in RASFF (notification number 2013.1685) the concentration of chloramphenicol as analysed was 9.4 µg/kg, the recommended number of servings per day was two and the weight of a single serving was 0.8 g. Accepting that the analytical result provided in the notification is of the supplement and not the enzyme preparation used for its production, the dietary exposure to chloramphenicol resulting from a single serving is 0.1 ng/kg b.w. (or 0.2 ng/kg b.w. per day).

6.1.2.3. Concluding comments

Based on the considered scenarios, mean and 95th percentile chronic dietary exposure to chloramphenicol in the adult population across Europe would range from 0.6 ng/kg b.w. per day (minimum for scenario 3) to 4.0 ng/kg b.w. per day (maximum for scenario 4), and from 1.1 ng/kg b.w. per day (minimum for scenario 3) to 7.2 ng/kg b.w. per day (maximum for scenario 4), respectively. A relatively high variation between the exposure estimates across the dietary surveys within each age group was observed. Overall, the age group with the highest chronic dietary exposure was toddlers (a) due to the higher intake of food per kg b.w. in this age group and (b) because the food category of milk and dairy products was a main contributor to the diet of this group.

Dietary exposure to chloramphenicol from enzyme-based food supplements, at the concentrations reported in RASFF notifications, ranged from 0.1 to 12 ng/kg b.w. per day.

6.1.3. Non-dietary exposure

In humans, there is potential for additional exposure to chloramphenicol from licensed medicines via oral, i.v. or topical ocular administration (see Section 1.3).

6.2. Dietary exposure assessment of chloramphenicol in animals

6.2.1. Exposure from compound feed consumption

Only limited chloramphenicol occurrence data in feed were available for this risk assessment (see Section 4.2). Therefore, the CONTAM Panel concluded that these data are too limited to carry out a reliable animal dietary exposure assessment. Instead, the CONTAM Panel estimated a possible worst-case exposure level.

Owing to the recent findings on occurrence of chloramphenicol in feed enzyme preparations, the CONTAM Panel considered the dietary exposure to chloramphenicol via feed enzymes for pigs and poultry. Final feed enzyme preparations contain between 5 and 25 % (w/w) enzyme concentrates from one or more fermentations and are added to compound feeds for pigs and poultry at a concentration of 50–500 mg/kg compound feed. Considering the highest chloramphenicol concentration observed in enzyme concentrates of 47 000 µg/kg, the highest inclusion level of enzyme concentrate in the final enzyme preparation (25 %) and the highest inclusion level of enzyme preparation in compound feed (500 mg/kg), a chloramphenicol concentration of 5.9 µg/kg compound feed was used for the exposure assessment. Table 6 provides estimated exposures of pigs and poultry to chloramphenicol from compound feeds.

Table 6: Estimated dietary exposure of pigs and poultry to chloramphenicol from compound feeds

Animal	Live weight (kg)	Feed intake (kg per day)	Exposure (µg per day)	Exposure (µg/kg body weight per day)
Piglets	20	1	5.9	0.3
Pigs for fattening	100	3	18	0.2
Sows	200	6	35	0.2
Chickens for fattening	2	0.12	0.7	0.4
Laying hens	2	0.12	0.7	0.4
Turkeys for fattening	12	0.4	2.4	0.2
Ducks	3	0.14	0.8	0.3

Note: In order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to 2 figures.

6.2.2. Exposure from straw consumption and soil intake

In the past, chloramphenicol was applied in ruminants as an injectable preparation at levels of more than 10 mg/kg b.w. but not used in medicated feeds because of degradation in the rumen (see Section 7.1.4). More recently, natural occurrence was observed, in particular, in herbs but also in straw and grass. The highest level detected in straw was 32 µg/kg (see Section 4.1.1). Straw is a common feature of dairy cow diets throughout Europe and consumption levels of 0.5 to 1 kg per day are not uncommon. This implies a potential dietary exposure of dairy cows of up to 34 µg, equivalent to less than 0.1 µg/kg b.w. per day, i.e. more than 100 000-fold lower than the formerly used therapeutic dose. Beef cattle in some countries reportedly eat larger amounts of straw, up to 8.5 kg per day for a 650 kg weighing beef cow, 4.5 kg per day for a store beef animal of 350 kg and 5.5 kg per day for a heifer of 350 kg. Again, based on the highest observed chloramphenicol level of 32 µg/kg, these straw intakes result in a daily exposure of 272, 144 and 176 µg chloramphenicol, respectively, or 0.4, 0.4 and 0.5 µg/kg b.w. per day; around 4–5 times higher than calculated for dairy cows.

Growing pigs receive straw, but are estimated to consume only a handful (few grams) per day. The amount may be higher when pigs are bedded on straw. Cole (1990) reported a straw intake of 0.5 kg per day for sows. Combining this with the highest level of chloramphenicol detected in straw, 32 µg/kg, dietary exposure of pigs just before slaughter could be as high as 16 µg or 0.2 µg/kg b.w. per day.

Chickens are provided with bedding which can contain straw, but it is estimated that they consume very little of the bedding.

The highest level observed in samples of grass from Mongolia was 1.2 µg/kg. There are no data available on chloramphenicol levels in European grasses. Using the concentration of 1.2 µg/kg, with an estimated grass intake of 80–100 kg per day (w.w.) by high-yielding dairy cows, this would amount to an intake of up to 96–120 µg chloramphenicol or up to 0.2 µg/kg b.w. per day, again about 100 000-fold lower than the formerly used therapeutic dose.

Another potential source of chloramphenicol is soil which can be consumed during grazing. Recently, chloramphenicol was detected in Mongolian soil by Berendsen et al. (2010), although at low levels (up to 0.2 µg/kg) and much lower than the worst-case levels used by JECFA (1 and 25 mg/kg, based on levels detected in soil under laboratory conditions but not in samples from pastures). The low levels can be explained by the degradation of chloramphenicol by soil bacteria. Estimated soil intake by cows is 1 to 18 % of the dry matter (Thornton and Abrahams, 1983) and up to 30 % by sheep (Abrahams and Steigmajer, 2003). Based on 20.7 kg dry matter intake per day, this would imply a daily soil ingestion of 0.2–3.6 kg or a maximal intake of chloramphenicol of up to 0.7 µg by cows. Based on 2.8 kg dry matter intake per day by sheep, soil ingestion would be up to 0.8 kg or up to 0.2 µg chloramphenicol. As such, intake by both cows and sheep from soil would be much lower than the potential uptake from straw. There are no data on soil uptake by laying hens, but amounts around 10 g per day seem feasible. Therefore, the intake of chloramphenicol by laying hens could amount to 0.002 µg per day.

7. Hazard identification and characterisation

7.1. Toxicokinetics

7.1.1. Introduction

The kinetics and, more specifically, the biotransformation profile and the tissue distribution of chloramphenicol have been the subject of a number of reviews (Glazko, 1966; Ambrose, 1984; Milhaud, 1985; Bories and Cravedi, 1994; FAO/WHO, 2004a, b). Most of the kinetic studies performed up to the late 1970s used microbiological or colorimetric methods. The latter are based on the generation of a diazo-derivative by means of the Bratton–Marshall reaction. Both were characterised by low specificity and sensitivity (LOD in the mg/kg range) and the inability to identify the structure of chloramphenicol metabolites. Therefore, the results need to be interpreted with caution. Only relatively recently, the development of MS techniques enabled identification of the main metabolites occurring in humans and in most animal species. The tissue residue pattern, however, has not yet been fully elucidated in most food-producing species.

Chloramphenicol is slightly soluble in water and freely soluble in organic solvents and may be used in humans as well as in farm and companion animals as such or as esters (e.g. palmitate, succinate) for oral or parenteral administration. In either case, esters are, in general, rapidly hydrolysed in the gastrointestinal tract (GI) or in the systemic circulation to release the active drug, i.e. chloramphenicol. With few exceptions, chloramphenicol is well absorbed upon oral administration in monogastric species and in pre-ruminant calves, while it appears to be extensively metabolised by rumen microorganisms. The bioavailability is, in general, good, particularly in mammalian species, although it may vary mainly according to the route of administration, the species, and the age of the treated individuals. The lipid solubility and the relatively low binding to plasma proteins (30–53 %) enable the drug to attain effective concentrations in most tissues and to cross the blood–brain barrier as well as the placental and the mammary barriers. As a consequence, the drug displays a relatively large volume of distribution (Vd) particularly in mammalian species, being in the range 0.5–2.5 L/kg b.w.

The biotransformation of chloramphenicol is a complex process, and in some cases the biochemical pathways involved in the generation of relevant metabolites have not been fully elucidated.

Chloramphenicol metabolism is an important determinant not only of its antibacterial activity and excretion rate but also of its bioactivation to reactive metabolites, which have been implicated in the generation of a number of adverse effects. The main biotransformation pathways are depicted in Figure 2. In most species, chloramphenicol undergoes extensive conjugation. Due to its two aliphatic hydroxyl groups, chloramphenicol can form two distinct monoglucuronides, with the 3-glucuronide largely prevailing over the 1-glucuronide (Chen et al., 2007); sulphate derivatives are formed to a much lesser extent than glucurono-conjugates. Both phase II metabolites are largely excreted via the urinary route.

Under certain conditions, chloramphenicol may enter the oxidative or reductive metabolic pathways involving the dichloroacetate or the nitrobenzene moiety, respectively. The former entails an oxidative dehalogenation mediated by a number of CYP isoforms, mainly belonging to 2B, 2C and 3A subfamilies (Bories and Cravedi, 1994) resulting in the formation of a hydroxydichloroacetamido intermediate, which spontaneously releases hydrogen chloride to generate a highly reactive oxamyl chloride. This, in turn, may react with the ε-amino group of a lysine residue in CYP inhibiting the enzymatic reaction progressively with time. Such a phenomenon has been called "suicide inhibition" and may explain most of the well-known interactions displayed by chloramphenicol in humans, farm and companion animals with a number of drugs being substrates of the inhibited CYPs. The oxamyl chloride derivative can also react with water to yield the hydrolysed product oxamic acid, which has been identified as a urinary metabolite in a number of species.

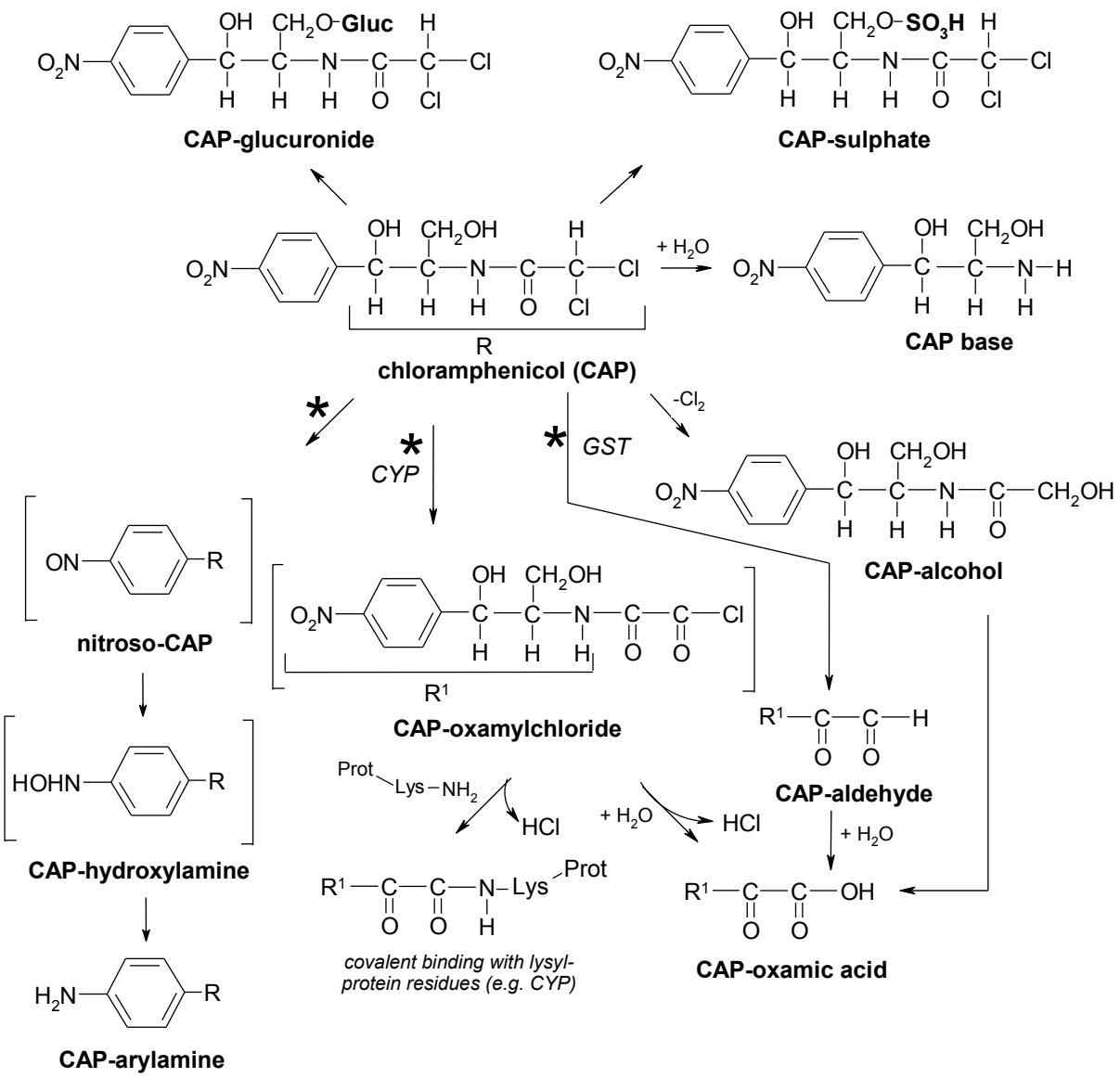

Figure 2: Main biotransformation pathways of chloramphenicol. Metabolites in brackets are unstable metabolites characterised by a remarkable reactivity; only nitroso-chloramphenicol has been identified as a tissue metabolite and only in chickens. Pathways marked with (*) are thought to generate free radicals (reactive oxygen and nitrogen species)

An alternative pathway leading to the formation of oxamic acid is mediated by cytosolic glutathione S-transferases and involves the glutathione-dependent chloramphenicol dechlorination with the generation of an aldehyde derivative (Martin et al., 1980; Holt et al., 1995b). A further relevant biotransformation is the hydrolytic dechlorination of the antibiotic, yielding the so-called chloramphenicol alcohol, which has been identified as a major *in vivo* metabolite in humans and in other mammalian and avian species (Bories and Cravedi, 1994).

Another important pathway which may be also mediated by enteric or rumen microorganisms is the nitro-reduction of chloramphenicol; in most species, such a process is thought to yield unstable intermediary nitroso- and hydroxylamine derivatives suspected to be involved in genotoxic and

cytotoxic effects (Yunis, 1988), particularly in the generation of aplastic anaemia, especially when produced in the bone marrow (Ambekar et al., 2000). The final product, an arylamine derivative, is then excreted by the urinary route. It is relevant to note that multiple biotransformation routes, i.e. the nitroreductive one, as well as the CYP-mediated oxidative dehalogenation and the glutathione-dependent dechlorination, are thought to generate oxygen and nitrogen radical species playing a pivotal role in a number of chloramphenicol-mediated adverse effects (Paez et al., 2008; Oyagbemi et al., 2010).

Additional biotransformation routes have been also documented. The hydrolysis of the amide function results in the formation of the so called chloramphenicol base (Figure 2), which has been identified in urine from certain animal species. Bacterial biotransformations are reported to generate further metabolites other than nitroso-chloramphenicol, including dehydro-chloramphenicol and dehydro-chloramphenicol base (Figure 3). Some authors referred to dehydro-chloramphenicol base as 2-amino-3-hydroxy-p-nitropropiophenone (NPAP) (Isildar et al., 1988b, Lafarge-Frayssinet et al., 1994; Robbana-Barnat et al., 1997). However, two papers mention a different structure for NPAP (nitrophenylaminopropanedione and p-nitrophenyl-2-amino-3-hydroxypropanone HCl; Anadón et al., 1994; Jimenez et al., 1987; respectively). The CONTAM Panel noted that these two papers reported a structure suggesting that HCl is covalently bound to the aminogroup of dehydro-CAP base. These metabolites have been implicated in the haematotoxicity of the antibiotic (Jimenez et al., 1987; Robbana-Barnat et al., 1997) and have been tested for their effects on DNA using the alkaline elution assay (Isildar et al., 1988b; Lafarge-Frayssinet et al., 1994) (see Section 7.2.7).

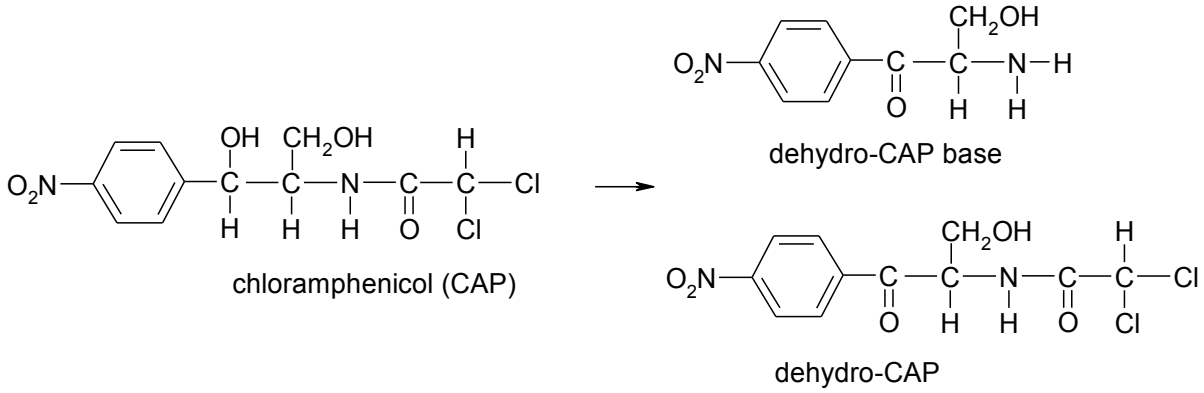

Figure 3: Other chloramphenicol metabolites reported to be involved in some toxic actions

In vitro studies have demonstrated the nitro-reduction of dehydro-chloramphenicol by human or rabbit bone marrow preparations as well as human liver homogenates under aerobic conditions (Isildar et al., 1988a, b). The relatively higher stability of dehydro-chloramphenicol compared with nitroso-chloramphenicol and its proven capacity to undergo nitro-reduction in the target tissue, strongly support the role of dehydro-chloramphenicol in chloramphenicol-induced haematotoxicity (Jimenez et al., 1987; Isildar et al., 1988 a, b).

7.1.2. Laboratory animals

Soon after the discovery of the drug, studies were started to examine the biotransformation in rats (Glazko et al., 1949, 1950, 1952). These authors were able to identify the 3-glucuronide and indirectly showed the presence of conjugated amines in rat urine. Uesugi et al. (1974) injected chloramphenicol (100 mg/kg b.w.) intraduodenally in bile duct-cannulated rats, rabbits and guinea pigs. Using thin-layer chromatography (TLC), they showed that, in the case of rats, most of the drug (70 %) was excreted as the glucuronide in the bile, and to some extent (11 %) in urine. They also detected small amounts of aryl amines in both bile and urine. This was contrary to what was found in guinea pigs and especially rabbits where most of the drug was excreted in the urine, again as the glucuronide. However, also in these species, small amounts of the arylamines were detected in urine and bile. This

study shows clear species differences in the excretion via bile or urine, which might be relevant with respect to the further degradation of metabolites in the GI tract.

Bories et al. (1983) used ^{3}H-labelled chloramphenicol (label in the propanediol moiety at C1) and GC/MS to identify and quantify various metabolites in 24 hours urine of rats injected intramuscular (i.m.). In addition to the parent compound (11.8 % of excreted radioactivity) and the glucuronide (7.8 %), the chloramphenicol base (25.7 %), the oxamic acid (15.4 %) and the alcohol (9.4 %) were detected, as well as the arylamine (4.3 %) and the acetylarylamine (19.1 %). Together these various compounds constituted 17 % of the injected radioactivity. In an additional study by a group with conventional and germ-free rats (Wal et al., 1983), treated by gavage with ^{3}H-labelled drug (label in the propanediol moiety at C1), it was clearly demonstrated that the arylamine and its acetylated metabolite, both resulting from reduction of the nitro-group, are primarily formed by bacteria in the GI tract. It was also shown that in rats, chloramphenicol is almost completely absorbed, glucuronidated in the liver and excreted via the bile into the GI tract, where it is deconjugated and partly metabolised by bacteria, followed by reabsorption. In conventional rats, 23.3 % of the label was recovered in the urine over four days and 31.6 % in the faeces, as compared with 15.2 and 37.9 % in germ-free rats. In urine, chloramphenicol constituted 8.6 %, the glucuronide 7.4 %, the oxamic acid 24.1 %, the base 7.5 %, the alcohol 5.4 % and the arylamine and acetylarylamine 22.7 and 15.2 %, respectively, with marked differences between the days. The (acetylated) arylamine was detected in germ-free rats but at much lower levels, indicating that some nitroreduction occurs in the tissues and organs of rats. In the faeces, metabolites could not be quantified individually but only after hydrolysis into the base or arylamine, in conventional rats showing contributions of 4.1 and 96.0 % respectively, as compared with 67.1 and 6.8 % in germ-free rats, again confirming the importance of the nitroreduction by bacteria.

The studies with rats indicate that metabolism is different from that in other species, due to the excretion of the glucuronide in the bile, allowing the reduction of the nitro group by bacteria in the GI tract followed by reabsorption and further metabolism.

7.1.3. Humans

Capsulated crystalline chloramphenicol and chloramphenicol palmitate are highly bioavailable (76–90 %) upon oral administration and reach plasma/serum peak values between 0.5 and 6 hours after dosing. Serum levels of 8–15 µg/mL have been measured in patients two to three hours after the oral administration of 7 mg chloramphenicol/kg b.w. (Bartlett, 1982). Chloramphenicol succinate is mainly used parenterally and appears comparatively less bioavailable with respect to the orally administered forms; in fact, the i.m. or i.v. dosing results in variable percentages (up to 25–30 %) of the drug escaping hydrolysis to the active (de-esterified) form and undergoing prompt renal excretion (Kramer et al., 1984), likely by active tubular secretion (Ambrose, 1984). The hydrolysis of chloramphenicol succinate to chloramphenicol has been recently demonstrated to occur also in the bone marrow (Ambekar et al., 2000). Serum protein binding has been reported to be around 53 % in healthy adults, while only about 32 % in premature neonates (Koup et al., 1979). Maximum concentration values ranging from 16.6 ± 4.23, 22.3 ± 7.64 and 22.8 ± 11.8 mg/L have been found in adults patients receiving chloramphenicol succinate i.v., chloramphenicol crystalline per os (p.o.), or chloramphenicol palmitate p.o., respectively (Kramer et al., 1984).

Chloramphenicol is readily distributed to a variety of tissues, including bone marrow and CNS, and also to breast milk. As assessed by a turbidimetric bioassay method, chloramphenicol concentrations in the range of 16–25 µg/mL and a plasma to milk ratio of 0.51–0.62 have been reported in orally treated women (Smadel et al., 1949; Chin et al., 2001). As measured by a chemical (presumably colorimetric) method, much lower milk concentrations (range 0.54–2.84 µg/mL) were detected in women receiving a prophylactic treatment (1 g chloramphenicol per day for 7–10 days) while concentrations in the range 1.75–3.10 µg/mL were present in women treated for mastitis (a total of 2 g (4 × 500 µg) chloramphenicol per day for eight days). As the corresponding milk levels measured with a microbiological method were about one-half those measured by the chemical method, the authors concluded that milk of chloramphenicol-treated women also contains a significant amount of

microbiologically inactive metabolites. In both cases, the chemical composition of the drug had not been indicated (Havelka et al., 1968).

Following the oral administration of a therapeutic dosage of chloramphenicol (2 g) to women during term labour, it was concluded that the antibiotic is able to cross the placental barrier, appearing in the fetal circulation in concentrations of the same order of magnitude to those occurring in the maternal one (Scott and Warner, 1950). Based on colorimetric assay methods, it was reported that the transfer rate of chloramphenicol to the fetus is high, concentrations of chloramphenicol (and possibly some of its metabolites) being high enough to be therapeutically effective in the fetus within 71 minutes of administration to the mother; clearance from the maternal blood may be completed in 24 hours.

Chloramphenicol is reported to efficiently cross the blood–brain barrier, reaching cerebrospinal fluid concentrations of about 50 % of the corresponding serum concentrations (Ambrose, 1984). A rough assessment of the brain transfer of the drug may be derived from a study in which patients suffering from cerebral pathologies (abscesses, tumours) were i.m. administered 2 g of chloramphenicol (compound not specified) and thereafter subjected to surgical removal of specimens of brain tissue close to the lesion (as near normal tissue as possible). A blood to brain tissue ratio of 1:9 was estimated (Kramer et al., 1969).

Hepatic biotransformations play a key role in chloramphenicol disposition and clearance. Glucuronidation is by far the major metabolic pathway. Chen et al. (2007) were able to unambiguously demonstrate the *in vitro* formation of two isomeric chloramphenicol monoglucuronides in pooled human liver microsomes. In a subsequent *in vitro* study using twelve different isoforms of human UDP-glucuronosyltransferase (UGT), it was shown that UGT2B7 has by far the highest activity to convert chloramphenicol to its 3- and 1-glucuronide (Chen et al., 2010). As with liver microsomes, formation of the 3-glucuronide was markedly faster than formation of the 1-glucuronide. A low activity for 3-glucuronidation was also observed for UGT1A9, 2B4, 2B17, 1A3, and 1A6 (Chen et al., 2010).

Factors reducing the rate of glucuronidation such as young age (fetuses, newborn infants, young children; for a review see Hines (2008)) or liver diseases (Narang et al., 1981) may increase serum levels of the unconjugated drug. The latter may lead to (reversible) bone marrow suppression (see Section 7.3.1) or enter oxidative and/or reductive pathways leading to the generation of reactive toxic metabolites resulting in genotoxicity and aplastic anaemia.

As described in the introduction (see Section 7.1.1), the oxidative CYP-mediated biotransformation of chloramphenicol may result in a mechanism-based “suicide” inhibition, in that the produced reactive metabolites (oxamyl derivatives) are able to covalently bind the apoprotein through acylation of a lysine residue, thereby inactivating the same CYP(s) involved in the metabolic reaction (Halpert et al., 1985). Inhibition studies performed with human liver microsomes and cDNA-expressed CYPs revealed that chloramphenicol is a strong inhibitor of CYP2C19 and CYP3A4 and a weak inhibitor of CYP2D6 (Park et al., 2003). This is believed to be the mechanism by which chloramphenicol prolongs the elimination half-life or increases serum drug concentrations of several widely used drugs with narrow therapeutic ranges, including cyclosporine, phenobarbital, phenytoin, tolbutamide and oral anticoagulants such as warfarin (for a review, see Pai et al., 2006). Alternatively, the oxamyl derivative can also react with water to yield the hydrolysed product oxamic acid and further oxidative metabolites, chloramphenicol aldehyde and chloramphenicol alcohol, which have been unequivocally identified in sera of young children and adults treated with chloramphenicol for serious infections (Holt et al., 1995a).

Reductive chloramphenicol biotransformations involving the *p*-nitrobenzyl moiety have been reported leading to the generation of unstable reactive intermediates (nitroso-chloramphenicol and hydroxylamino-chloramphenicol) and of stable amino-derivatives; the latter have been also identified in sera from treated patients (Holt et al., 1995a). This pathway, which has been associated with the onset of fatal aplastic anaemia and possibly DNA damage in susceptible patients (Yunis, 1988), was

originally thought to occur mainly—if not solely—in the gut and be catalysed by bacterial nitroreductases (Smith and Worrel, 1950). More recently (Ambekar et al., 2000), it has been shown that the incubation of chloramphenicol succinate (20 µg) with human bone marrow samples (n = 75), each belonging to a different donor, resulted in the slow release of the active principle (chloramphenicol) in the large majority of samples (n = 72); a faster rate of chloramphenicol hydrolysis and the nicotinamide adenine dinucleotide phosphate (NADPH)-independent generation of nitroso-chloramphenicol and a further unidentified metabolite could be detected in a small number (n = 3) of samples, suggesting a higher expression/activity of chloramphenicol-metabolising enzymes possibly resulting from enzyme induction. However, enzyme induction possibly arising from exposure to drugs, could not be demonstrated except in the case of one donor, who took a single dose of traditional Chinese Medicine 10 days prior to bone marrow donation. In addition, the nitro-reduction of dehydro-chloramphenicol, a further metabolite originated by intestinal microflora (Smith and Worrell, 1950) under aerobic conditions has been demonstrated in bone marrow and liver preparations.

In further *ex vivo* experiments, the incubation of chloramphenicol succinate with human bone marrow preparations resulted in the slow release of the free (de-esterified) drug; this metabolic reaction was enhanced by addition of flavine adenine dinucleotide (FAD) and inhibited by addition of high concentrations of malonate and the specific succinic dehydrogenase inhibitor 3-nitropropionic acid. This suggests that chloramphenicol succinate may be oxidised to chloramphenicol by succinic dehydrogenase; in turn, succinic dehydrogenase could be inhibited by chloramphenicol—possibly via a feedback mechanism—representing a further mechanism contributing to chloramphenicol-mediated reversible bone marrow depression (Ambekar et al., 2004).

Early studies in human subjects with normal hepatic function demonstrated that 75–90 % of a single chloramphenicol dose was excreted in the urine within 24 hours, while only 5–10 % of the administered dose was found as the parent compound (Glazko, 1966). As mentioned before, chloramphenicol-glucuronide is the principal metabolite, being excreted in urine likely by an active tubular secretion, together with variable percentages (5–10 %) of the active drug; up to 30 % of the chloramphenicol succinate may be excreted unchanged after parenteral administration (Ambrose, 1984). Biliary excretion is negligible in humans. Minor urinary metabolites such as chloramphenicol base and chloramphenicol oxamyl derivatives have been also reported in old studies (Corpet and Bories, 1987). More recently, in a study performed with more sophisticated analytical techniques (HPLC and GC–MS), chloramphenicol-oxamylethanolamine was isolated in urine samples from a human volunteer orally administered with [^{3}H]-chloramphenicol (10 mg/kg b.w.; labelled in the phenyl ring at positions 3 and 5); the recovery of 65 % of the ingested ^{3}H in urine eight hours after dosing (Cravedi et al., 1995) confirms the rapid excretion of the drug and its metabolites (90 % in 24 hours) already assessed in older studies (Glazko et al., 1949).

In conclusion, chloramphenicol is highly bioavailable upon oral exposure, and may easily cross both placental and mammary barriers. Under normal conditions, the drug is extensively biotransformed and rapidly eliminated, mainly as glucuronide derivatives. However, conditions known to depress the glucuronidation rate (young age, liver diseases, etc.) may allow the drug to enter reductive and/or oxidative pathways yielding toxic metabolites. Those resulting from nitroreduction have been implicated in the generation of blood dyscrasias and possibly genotoxicity, while the CYP-mediated reactive metabolites are responsible for CYP destruction and a number of clinically relevant drug–drug interactions.

7.1.4. Livestock

7.1.4.1. Ruminants

A large number of studies were carried out with cows and calves to investigate the kinetics of chloramphenicol by measuring blood levels after oral, i.v., i.m., subcutaneous (s.c.) and even intramammary injection of various preparations (De Corte-Baeten and Debackere, 1975; Nouws and

Ziv, 1978, 1979, 1982; Burrows et al., 1984, 1988; Epstein et al., 1986; Nouws et al., 1986; Sanders et al., 1988; Guillot et al., 1989; Gassner and Wuethrich, 1994). Initially colorimetric and microbiological methods were used to determine chloramphenicol levels, later on HPLC with UV detection was introduced. An important parameter in these studies was the time interval during which plasma levels exceeded the level of 5 µg/mL, a critical concentration in terms of effectiveness against bacterial infections. Dosages up to 50 mg/kg b.w. were required to achieve such levels for a certain time period, preferentially applied i.m. Some studies also determined levels in milk or meat. However, information on metabolites is rather limited.

De Corte-Baeten and Debackere (1975) investigated plasma levels in horses, cattle and sheep after oral and i.m. treatment with a single dose of 10 mg chloramphenicol per kg b.w. Using a colorimetric method, levels in plasma of six cows treated i.m. reached average levels up to 1.7 µg/mL. However, after oral treatment, no chloramphenicol could be detected (LOD of 0.16 µg/mL). Similar was the case for sheep but in the case of horses chloramphenicol could be detected after oral treatment, although at lower levels than in the case of i.m. treatment. This was one of the few studies indicating that oral treatment of ruminants did not result in significant levels of the drug in plasma, probably due to the extensive degradation by the ruminal flora.

Nouws and Ziv (1978) applied microbiological and chemical assays to determine chloramphenicol in blood, kidney, liver, muscle, bile and urine of dairy cows after a single i.m. dose of two different formulations and dose levels of the drug. The microbiological assay showed in general much lower levels than the chemical assay, except for serum, which can be explained by the fact that the chemical assay also determines metabolites. In fact, in tissues and organs no antimicrobial activity could be measured at 46 and 65 hours. After application of the highest dose of around 43 mg/kg b.w., levels in liver and kidney determined with the chemical assay were around 50 µg/g at 18 hours after injection, decreasing to 25 and 10 µg/g after 46 and 65 hours. Urine levels were highest, being 1 000, 245 and 135 µg/mL at 18, 46 and 65 hours, but also bile contained detectable levels (23, 30 and 13 µg/g), indicating some excretion of metabolites and parent drug (as detected by microbiological assay) via this route. Serum levels were in general lower than those in livers and kidneys but comparable to muscle levels (around 1 µg/g after 65 hours). The chemical assay could discriminate metabolites resulting from nitroreduction from other metabolites, but showed no positive findings for these metabolites in serum and muscle. In urine 2–9 % was estimated to be reduced metabolites, whereas in liver and kidney this amounted to 61 and 94 %. A large portion of the injected dose was detected in injection sites at slaughter, amounting to 49 % at 8 hours and 25 % at 18 hours (other formulation). The authors estimated withdrawal times of around 78 hours for muscle and 165 hours for kidney to obtain levels below 0.5 µg/g, and 7.7 and 14.1 days to reach a level of 0.002 µg/g (estimated half-lives of 14 and 22 hours, respectively). Again, this probably concerns metabolites rather than the parent drug.

In a follow-up study Nouws and Ziv (1979) treated cows, sheep and goats with i.m. injections with different products containing chloramphenicol. As before, levels in blood were analysed with both a chemical and microbiological assay, the latter one again showing lower levels in the blood. A dosage of 36 mg/kg b.w. in cows did not result in blood levels above the desired 5 µg/mL. Such levels were obtained with ewes and goats treated i.m. with 50 mg/kg b.w. Half-lives in cows, goats and ewes were comparable, varying between 88 and 643 minutes depending on the preparation and plasma levels up to 20 µg/mL. It was concluded that the overall absorption from the injection site was relatively poor. Therefore, Nouws and Ziv (1982) performed a study with intramammary infusion of different dosages of chloramphenicol (5, 12.5 and 25 g) using dairy cows of 475–525 kg. This resulted in a dose-related increase in serum levels with maximum levels of 6, 16 and 37 µg/mL, respectively, as determined with the microbiological assay. Corresponding half-lives were 146, 213 and 285 minutes, being much shorter than after i.m. treatment with the same dosage. Depending on the performance of the udders, milk levels at 2 hours were 113 and 1 836 µg/mL for fully and partly functioning quarters, but decreased to less than 2 µg/mL at 9 hours in the active quarter and 4.7 µg/mL at 20 hours in the partly active quarter. Levels in the non-injected quarters were much lower.

Using HPLC–UV and the microbiological assay, Nouws et al. (1986) studied the fate of chloramphenicol in five dairy cows and eight ruminant calves, after a single i.m. injection of chloramphenicol or chloramphenicol sodium succinate (50 mg chloramphenicol eq./kg b.w.). The use of β-glucuronidase allowed for estimation of the glucuronide. The HPLC–UV method and the microbiological assay showed excellent agreement, confirming that the latter only detects the parent drug. In the dairy cows plasma and milk levels of chloramphenicol were similar and followed a similar pattern, with a half-life of 10 hours. The highest level in plasma was 13 µg/mL, and in milk was 10 µg/mL. The glucuronide was detected in plasma at a highest level of 5 µg/mL and levels became similar to those of the parent drug after 30 hours. In milk no glucuronide was detected. Chloramphenicol sodium succinate showed similar kinetics but somewhat lower levels and half-life. This preparation also caused more irritation at the injection site. Treated calves were slaughtered after seven days but no chloramphenicol could be detected in liver, kidney and muscle (< 0.002 µg/g). The injection sites contained 0.001–0.04 % and 0.002–0.004 % of the applied dose of chloramphenicol or the ester. This would amount to levels around 1–4 µg/g (assuming 1–4 kg meat from the injection site).

Burrows et al. (1984) treated calves of different ages i.v. with 25 mg/kg b.w. chloramphenicol. They observed an age-dependent decrease in plasma half-lives, varying between 450 minutes at age one to three days and 150 minutes at age 275 days. When calves were given a single dose either i.v., i.m. or s.c., plasma levels of 80, 11 and 7 µg/mL were observed, respectively, as determined by a microbiological assay. Similar results were obtained in a second study (Burrows et al., 1988).

Epstein et al. (1986) treated calves with an i.m. dose of 6.8 or 13.6 mg/kg b.w for two days. Animals (two per group at each time point) were slaughtered 2, 6, 24, 48 and 72 hours after the last treatment and the injection site and two muscles (shoulder and gluteal) were sampled. Levels of chloramphenicol in the muscle tissue, as determined by GC, were initially two and six hours in the range 1–13 mg/kg but decreased to 0.2–1.4 mg/kg at 72 hours (one higher level of 7.7 mg/kg). Levels in the injection site were initially very high (911/918 and 2 250/3 390 mg/kg at two hours in the 6.8 and 13.6 mg/kg b.w. groups, respectively) but decreased to 19.4/1.8 and 1.8/23.9 mg/kg at 72 hours. Treatment of one calf with 13.6 mg/kg b.w. twice in 24 hours via i.v. injection resulted initially in much higher levels in muscle, which then decreased rapidly to 0.37 and 0.08 µg/g in the two muscle tissues.

To investigate the time-related decrease in residue levels in various tissues, Korsrud et al. (1987) treated calves i.m. with 11 mg chloramphenicol/kg b.w. twice daily for three days. Using a GC method with an LOQ of 5 ng/g, varying levels (< 5 to 218 ng/g) were observed in muscle tissue of four calves slaughtered five days after the last treatment and levels up to 95 µg/g in injection sites. Levels in liver and kidney were below the LOQ in most animals. Animals killed after 21 days showed levels in injection sites of 9–51 ng/g, whereas levels in other tissues were below or around the LOQ. When slaughtered after 70 days no residues were detectable in any of the tissues.

Sanders et al. (1988) treated six yearling bullocks (235 ± 35 kg) with a long-acting preparation of chloramphenicol. Animals were first treated by i.v. injection of 40 mg/kg b.w. After two weeks, they were injected either i.m. or s.c. with a dose of 90 mg/kg, which was repeated after 48 hours. After another three weeks the same treatment was performed but switched between animals (cross-over). After i.v. injection plasma levels rapidly increased to around 80 µg/mL and declined within 48 hours to 7–28 ng/mL. The half-life was around four hours. Plasma concentrations after s.c. or i.m. treatment increased more slowly and reached a plateau after 9–12 hours with levels of 10 to 15 µg/mL. Levels decreased slowly, still being around 1 µg/mL after three days.

Guillot et al. (1989) treated five Friesian dairy cows with i.m. injections of 20 mg chloramphenicol/kg b.w. and 5 mg tetracycline/kg b.w. every 12 hours for three days. Nine crossbred bullocks were treated with similar doses and slaughtered 14, 21 and 35 days after treatment. Chloramphenicol levels in the milk of the dairy cows during the first six milkings were between 2.3 and 3.7 µg/mL, followed by a rapid decline in the next three milkings. The elimination rate was calculated to be 4.7 hours.

Chloramphenicol could not be detected in liver and muscle 14, 21 or 35 days after treatment. Some kidneys at day 14 contained detectable levels (5.3 ± 9.1 ng/g). Injection sites contained even nine-fold higher levels at day 14 and 3.7 ± 3.7 ng/g at day 21, but no detectable levels at day 35.

Delépine and Sanders (1992) treated three one-month-old calves with an i.v. dose of 25 mg/kg b.w. and slaughtered them after 48 hours. Using a newly developed LC/MS method, the parent drug could be detected in meat at levels of 6–11 µg/kg. Although the LC/MS method included nitrophenylaminopropanediol, dehydro-chloramphenicol and nitroso-chloramphenicol, it was not mentioned whether these metabolites were covered by the extraction method and whether they were actually detected in the meat.

Gassner and Wuethrich (1994) treated four female beef-type calves (208–219 kg) with four oral doses of chloramphenicol palmitate, 25 mg/kg b.w. at 12-hour intervals. In contrast to the previous study by De Corte-Baeten and Debackere (1975), chloramphenicol was detected in plasma by HPLC–DAD analysis showing levels rising to about 6 µg/mL between 36 and 48 hours, followed by a rapid decrease with a half-life of 4.5 hours. Based on a similar study with i.v. injection the authors estimated that 30 % of the dose was absorbed as the parent drug. In addition also dehydro-chloramphenicol was reported in blood of all four animals at levels of 3 to 7 ng/mL. Electrochemical detection was used and reported levels were close to the LOD. The CONTAM Panel noted that this is the only study indicating the formation of a reactive intermediate resulting from reduction of the nitro-group, potentially by bacteria in the rumen. However, identification was not based on methods suitable for this purpose.

FAO/WHO (2004b) prepared a report on data obtained from industry for the JECFA evaluation. Studies with ^{14}C-labelled drug (position of the label not specified) applied orally to cattle (50 mg/kg b.w.) revealed that after 96 hours 56 % of the label was excreted via urine and 6 % via faeces. At five hours after the treatment, highest levels of chloramphenicol and metabolites were detected in liver and kidney (77 and 63 µg/g), followed by muscle (31 µg/g), plasma (18 µg/g) and fat (12 µg/g). In plasma, muscle and fat, the parent drug contributed most, while in the liver and kidney also the glucuronide and chloramphenicol alcohol (hydroxyamphenicol) contributed significantly. In addition, small amounts of chloramphenicol base were observed but not the nitroso-chloramphenicol, dehydro-chloramphenicol and dehydro-chloramphenicol base. Protein bound residues were not determined.

In addition to rats and humans, Bories et al. (1983) also studied the metabolism of chloramphenicol in three goats injected i.m. with radiolabelled drug (^{3}H-labelled at C1 in the propanediol moiety). In the urine collected during the first 24 hours, the unchanged drug and the glucuronide were predominant, followed by the acetylarylamine, the oxamic acid, the base and the alcohol. Two major metabolites could be identified in a follow-up study (Wal et al., 1988); one was the 3-sulphate, the other one most likely being a 3-phosphate conjugate. Overall, the percentage of chloramphenicol was 14.2 %, of the glucuronide 36.5 %, of the sulphate 22.4 %, of the presumed phosphate 7.9 %, of the oxamic acid 7.8 %, of the alcohol 2.4 %, of the base 3.1 % and of the acetylarylamine 4.9 %.

Nijmeijer et al. (1990) treated dwarf goats orally with a dose of 50 mg/kg b.w. of chloramphenicol or chloramphenicol palmitate. Blood levels remained just below the desired 5 µg/mL but decreased much more rapidly in the case of the free drug compared with the ester. Based on comparison with other studies, it was concluded that only a small part of the drug was absorbed, due to degradation in the rumen. This was supported by incubation of the drug with ruminal fluid from the same animals, showing a rapid degradation.

Etuk and Onyeyili (2005a, b) examined plasma and tissue levels in Sokoto red goats after a single i.v. dose of 25 mg/kg b.w., applying a colorimetric method. Plasma levels around 30 µg/mL were observed after five minutes following a biphasic decrease with half-lives of 0.13 and 3.6 hours. Initial levels in liver, kidney, lung, heart, spleen and bone marrow were in a similar range as plasma, demonstrating the wide distribution of the drug in the body. Half-lives in these tissues were one to four hours, similar to the half-life in plasma for the second phase. Levels in muscle and brain were much

lower (maximum 4.6 and 0.6 µg/g) but, contrary to the other tissues, showed an increase in the levels during the first hour and much longer half-lives of 24 and 21 hours, respectively. Muscle levels were still around 0.12 µg/g after 10 days but non-detectable at 11 days. Detection limits and specificity of the method towards parent drug and potential metabolites were not discussed.

Dagorn et al. (1990) treated sheep (54 kg) i.v., i.m. or s.c. with a single dose of 30 mg per kg b.w. Half-lives for plasma levels, determined by HPLC–UV) were 1.7, 2.7 and 17.9 hours after i.v., i.m. and s.c. treatment with maximum concentrations of 90, 14 and 3 µg/mL. Animals i.m. treated were slaughtered at different time points to determine tissue levels. Highest levels were observed in the injection site after four hours with a level up to 1 mg/g, decreasing to 100 ng/g after 14 days. Muscle levels were around 10 µg/g at four hours and less than 10 ng/g at 14 days. Levels in other tissues were much lower.

In conclusion, the studies in ruminants, primarily performed by i.v., i.m., s.c. or intramammary injection, show that chloramphenicol is widely distributed in tissues of ruminants, initially resulting in detectable residues in various tissues. However, these studies apply dosages at the mg/kg b.w. level and thus much higher than the potential exposure through feed or straw. Furthermore, studies indicate that oral exposure results in a relatively low bioavailability of the parent drug due to extensive metabolism by the ruminal flora. Relatively little information is available on the metabolites formed by ruminants and in particular the ruminal flora, which are probably capable of reducing the nitro group. It is unclear whether reactive intermediates resulting from nitro reduction could be absorbed, resulting in residues in milk or meat. The limited information indicates that the glucuronide is the most important metabolite. Unpublished studies delivered to FAO/WHO reported that metabolites thought to be involved in the adverse effects of chloramphenicol, were not detectable in various tissues of treated calves.

7.1.4.2. Pigs

There is scant information on chloramphenicol kinetics in swine and most of these data derive from old studies performed with analytical techniques of poor sensitivity. The i.v. administration of 22 mg chloramphenicol/kg b.w. to seven-week-old pigs resulted in a plasma half-life of 55 minutes (Rao and Clarenburg, 1977). A longer half-life (2.6 ± 1 hour) was reported for 12- to 16-week-old crossbred pigs (20–40 kg b.w.) treated with the same dose by the same route (Mercer et al., 1978). The effects of diet composition on the oral bioavailability of chloramphenicol palmitate was studied in 6.5-month-old Large White pigs. Plasma levels of chloramphenicol were measured with a HPLC method 2, 12, and 24 hours after the animals were offered different meals (standard diet, milk or milk + bran) to which 8 g chloramphenicol (corresponding to 200 mg/kg b.w.) was added. The lowest bioavailability was observed in swine fed on the standard diet. When bran was added to milk, remarkably higher plasma levels (up to 17-fold) were detected with respect to those measured in pigs fed milk alone (Bueno et al., 1984). The Vd after i.v. administration to adult Hampshire pigs (22 mg chloramphenicol/kg b.w.) (Davis et al., 1972) or crossbred Swedish Landrace × Yorkshire piglets (25 mg chloramphenicol/kg b.w.) was in the order of 1 L/kg b.w. (Martin and Wiese, 1988). In the cited experiment of Mercer et al. (1978), in which pigs were subjected to a single i.v. dosing (22 mg chloramphenicol/kg b.w.), the elimination half-life in tissues varied from 1.25 hours in kidney to 5.89 hours in fat, ranging from 2.0 to 5.0 hours in most major organs. Significant correlations were found to exist between plasma concentrations and tissues. Tissue chloramphenicol concentrations measured with a fluorometric method (assay sensitivity 0.2 µg/mL) for liver, kidney and muscles averaged 92.4, 85, and 28.5 mg/kg, respectively, in animals sacrificed five minutes after dosing and fell to 7.26, 24.14, and 2.82 mg/kg, respectively, 6 hours post-dosing; 18 hours after treatment, measurable levels of chloramphenicol could be found in muscles only (1.18 mg/kg). Eight hours after the i.v. dosing of eight newborn pigs with 0.52 mg/kg b.w. ^{14}C-labelled chloramphenicol (dichloroacetyl-1,2-^{14}C,D(-)-threo form), liver, kidney, and skeletal muscle radioactivity levels were 2.27-, 2.02-, and 1.09-fold higher than those found in blood, respectively (Appelgren et al., 1982).

Data on the kinetics and metabolite formation of chloramphenicol in pigs may be derived from an unpublished study reported in the FAO/WHO evaluation (FAO/WHO, 2004b). After the administration of a single oral dose of 50 mg of [^{14}C]-chloramphenicol/kg b.w. (position of the label, number of animals, breed and weight not specified), the peak concentration (5.1 mg/L) was reached after 3 hours; 96 hours after dosing, only about 60 % of the administered drug was excreted in urine (53.5 %) and in faeces (5.7 %) indicating a tendency for chloramphenicol to be accumulated in tissues. Three hours after dosing, the parent drug was the most abundant compound in muscles[35] (about 5 mg/kg), while chloramphenicol glucuronide predominated in fat and kidney, where it amounted to nearly 80 % of total residues, reaching concentrations around 4 and 1 mg/kg, respectively. Chloramphenicol base (deacetylated chloramphenicol) and chloramphenicol alcohol were also detected in muscle, liver and kidney, but only in the latter at concentrations higher than 1 mg/kg.

In a tissue depletion (unpublished) study included in the FAO/WHO evaluation (FAO/WHO, 2004b), 12 pigs (average weight 27 kg) were fed twice daily with an in-feed dose of 25 mg unlabelled chloramphenicol/kg b.w. Tissue residues of the parent drug as well as chloramphenicol base and chloramphenicol glucuronide were measured with a HPLC–UV method in samples of muscle liver, kidney, and fat from animals slaughtered 3, 7, 10, or 21 days (n = 3 for each time point) after withdrawal of treatment. The results, presented as range values, are difficult to interpret because of a relatively wide scatter and low number of experimental animals. Chloramphenicol residues in the µg/kg range (10–50) were detected at all time points in the liver only. In muscle, residues appeared to increase with time, reaching the highest concentrations (40–270 µg/kg) 10 days after withdrawal of treatment, and a similar trend was observed in fat, where measurable levels (> 5 µg/kg) could be observed only in pigs seven days or more after withdrawal of treatment. Unlike what was observed after a single i.v. administration with labelled chloramphenicol (FAO/WHO, 2004b), chloramphenicol glucuronide accumulated to a greater extent in liver and kidney (up to 430 and 370 µg/kg, respectively) irrespective of the day of slaughtering; chloramphenicol base (20–180 µg/kg) was still present in muscle, liver, and fat specimens collected 21 days after withdrawal of treatment.

A survey was performed at three Canadian abattoirs on 279 hogs showing evidence of infection at the time of slaughtering, on the assumption that they might have been treated with antibiotics (Salisbury et al., 1988). Samples of injection sites, muscle, and kidney were analysed for chloramphenicol by a GC method and further confirmed by a GC–MS method. Of 279 pigs tested, 31 (11 %) were found to contain chloramphenicol residues in one or more tissues ranging from 1 to 5 727 µg/kg (found in an injection site). Of the four muscle samples containing chloramphenicol residues, one was in the range 5–10 µg/kg, two in the range 10–50 µg/kg, and one in the range 100–500 µg/kg.

Overall, based on studies performed in pigs of different ages treated with chloramphenicol at doses formerly used therapeutically, the data indicate that chloramphenicol is widely bioavailable by the oral route and is distributed in all edible tissues. According to a limited dataset, residues of the parent drug and its main metabolites (chloramphenicol base and chloramphenicol glucuronide) are slowly depleted and may be still detected in the µg/kg range several days after withdrawal of treatment. No peer-reviewed publications are available on the generation and/or the presence of reactive metabolites in edible tissues or the kinetics of the drug after oral exposure to very low concentrations. However, unpublished studies delivered to FAO/WHO reported that metabolites thought to be involved in the adverse effects of chloramphenicol, were not detectable in various tissues of treated pigs.

7.1.4.3. Poultry

For therapeutic purposes, amphenicols[36] are usually administered to poultry in feed or water (Botsoglou and Fletouris, 2001). Despite that, data on chloramphenicol kinetics following the exposure by the oral route are scant in avian species, and very few reports are available for species

[35] All figures from the cited study derived by eye from a bar graph.

[36] Amphenicols are a group of antibiotics with a phenylpropanoid structure; besides chloramphenicol, they include florfenicol and thiamphenicol.

other than chickens. Based on comparative data obtained after i.v. administration of comparable dosages, remarkable differences in the kinetics of chloramphenicol occur between farmed mammalian and avian species, which show shorter plasma half-life, smaller volume of distribution and higher clearance rates compared with the former (Dorrestein et al., 1984).

The bioavailability, pharmacokinetics and residues of chloramphenicol in 40 day-old male broiler chickens have been studied by Anadón et al. (1994) by means of a HPLC method. Orally administered chloramphenicol is rapidly but incompletely absorbed in broiler chickens. At a single oral dose of 30 or 50 mg/kg b.w., the drug reached the maximum plasma concentration at 0.72 hours or 0.60 hours, and was eliminated with a mean half-life of 6.87 or 7.4 hours. A chloramphenicol concentration > 5 µg/mL was achieved in plasma at 15 minutes, and persisted up to two or four hours after drug administration The disappearance of chloramphenicol from the plasma of chickens could be described by the two-compartment open model. A limited bioavailability (29–38 %) was determined, pointing to a low absorption rate and an extensive first-pass effect. The levels of the parent drug and of three of the main metabolites, i.e. dehydro-chloramphenicol, NPAP and nitroso-chloramphenicol were measured in chickens receiving an oral dose of chloramphenicol at 50 mg/kg b.w. once daily for four days. One day after the last dosing, the sum of the three metabolites measured in plasma was almost 50 % higher than that of the active drug. The study found a slow clearance of residues from kidney, liver, and muscle as measurable levels of the drug and much higher concentrations (10- to more than 350-fold) of NPAP and nitroso-chloramphenicol (at µg/g level) were found up to 6 days and, for metabolites only, even 12 days after the last dose. Evidence has been presented supporting an active role of such metabolites and dehydro-chloramphenicol in mediating the haematotoxicity of the drug (Isildar et al., 1988a, b; Yunis, 1988). Five hours after a single intracrop dosing of 100 mg of [^{14}C]-chloramphenicol (dichloroacetyl-1,2-^{14}C,D(-)-threo form), corresponding to about 66 mg/kg b.w., in White Leghorn chickens (n = 2), 70 % of the administered dose was eliminated and average tissue chloramphenicol equivalent residues in the µg/g range were found in kidney (69.4 µg/g), liver (61.5 µg/g), thigh muscle (13.30 µg/g), breast muscle (14.05 µg/g), and ovarian yolk (11.57 µg/g) (Akhtar et al., 1996). The main metabolites that could be recovered in tissues were chloramphenicol glucuronide and, to a far lesser extent, chloramphenicol base, while the generation of chloramphenicol alcohol represented only a minor pathway. The presence of other metabolites (dehydro-chloramphenicol, NPAP or nitroso-chloramphenicol) was apparently not identified.

In an earlier study (Sisodia and Dunlop, 1972) seven-week-old broiler chickens were given water containing chloramphenicol (40 mg/L, corresponding approximately to 4.8–7.2 mg/kg b.w. per day, assuming a drinking water consumption of two to three times the feed intake (EFSA FEEDAP Panel, 2010), a feed intake of 120 g per day and a body weight of 2 kg for broilers (EFSA FEEDAP Panel, 2012)) for five days. As measured with a colorimetric method (LOQ = 0.1 µg/g), chloramphenicol levels of about 0.2 µg/g in muscle, liver and skin + fat, and about 0.6 µg/g in kidney were detected at the end of the study. The antibiotic was not detectable after 48 hours in the muscle and liver, and after eight hours in skin + fat, but was still present in measurable concentrations (around 0.3 µg/g) in the kidney 72 hours after removing the medicated water.

Data provided by industry on the kinetics and metabolite formation of chloramphenicol in poultry may be derived from an unpublished study reported in the FAO/WHO evaluation (FAO/WHO, 2004b). In a single administration study, birds received ^{14}C-labelled chloramphenicol (100 mg/kg b.w.; position of the label not specified). After 24 hours, a rapid excretion of the drug could be demonstrated (94 % and 82.5 % in male and female excreta, respectively). At five hours post-treatment, highest levels of chloramphenicol equivalents were found in liver (114 µg/g) and kidney (106 µg/g), followed by muscle (43 µg/g), plasma (35 µg/g) and skin (28 µg/g). Chloramphenicol alcohol was the most abundant metabolite, with remarkable amounts (around 20 µg/g)[37] in liver, kidney and plasma, while chloramphenicol glucuronide could be detected only in liver and kidney. Lower amounts of chloramphenicol base (in the range 2.5–7 µg/g) and of two unidentified metabolites could be found in liver, kidney and muscle. Nitroso-chloramphenicol, dehydro-chloramphenicol and dehydro-

[37] As derived by eye from a bargraph.

chloramphenicol base were not detected at the level of sensitivity of the assay. Protein-bound residues were not determined.

Data from a tissue depletion study were also cited in the same report (FAO/WHO, 2004b). The birds received 100 mg chloramphenicol/kg b.w. per day for 4 days and were slaughtered 1, 3, 10, or 17 days after treatment was stopped; residues of the parent compound, of the glucuronide derivative, and of chloramphenicol base were measured in muscle, liver, kidney, fat and skin by a HPLC–UV method. Chloramphenicol alcohol (the most abundant metabolite in the single dosed birds) or other toxic metabolites were not measured. Contrary to what was observed in the single administration study, where residues in the µg/kg range could be found in all tissues five hours after dosing, as early as one day after treatment withdrawal, no measurable residues (< LOQ) of chloramphenicol and the selected metabolites were found in all tissues except for skin, where residues up to 1 340 µg/g persisted up to 17 days after cessation of treatment. No explanation for this finding has been provided.

The effects of disease on chloramphenicol kinetics has been studied by Atef and co-workers (1991), who reported a prolonged elimination half-life (26.21 ± 0.2 vs. 8.32 ± 0.5 hours) and a reduced body clearance (0.24 ± 0.01 vs. 0.75 ± 0.03 mL/kg per minute) in *Escherichia coli*-infected Hubbard chickens versus non-infected ones following the i.v. administration of 20 mg chloramphenicol succinate/kg b.w.

The excretion of antimicrobials in eggs (including chloramphenicol) has been reviewed (Kan and Petz, 2000; Goetting et al., 2011). The limited number of studies performed in laying hens demonstrate that chloramphenicol residues at µg/g level are found in both yolk and albumen during the treatment and up to for several days after withdrawal of treatment. A trial was performed on 12-month-old laying hens, which were fed (a) 200, (b) 500, (c) 800 or (d) 1 000 mg chloramphenicol (chemical form not specified)/kg diet for five days (Samouris et al., 1998). As measured by a HPLC method, chloramphenicol residues were detected in the albumen from the first day of administration and in the yolk from the second day and persisted up to four and nine days after termination of treatment in albumen and yolk, respectively. Mean chloramphenicol concentrations of 0.052, 0.52, 0.46 and 1.23 mg/kg were measured in the albumen and 0.37, 1.69, 2.02, and 3.69 mg/kg in the yolk from birds treated with the a, b, c or d diet, respectively.

As reported for other species, the concurrent administration of chloramphenicol and other drugs may result in the inhibition of the biotransformation rate of such drugs. Five-day-old broiler chicks were offered a diet supplemented with chloramphenicol (500 mg/kg) and lasalocid (80 mg/kg), an ionophore antibiotic widely used as a coccidiostat in avian species. Clinical signs suggestive of neuromuscular toxicity (impaired gait and leg weakness) were recorded by day 12 of treatment in about half of the chicks (Perelman et al., 1986), resembling those already described in broilers upon the treatment with a combination of ionophores (i.e. monensin) and other known CYP inhibitors such as tiamulin (Umemura et al., 1984a) and oleandomycin (Umemura et al., 1984b), respectively. Likewise, pre-treatment with 100 mg chloramphenicol/kg i.m. significantly increased the duration of xylazine + ketamine anaesthesia in six- to seven-week-old broiler chickens (Roder et al., 1993).

Data on the kinetics of chloramphenicol in turkeys may be derived from a comparative study in which 12-week-old female broiler turkeys were administered a single i.v. or oral (p.o.) dose (30 mg/kg b.w.) of florfenicol, thiamphenicol or chloramphenicol, respectively (Switala et al., 2007). Plasma concentrations were measured with a HPLC method, and the pharmacokinetic parameters calculated according to a non-compartmental model. Higher plasma clearance and lower volume of distribution values, but a similar oral bioavailability were found in turkeys as compared with the results from a study performed in chickens using the same administration routes and the same chloramphenicol dosage (30 mg/kg b.w.) (Anadón et al., 1994). No published reports concerning tissue distribution or residue depletion could be identified.

Three adult male Muscowy ducks (3.5 ± 0.5 kg b.w.) were s.c. injected with 500 mg [^{3}H]-chloramphenicol (labelled in the phenyl ring at positions 3 and 5) and excreta were collected for

the subsequent 24 hours. Metabolites were quali-quantitatively determined with various chromatographic and MS techniques. The elimination of the radiolabel occurred extensively within the 24-hour period (65 %); the main metabolites were chloramphenicol oxamic acid and chloramphenicol alcohol, which together accounted for about one-third of the recovered radioactivity. The remainder radioactivity was due to the parent compound (15 % of the dose) and chloramphenicol base (5 % of the dose), and to several other minor metabolites. Among the latter, chloramphenicol glucuronide and chloramphenicol sulphate (already detected in mammals) were identified together with a number of previously unidentified derivatives including chloramphenicol oxamylglycine, chloramphenicol oxamylethanolamine and metabolites resulting from acetylation of chloramphenicol or chloramphenicol base (Cravedi et al., 1994). No data were provided about tissue disposition and residue depletion.

The tissue and egg distribution of [^{14}C]-chloramphenicol (dichloroacetyl-1,2-^{14}C,D(-)-threo form) after a single i.v. (approximately 0.8 mg/kg b.w.) or oral (approximately 0.7 mg/kg b.w.) chloramphenicol administration were investigated in the Japanese quail (*Coturnix coturnix japonica*) by Appelgren and co-workers (1985) using autoradiography. Animals were sacrificed at different time points, i.e. 20 minutes, one hour, four hours, 4 days, or 10 days after treatment. A good agreement was found between the qualitative distribution patterns after i.v. and oral administration. On the whole, liver and kidney displayed higher ^{14}C concentrations than muscle, which, after 10 days, appeared almost free of radioactivity. Egg yolk and albumen showed highest ^{14}C concentrations 2–4 days and 1–2 days after treatment, respectively, and measurable radioactivity could still be found in the yolk after 11 days.

In conclusion, a limited number of studies are available concerning the kinetics, tissue distribution, and residue depletion after oral exposure to chloramphenicol in avian species, and most of these were performed in chickens treated with chloramphenicol at doses formerly used therapeutically or at lower doses. In such species, oral dosing with chloramphenicol is characterised by a limited bioavailability (35–45 %) and a remarkable first-pass effect. The parent drug and different metabolites have been detected in liver, muscles and eggs up to several days after cessation of treatment. The presence of toxic metabolites in plasma and edible tissues of chickens administered with chloramphenicol at doses formerly used therapeutically for four consecutive days has been reported in one study. However, unpublished studies delivered to FAO/WHO (2004b) reported that metabolites thought to be involved in the adverse effects of chloramphenicol, were not detectable in various tissues of broilers.

7.1.5. Horses

Before the ban on the use of chloramphenicol in the horse as a food-producing species, commercial preparations for oral, i.m. or i.v. administration were widely available. However, after reviewing the published literature, Page (1991) concluded that the short half-life of chloramphenicol in the horse, together with the broad range of minimum inhibitory concentrations for target pathogens did not support the routine use of chloramphenicol as an appropriate antibiotic for systemic use in the equine species. Accordingly, i.v. administration of chloramphenicol is not indicated for horses due to the short half-life (less than one hour, Sisodia et al., 1975), which precludes achieving therapeutic plasma concentrations for most pathogens. Injections of chloramphenicol i.m. are associated with severe pain in horses and are not recommended. Therefore, it seems unlikely that chloramphenicol is used in horses. Unlike that reported for ruminants, in which no measurable plasma levels are noticed after oral application due to a remarkable ruminal degradation (see Section 7.1.4.1), a single oral administration of chloramphenicol (10 mg/kg b.w.) to adult horses results in a rapid absorption reaching peak plasma levels in about half an hour (De Corte-Baeten and Debackere (1975). A rapid and extensive absorption (83 %) after p.o. administration has been reported in foals administered 50 mg chloramphenicol/kg b.w., with mean peak serum concentrations of 6.1 µg/mL and a serum half-life of 1.44 hours (Buonpane et al., 1988). Similar half-life values (1.8 hours) but much higher serum peak concentrations (18.0 µg/mL) were documented in adult horses receiving chloramphenicol intragastrically at the same dose rate (Gronwall et al., 1986). As in other species, chloramphenicol distributes widely throughout the body. The highest drug levels are attained in the liver and kidneys, but effective drug concentrations are attained in most tissues and fluids, including the ocular humours

and synovial fluid, as well as cerebrospinal fluid where it may achieve levels of up to 50 % or even more (in the case of meningitis) of plasma concentrations. The Vd of chloramphenicol is 1.41 L/kg b.w. in adult horses and 1.6 L/kg b.w. in neonatal foals. Hepatic metabolism (glucuronic conjugation) has been documented followed by active renal tubular secretion, with only 5–15 % of the drug being excreted unchanged (glomerular filtration) in urine (Dowling, 2004). Based on the known inhibitory effects on a number of CYPs, a variety of drug interactions have been reported in chloramphenicol-treated horses concurrently administered with drugs that are substrates of the inhibited enzymes, such as xylazine (Grubb et al., 1997), or phenytoin, phenobarbital, pentobarbital and cyclophosphamide (Dowling, 2004).

In conclusion, when administered by the oral route to ponies or adult horses, chloramphenicol was rapidly and extensively absorbed and widely distributed to tissues. However, no specific studies are available on tissue disposition and carry-over of the drug in the equine species.

7.1.6. Fish

Cravedi and Baradat (1991) studied the metabolic pathways of chloramphenicol in rat and trout hepatocytes. The metabolic profiles were similar, however the biotransformation rate was considerably slower in trout hepatocytes (ca 25 % of the dose vs. ca 85 % of the dose in rat hepatocytes after two-hour incubation).

The urinary and faecal excretion, tissue distribution and metabolism of ^{3}H-labelled chloramphenicol (label in the propanediol moiety at C1) were measured in rainbow trout (*Oncorychus mykiss*) after a single 50 mg/kg b.w. intragastric dose (Cravedi et al., 1985). The major route of excretion was faecal (64.3 % of the dose), with approximately 16 % in the urine in five days. Radioactivity was widely distributed in trout tissues and organs, the highest concentrations were in the bile and intestine. At 48 hours after dosing, the radioactivity remaining in the liver, the muscle and the perigastric adipose tissue was as chloramphenicol-derived compounds bound to tissues. In addition to unchanged chloramphenicol (4.3 % of the dose after 96 hours), the other metabolites excreted in the urine were chloramphenicol base (5.2 %), chloramphenicol alcohol (4.0 %) and chloramphenicol glucuronide (1.8 %).

Bilandžić et al. (2012) examined the depletion of chloramphenicol from muscle of rainbow trout following four days of oral administration with two doses (42 and 84 mg/kg b.w. per day). Sampling was conducted during treatment and for 35 days following the end of treatment. Concentrations measured during treatment exceeded 300 µg/kg muscle tissue, and were dose dependent. A significant elimination occurred within nine days following the cessation of treatment in both groups and chloramphenicol was not detectable after 13 and 15 days. In contrast, Biancotto et al. (2009) found considerably slower depletion from muscle of rainbow trout fed a diet containing 73.9 mg/kg b.w. per day and administered for 10 days, with concentrations in the range of the RPA up to 31 days after the end of treatment.

The pharmacokinetics of chloramphenicol in carp (*Cyprinus carpio* L.) has been investigated following i.m. injection of 80 mg/kg b.w. (Huang et al., 2006). Chloramphenicol was readily assimilated and the order of absorption rate constant was liver > serum > gill > kidney > muscle. Elimination half-lives were 22.3, 15.5, 14.9, 9.3 and 5.3 hours for the liver, serum, gill, muscle and kidney, respectively. The elimination half-lives after five days of multi-doses of repeated chloramphenicol injection with 40 mg/kg b.w. were 26.4, 21.4, 20.9, 11.5 and 14.9 hours for the five tissues, respectively. In the warm water species gilthead seabream (*Sparus aurata*) after an i.v. injection of 10 mg/kg b.w. the distribution half-life and elimination half-life were 1.6 and 69 hours, respectively, for muscle tissue (Tyrpenou et al., 2003).

In conclusion, metabolism of chloramphenicol in fish is dependent on species and a variety of environmental factors, such as water temperature and water flow.

7.1.7. Companion animals

In dogs, chloramphenicol crystalline or chloramphenicol palmitate are administered via the oral route (50–150 mg/kg b.w. every 8–12 hours); both are rapidly absorbed, reach serum peak levels approximately 30 minutes after dosing and are widely distributed in most tissues and fluids, including the aqueous and vitreous humour, the synovial fluid, and the CNS. The ester is rapidly hydrolysed to release the active drug. The Vd has been reported as 1.8 L/kg b.w. (Plumb, 2011). The principal biotransformation pathway is liver glucuronidation, resulting in the formation of chloramphenicol glucuronides which, unlike the situation in humans, are excreted to a considerable extent (50 %) also via the bile together with other metabolites (mainly amino derivatives originating from the nitro-reduction of chloramphenicol) and possibly chloramphenicol, giving rise to enterohepatic circulation (Danopoulos et al., 1954). Eight hours after the oral administration of 99 mg of chloramphenicol sodium succinate/kg b.w., dogs excrete on average 6.3 % of the unchanged drug in the urine as measured by a microbiological method (Ling et al., 1980). The elimination half-life has been reported as 1.1–5.0 hours (Plumb, 2011). The chloramphenicol-mediated "suicide" selective inhibition of CYP2B11, the major phenobarbital-inducible liver CYP, has been described in dogs (Ciaccio et al., 1987; Hay Kraus et al., 2000), with the potential for the inhibition of other CYPs. This entails a number of *in vivo* drug–drug interactions which are of clinical relevance since many anaesthetic and neuroleptic agents (e.g. barbiturates, propofol, ketamine and midazolam) are known CYP2B substrates (Baratta et al., 2009). For example, the i.v. dosing of greyhound dogs with 50 mg of chloramphenicol sodium succinate/kg b.w. 30 minutes before a bolus i.v. injection of propofol (10 mg/kg b.w.) followed by a two-hour i.v. infusion of propofol (0.4 mg/kg per minute) resulted in a reduced clearance of the anaesthetic (about 50 %) and a dramatic increase in the recovery times (about 10 times) (Mandsager et al., 1995). Similar interactions have been described with thiopentone (Aravindakshan and Cherian, 1984) or phenobarbital (Houston et al., 1989).

Less information is available for cats. As for the dog, the oral route would be preferred over the parenteral one owing to a relatively higher bioavailability of the drug, which is easily distributed in tissues and body fluids showing Vd values (2.4 L/kg b.w.) similar to those found in the canine species. Owing to the lack of UGT1A6 and UGT1A9, the consequent poor capacity in the cat for efficient glucuronidation of several drugs, including chloramphenicol (Court, 2013) results in a relatively higher amount of the drug remaining unconjugated compared with other species. This is consistent with the higher peak plasma values compared to other domestic animal species investigated, the relatively long elimination half-life (four to eight hours) and the high extent of the urinary excretion of the unchanged drug (25 % or more) (Davis et al., 1972). In addition, the appreciable amount of chloramphenicol escaping conjugation is expected to enter the bioactivating reductive and/or oxidative pathways and would consequently predispose the cat to develop chloramphenicol toxicosis. Accordingly, the cat is considered the most sensitive domestic species to the haematological effects of the antibiotic (Watson, 1980). Prolongation of the anaesthetic effects of pentobarbital and other compounds have been reported in therapeutically treated cats (Trepanier, 2006).

7.1.8. Carry-over and potentially toxic and bound residues

7.1.8.1. Cows

In ruminants most studies were performed with injections of chloramphenicol. Residues in various tissues were described, in most cases of the parent compound only. However, these studies seem less relevant for the oral exposure of ruminants, where early studies indicated complete degradation in the rumen and no absorption of the parent compound (De Corte-Baeten and Debackere, 1975). A more recent study by Gassner and Wuethrich (1994) with chloramphenicol palmitate indicated that 30 % of the orally applied drug was absorbed intact. The unpublished studies submitted for assessment to FAO/WHO (2004b) confirmed that at least part of the orally applied drug is absorbed intact and can be recovered in the muscle, fat, liver and kidney. A dose of 50 mg/kg b.w. radiolabelled chloramphenicol (position of the label not specified) led to muscle, liver, kidney and fat levels of 31, 77, 63 and 12 mg/kg in animals slaughtered five hours after the treatment, expressed as chloramphenicol equivalents. The parent compound represented about 75, 25, 20 and 65 % of the

radiolabel in muscle, liver, kidney and fat, respectively. Metabolites detected were the glucuronide, chloramphenicol base, chloramphenicol alcohol and some unidentified compounds. However, the nitroso-chloramphenicol, dehydro-chloramphenicol and dehydro-chloramphenicol base were not detected. From the description of this study, the age of the calves is unclear and also whether the rumen was fully developed. The slow plasma clearance of the radiolabel reported in that study might suggest the occurrence of some bound residues, but no specific data were provided.

Chloramphenicol has been detected in milk after i.m. treatment (50 mg/kg b.w.) with levels up to 10 µg/mL (Nouws et al., 1986). Guillot et al. (1989) observed levels between 2.3 and 3.7 µg/mL after i.m. treatment of Friesian cows with 20 mg/kg b.w. No studies were identified with oral application of the drug.

7.1.8.2. Pigs

FAO/WHO (2004b) also describes a study performed with pigs given an oral dose of 50 mg/kg b.w. of radiolabelled chloramphenicol (position of the label not specified). As in calves most of the radiolabel was excreted in the urine. Levels of the radiolabel expressed as chloramphenicol equivalents in tissues collected three hours after the treatment were 6, 19, 102 and 5 mg/kg in muscle, liver, kidney and fat, respectively, with contribution of the parent drug of roughly 75, < 1, 4 and 25 %. Metabolites detected were the glucuronide, chloramphenicol base, chloramphenicol alcohol and some unidentified compounds. Again, the nitroso-chloramphenicol, dehydro-chloramphenicol and dehydro-chloramphenicol base were not detected. Since only about half of the administered dose was cleared from the plasma in four days, the accumulation of some bound residues might be suggested, but no specific data were provided.

A second study described by FAO/WHO (2004b) concerned 12 pigs of about 27 kg treated via feed twice daily at a dose of 25 mg/kg b.w. for 3.5 days. Animals were slaughtered 3, 7, 10 and 21 days after the last treatment. In muscle, the parent compound could only be detected in animals killed after 10 days (40–270 µg/kg, LOD 10 µg/kg). In liver and fat, chloramphenicol could be detected in some animals even at 21 days, whereas in kidney this was only the case at day 3. Both the glucuronide and chloramphenicol base were detected in liver and kidney, at higher levels than the parent compound, in livers up to day 21. For kidney these results were more variable. In general, the results were not very clear in terms of time.

7.1.8.3. Broilers

Anadón et al. (1994) treated male broilers orally by gavage with 50 mg/kg b.w. for four consecutive days. Animals were slaughtered 8 hours and 1, 2, 6 and 12 days after the last treatment. Residues of the parent compound reached highest levels in muscle, liver and kidney of, respectively, 1.8, 1.1 and 1.9 mg/kg after one day and then decreased to 0.43, 0.026 and 0.043 mg/kg at six days and below LODs (5 µg/kg) after 12 days. The levels at six days are still much higher than the current RPA of 0.3 µg/kg. In addition also the nitroso-chloramphenicol, dehydro-chloramphenicol and NPAP were detected in the various tissues at six days and in liver and muscle even at 12 days. Interestingly, the levels of NPAP and nitroso-chloramphenicol increased in time and reached highest levels at six days, being, respectively, 6.1 and 6.1 mg/kg in muscle, 9.7 and 8.1 mg/kg in liver and 3.5 and 3.3 mg/kg in kidneys. After 12 days, the levels of NPAP decreased by a factor 2.2, 3.2 and 1.7 in muscle, liver and kidney, respectively, still being in the 2 to 3 mg/kg range. The levels of nitroso-chloramphenicol decreased by factors of 23, 11 and 1.6 in muscle, liver and kidney, respectively, thus showing a more rapid decline especially in muscle and livers.

FAO/WHO (2004b) described an unpublished study where poultry was treated orally with radiolabelled chloramphenicol (position of the label not specified) at a dose of 100 mg/kg b.w. In animals slaughtered five hours after the treatment, this resulted in levels of radiolabel (chloramphenicol equivalents) of 43, 114, 106, 10 and 28 mg/kg in muscle, liver, kidney, fat and skin, with the parent drug representing roughly 75, 30, 20, 60 and 50 % respectively. Metabolites detected were the glucuronide, chloramphenicol base, chloramphenicol alcohol and some unidentified

compounds. The nitroso-chloramphenicol, dehydro-chloramphenicol and dehydro-chloramphenicol base were not detected.

Five hours after a single intracrop dosing of 100 mg of [^{14}C]-chloramphenicol (dichloroacetyl-1,2-^{14}C,D(-)-threo form) (corresponding to about 66 mg/kg b.w.) to White Leghorn chickens (n = 2), less than 50 % of the radioactivity in liver was extractable and less than 30 % in the kidney (Akhtar et al., 1996), indicating the potential for bound residues.

7.1.8.4. Laying hens

Samouris et al. (1998) provided laying hens with feed containing 200, 500, 800 or 1 000 mg chloramphenicol/kg for five days. Eggs were analysed for the parent compound, showing about three-fold higher levels in the yolk than in the white. In addition, the levels in the yolk decreased much more slowly after a switch to clean feed, where it took about 10 days to obtain eggs without the drug. This is clearly related to the time period of up to 10 days required to produce an egg. Highest levels in the egg yolk were, respectively, 0.15, 1.7, 2.0 and 3.6 mg/kg for the four different feed levels. The results are in line with the previous study by Petz (1984) with laying hens that obtained medicated feed (400 mg/kg chloramphenicol) for 14 days. This resulted in maximum eggs levels around 1 mg/kg (total egg), which decreased to below the LOQ (0.02 mg/kg) in about eight days. Egg yolk contained three-fold higher levels than egg white.

7.1.8.5. Potentially toxic metabolites

There is limited and controversial information about the generation of potentially toxic metabolites in food-producing species and their eventual accumulation in edible tissues and animal products, most of the available data on residues being restricted to the parent molecule. In addition, the apparent contradiction between the reactivity of certain metabolites (e.g. nitroso-chloramphenicol) and their detection in animal tissues (Anadón et al., 1994), as well as the origin of such metabolites (i.e. from tissue or gastro-enteric bacteria), are still a matter of debate or are simply not known. Compared with other metabolites, nitroso-chloramphenicol, dehydro-chloramphenicol and, to a lesser extent, dehydro-chloramphenicol base and NPAP are characterised by higher cyto- and genotoxic potency (Lafarge-Fraissinet et al., 1994, Jimenez et al., 1987; Isildar et al., 1988b; Robbana-Barnat et al., 1997). The presence of all such metabolites except dehydro-chloramphenicol base has been demonstrated in kidney, liver and muscle samples from broiler chickens orally administered 50 mg chloramphenicol/kg b.w. once a day for four consecutive days, nitroso-chloramphenicol and NPAP being still detectable in all examined tissues at mg/kg level (0.26–3.04) 12 days after the last dosing (Anadón et al., 1994). Comparative unpublished studies in poultry, calves and pigs on tissue distribution of chloramphenicol and its metabolites have been reported by JECFA (FAO/WHO, 2004b). Animals were treated with a single oral dose of 50 (calves, pigs) or 100 (poultry) mg chloramphenicol/kg b.w. and sacrificed after three or five hours. None of the abovementioned metabolites could be detected "at the level of sensitivity of the assay" (not specified in FAO/WHO, 2004b).

7.1.8.6. Concluding comments

It can be concluded that exposure of animals to chloramphenicol at doses formerly used therapeutically (typically 25-50 mg/kg b.w. per day) results in residues of chloramphenicol in meat, organs, eggs and milk. In meat, various metabolites were also detected. For broilers, one study also reported the presence of metabolites that are thought to be involved in the adverse effects of chloramphenicol. However, this was not confirmed in other studies reported to FAO/WHO. Assuming a linear relationship between levels of exposure and residue levels in edible tissues/products, it can be calculated that exposure of animals to 1 µg/kg b.w. per day or lower (i.e. more than 25 000 fold lower than doses formerly used therapeutically) is unlikely to result in residue levels in meat, organs, milk and eggs exceeding the current RPA. Various metabolites were identified in carry-over studies with doses of chloramphenicol formerly used therapeutically. There is uncertainty about potential occurrence of residues of genotoxic metabolites in animals.

7.2. Toxicity in experimental animals

7.2.1. Acute toxicity

Smith et al. (1948) investigated acute toxicity of chloramphenicol (chemical form not specified) in mice and dogs. Oral treatment of mice with 1 250 mg/kg b.w. caused tremors and prostration, but the mice recovered after termination of treatment. The median lethal dose (LD_{50}) in mice after i.v. administration of chloramphenicol in propyleneglycol was estimated to 245 mg/kg b.w. Dogs i.v. treated with 50 and 100 mg/kg b.w. chloramphenicol in propyleneglycol. The only effect noticed was a reversible increase in body temperature.

Acute toxicity of chloramphenicol (chemical form not specified) in mice, rats, rabbits and dogs was investigated by Gruhzit et al. (1949). In mice, the oral LD_{50} was 2 640 mg/kg b.w. (n = 270) and the intraperitoneal (i.p.) LD_{50} was 1 320 mg/kg b.w. (n = 450). The LD_{50} after i.v. treatment of mice with chloramphenicol in different formulations was about 110 mg/kg b.w. (fermentation in propylene glycol, n = 455), about 195 mg/kg b.w. (fermentation in water, n = 954) and about 200 mg/kg b.w. (synthetic in water, n = 375). The main findings in mice at doses close to the LD_{50} were incoordination, flaccid prone position and dyspnoea. In rats (n = 1 335), the i.v. LD_{50} was between 170 and 280 mg/kg b.w., depending on formulation (propylene glycol or acetamide) and whether the substance was produced by fermentation or synthetically. In dogs, the oral LD_{50} was > 300 mg/kg b.w. (n = 7), i.m. LD_{50} was > 100 mg/kg b.w (n = 3) for chloramphenicol in peanut oil suspension, > 46.5 mg/kg b.w. (n = 8) for chloramphenicol in propylene glycol and the i.v. LD_{50} was 150 mg/kg b.w (n = 7) for chloramphenicol in propylene glycol. In dogs, the main findings were vomiting, diarrhoea, occasional spasticity and convulsive seizures. In rabbits, only the i.v. LD_{50} was estimated to be about 120 mg/kg b.w. (n = 25) for chloramphenicol in propylene glycol.

Pregnant and non-pregnant mice (Charles River CD-1) were treated with chloramphenicol succinate by the i.v. route in at least four dose groups (n = 5). No signs of toxicity were found. The LD_{50} was determined to be 1 530 (1 260–1 840) mg/kg b.w. for non-pregnant mice and 1 210 mg/kg b.w. for pregnant mice (Beliles, 1972). The fetal LD_{50} was calculated to be 679 (643–716) mg/kg b.w., based on the maternal dose.

Mice (hybrid) were i.v. treated once with 187 (n = 7), 250 (n = 8) or 375 (n = 5) mg/kg b.w. chloramphenicol (chemical form not specified) in dimethylacetamide solution and the mice were killed 18 hours later, except for three animals in the highest dose group, that died within one minute (Lepper et al., 1951). In liver, microscopic changes increased with dose in the two lowest dose groups. The two remaining mice in the highest dose group had moderate changes in the liver. The LD_{50} was not determined in this study.

In summary, the oral LD_{50} was estimated in mice to be 2 640 mg/kg b.w. and neurotoxic effects were observed after acute dosing at 1 250 mg/kg b.w. and higher. In dogs, neurotoxic effects were observed at 300 mg/kg b.w. (orally). Other routes (i.v., i.p., i.m.) of administration to mice, rats, dogs and rabbits resulted in much lower LD_{50} values.

7.2.2. Repeated dose toxicity

Smith et al. (1948) investigated the toxic effects of chloramphenicol (chemical form not specified) in mice treated with 360 and 1 290 mg/kg b.w. per day orally through feed for 14 days. No effects were seen in the low dose but in the high dose the mice lost approximately 15 % of their body weight. The only effects of chloramphenicol (chemical form not specified) in dogs treated orally (n = 1) with 143 mg/kg b.w. and i.m. with 72 to 88 mg/kg b.w. (n = 3) twice daily, five days a week for a 24-day period was a decreased body weight. I.m. treatment caused anaemia in varying degrees in the three animals.

Toxicity of chloramphenicol succinate in newborn mice was compared with adult mice (Kent et al., 1960). Forty-four litters, containing 394 newborn mice (CFW strain), were divided in control groups

and experimental groups. The newborn mice were treated s.c. with doses of 100 to 1 600 mg/kg b.w. per day during five consecutive days (n = 27 to 37). In addition, adult mice were treated s.c. with 230 to 2 400 mg/kg b.w. per day for five consecutive days. The LD_{50} after five days treatment was estimated to be 315 and 1 675 mg/kg b.w. for pups and adults, respectively. It was concluded that newborn mice are much more sensitive to chloramphenicol than adult mice.

Gruhzit et al. (1949) investigated the effects of chloramphenicol in mice, guinea pigs and dogs. Mice were treated orally with chloramphenicol (chemical form not specified) in an acacia–water suspension, in doses of 215 or 311 mg/kg b.w. twice daily for four weeks. The highest dose caused death in 30 % of the animals. Guinea pigs were treated orally with increasing doses of chloramphenicol, 90 mg/kg b.w. per day to 256 mg/kg b.w. per day, during three weeks. The maximum tolerated dose was estimated to be 250 mg/kg b.w. per day. Dogs were treated orally with 75 mg/kg b.w. (n = 3) chloramphenicol (fermentation product, crystalline form), twice daily on six days a week, for 39 days (66 doses) and with 50, 75 and 100 mg/kg b.w. of chloramphenicol (synthetic), twice daily on five days a week, for 133 days (194 doses). All animals were in good condition with no effects on weight gain, behaviour or GI disturbances. Only few deviations in the haematology parameters were found and hydropic changes in capillaries of the glomerula in dogs treated orally with 100 to 200 mg/kg b.w. per day.

Chloramphenicol (chemical form not specified) toxicity was studied in albino Wistar rats (males) treated with chloramphenicol via drinking water for four days (Effiong et al., 2010). The intention of the study was to investigate if coconut water could reduce the toxicity of chloramphenicol in rats. The rats, five in each group, were treated with 0, 50 or 100 mg chloramphenicol/kg b.w. per day. In addition, one group was treated with both coconut water (20 mL/kg b.w. per day) and chloramphenicol (50 mg/kg b.w. per day) and one group was treated only with coconut water (20 mL/kg b.w. per day). The rats were sacrificed the day after the last treatment. Chloramphenicol treatment caused increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) at both dose levels tested, showing that chloramphenicol induced acute effects in liver, kidney and heart in rats after four days treatment. However, no histopathological investigations were performed that could confirm the toxicity.

Chloramphenicol (chemical form not specified) caused a dose-dependent toxicity in liver and small intestine in rats treated with 0, 200, 400 or 600 mg/kg b.w. per day for seven consecutive days (route of administration not mentioned) (Ahmadizadeh et al., 2013). Groups of rats were also treated with 80 mg/kg b.w. phenobarbital or saline 30 minutes before treatment with chloramphenicol in doses of 200, 400 and 600 mg/kg b.w. per day for seven consecutive days. Dose-dependent degenerative changes in hepatic parenchymal cells were found by light microscope. An increase in AST and ALT in serum was found in the rats treated only with chloramphenicol, which confirmed the liver toxicity found at histopathology. When rats were pre-treated with phenobarbital, before chloramphenicol treatment, ALT and AST in serum was lower than with the treatment with chloramphenicol alone. It appeared that phenobarbital protected liver cells from chloramphenicol injury as rat hepatocytes were normal in the groups pre-treated with phenobarbital compared with groups treated with chloramphenicol. Furthermore, chloramphenicol treatment caused damage in enterocytes.

Farombi et al. (2002) investigated the effect of chloramphenicol succinate on the microsomal drug oxidising system *in vivo* and *in vitro*. Male Wistar albino rats (n = 5) were treated orally with chloramphenicol succinate at doses of 28, 57 or 86 mg/kg b.w. per day for 10 consecutive days and sacrificed within 24 hours after end of treatment. Chloramphenicol treatment resulted in a dose-dependent decrease in bodyweight, absolute and relative liver weight, and in protein content of the blood. The cholesterol/phospholipid molar ratio in blood was increased. The activity of a number of CYP-enzymes (aniline hydroxylase, aminopyrine N-demethylase, *p*-nitroanisole O-demethylase, ethoxyresorufin O-deethylase) was dose-dependently decreased. In an *in vitro* study, reported in the same paper, chloramphenicol at concentrations between 0.001 and 0.1 mM inhibited the activity of the same enzymes, with the exception of ethoxyresorufin O-deethylase. In an additional study, in which rats (n = 5) were treated orally with 28 mg/kg chloramphenicol succinate for 10 consecutive days, an

increase in liver microsomal malondialdehyde and lipid hydroperoxide was observed, indicating induction of lipid peroxidation by chloramphenicol (Farombi et al., 2002).

Ebaid et al. (2011) treated rats orally with either saline (control), chloramphenicol sodium succinate (86 mg/kg) for 21 days, chloramphenicol sodium succinate (86 mg/kg) for 21 days followed by *Nigella sativa* oil for 30 days or combined exposure of chloramphenicol sodium succinate (86 mg/kg) and *N. sativa* for 21 days. A reduction of red blood cell parameters (erythrocytes, haemoglobin, haematocrit) and an increase in total leucocytes was found (not all parameters were statistically significant affected). For leucocytes an increase in circulating immature stages (e.g. myeloblasts, myelocytes) was reported. Histology analyses revealed that chloramphenicol caused damage to the spleen and thymus. Spleen histology indicated thickening (fibrosis) of capsule/trabeculae, and shift in cell populations, such as macrophages, neutrophils and megakaryocytes. In conclusion, the study showed signs of haemolytic anaemia and inhibitory action on the bone marrow, which resulted in an increase of the immature leucocytes in the peripheral blood. However, no details of the rats' ages, number, strain or sex were provided and only one dose was used.

Toxicity of chloramphenicol was investigated in rats (Wistar rats, both sexes) treated orally for 16 days with 25 mg/kg b.w. per day chloramphenicol palmitate (n = 10) or saline (n = 6) (Saba, 2000). The activity of the serum enzymes AST and ALT was significantly increased. In addition, hyperbilirubinaemia was observed and up to 80 % bilirubin was conjugated. Degenerative changes of hepatic parenchymal cells indicated that chloramphenicol had a hepatotoxic effect in rats. Only slightly increased serum urea levels were found but no histopathological changes in the kidney were observed.

Swiss albino mice received 100 or 200 mg/kg b.w. per day of chloramphenicol (chemical form not specified), for seven days in drinking water (Ege et al., 2008). Blood samples were taken on days 0, 1, 3, 7 and 14 of the study. Changes in ALT, ALP and AST were found, indicating effects on the liver. However, the effects were reversible and no statistically significant increases in the parameters could be connected with dose and time. No histopathology analysis was conducted.

No longer-term repeated-dose toxicity studies in experimental animals (e.g. 90-day study) were identified.

In conclusion, repeated-dose studies show that oral treatment with chloramphenicol caused hepatotoxicity in rats and mice. Chloramphenicol also caused a concentration dependent inhibition of the activity of some CYP-enzymes in rat liver microsomal fractions. In a limited study in rats with only one dose level (86 mg/kg b.w. per day), signs of haemolytic anaemia as well as an inhibitory action of the bone marrow were found. In addition, histopathological changes in the spleen and thymus were observed. No information about whether the parent compound, chloramphenicol or metabolites are responsible for the liver toxicity could be identified. The most sensitive endpoint in these studies was liver toxicity, with effects found at the lowest tested dose of 25 mg/kg b.w. per day in rats. Consequently, a no observed adverse effect level (NOAEL) for repeated-dose toxicity could not be identified from these studies.

7.2.3. Immunotoxicity based on immune function tests

Few experimental studies have been published on immune disturbance of chloramphenicol, and these studies did not follow the systematic immunotoxicity protocols as is currently customary for immunotoxicity risk assessment, including quantitative aspects (WHO, 2012).

Madan et al. (2007) studied antibody production in male albino rats (8–12 weeks of age) after s.c. bovine serum albumin (BSA) immunisation, following treatment with ciprofloxacin (i.m. administration of 0.33 mg/kg b.w. at 12-hour intervals for five consecutive days) or chloramphenicol (i.m. administration of 0.8 mg/kg b.w. at six-hour intervals for three consecutive days). Compared

with the controls, ciprofloxacin-treated rats showed a reduced immunoglobulin G response during and following treatment; however, no effects were observed for chloramphenicol.

Yuan and Shi (2008) exposed mouse splenocytes *in vitro* to a stimulant (anti-CD3 or Staphylococcal enterotoxin B) with or without chloramphenicol and monitored survival and development (morphology and gene expression). When co-exposed to chloramphenicol the cells developed into cells expressing CD7, a marker for immature cells, as were Cyclin B1, Myc and CDC25A, indicative of inhibition of apoptosis. This study shows the potential of chloramphenicol to influence differentiation in mice *ex vivo*; however, the relevance for the human condition of aplastic anaemia remains unclear given the absence of a suitable *in vivo* animal model for these conditions.

Ebaid et al. (2011) studied the effects of *Nigella sativa* oil on tissue damage in rats attributed to chloramphenicol. See Section 7.2.2 for further details. Thymus histology showed a change in corticomedullary ratio, and vascular changes (dilatation, haemorrhage). In immune tests (plaque/rosette forming assay, passive haemagglutination test), a decreased response was observed after chloramphenicol sodium succinate exposure. From this study it is concluded that chloramphenicol may influence some immunological endpoints although the relevance for (low level) oral exposure in humans remains unclear.

Laval et al. (1988) studied immune effects in young chickens given chloramphenicol in drinking water (1 g/L for six days, thereafter 0.5 g/L for 15 days, corresponding to approximately 130 and 65 mg/kg b.w. per day, respectively, according to the authors). On day 24 they were immunised with sheep red blood cells (SRBC). Endpoints studied were body and spleen weight, antibody titres (haemagglutination), plaque-forming assays and graft versus host reaction. The results indicated that chloramphenicol had suppressive effects on these parameters.

Nara et al. (1982b) studied possible effects of chloramphenicol in young dogs on the vaccination efficacy against canine distemper virus (CDV). Beagle pups were given chloramphenicol orally (50 mg/kg b.w., three times per day). After one week they were vaccinated against CDV (including the non-medicated control group) and challenged with virulent virus 20 days after vaccination. Blood samples were collected at regular intervals for red and white blood cell analysis, lymphocyte blast transformation and CDV neutralisation tests. In the non-medicated vaccinated group, haematology was normal; the only significant difference between the medicated and non-medicated (vaccinated and challenged) groups was transient changes in blood morphology, while immunological and clinical parameters (including response upon challenge) were not affected. Therefore, it is concluded that despite the haematological changes related to chloramphenicol medication no adverse functional effects were seen in vaccination efficacy, including survival after challenge.

Overall, in a limited number of studies in various species addressing various endpoints, the results were indicative of chloramphenicol having effects on immune function. The CONTAM Panel noted, however, that it remains difficult to conclude on functional effects at low oral doses since these studies do not provide a coherent picture of the effects on the immune system.

7.2.4. Haematotoxicity

Shortly after its introduction as antibiotic, the haematological effects of chloramphenicol were studied in tests with laboratory animals. Saslaw et al. (1954) reported that long-term oral chloramphenicol treatment (up to 15 months) of monkeys (*Macaca mulatta*) either on a normal diet, suffering nutritionally induced cytopenia or previous irradiation treatment yielded no effect on peripheral blood parameters or bone marrow. Radomski and co-workers (1955) reported decrease of granulocyte counts in blood suggestive of bone marrow depression upon repeated oral application of chloramphenicol in dogs. Decreases in normoblasts and maturation of metamyelocytes in bone marrow, which returned to normal with continuation of treatment, were seen in macaques upon both short-term and chronic exposure to chloramphenicol (Hrenoff and Anderson, 1961; Hrenoff, 1962).

The most recent comprehensive evaluation of the available evidence on chloramphenicol-induced haematotoxicity in laboratory animals has been provided by JECFA (FAO/WHO, 2004a).

Turton et al. (1999) gavaged CD-1 mice with chloramphenicol succinate at doses from 800 to 2 000 mg/kg b.w. per day for seven days and observed reduction of reticulocytes in the medium-dose group (1 700 mg/kg b.w. per day) and reduced red blood cell count, haematocrit and haemoglobin levels in the high-dose group (2 000 mg/kg b.w. per day) while both platelet and white blood cell counts remained unaffected. In a further experiment, animals were dosed with 1 400 mg/kg b.w. per day of the compound for 10 days in order to investigate reversibility of effects. At day 1 post dosing, similarly to the first experiment, reduced red blood cell count, haematocrit and haemoglobin levels were observed. All parameters returned to normal by day 4 after treatment while only haematocrit levels rose slightly again at day 15. In Wistar Hannover rats dosed at 2 000–4 000 mg/kg b.w. per day for 19 days, haemoglobin levels were reduced only at the high dose, while the number of reticulocytes, white blood cells and platelets remained stable.

The same authors reported in a further study that BALB/c mice dosed by gavage with 2 000 mg/kg chloramphenicol for 17 days developed signs of a mild reversible anaemia evident as reduced red blood cells, haematocrit and haemoglobin levels returning to normal at the end of treatment. Changes involving bone marrow aplasia were not observed (Turton et al., 2000).

Administration of chloramphenicol succinate by gavage to guinea pigs at doses of 333–1 000 mg/kg b.w. per day for 13 days led to clear dose-related reduction of erythrocyte and reticulocyte numbers and bone marrow depletion. Decreased erythrocyte and reticulocyte counts and bone marrow depletion seen at doses of 825 mg/kg b.w. per day for 16 days returned to normal by, at the latest, day 63 after treatment. The authors concluded that the absence of chronic bone marrow depression corroborates the notion that rodents, in contrast to humans, are not susceptible to chloramphenicol-induced aplastic anaemia (Turton et al., 2002).

In order to investigate strain selective susceptibility towards chloramphenicol-induced haematotoxicity, Festing et al. (2001) dosed inbred C3H/He, CBA/Ca, BALB/c, C57BL/6 and outbred CD-1 mice by gavage with 500–2 500 mg/kg b.w. per day chloramphenicol for seven days. The compound caused anaemia and reticolocytopenia in all strains at high doses. Alteration of one or more blood parameters was seen in all inbred strains at 1000 mg/kg b.w. per day. In C3H/He and CBA/Ca mice some blood parameters effects were seen already at 500 mg/kg b.w. per day. Leucopenia was only observed in inbred strains, suggesting that these strains are more sensitive. Overall the results confirm earlier reports of dose-related reversible anaemia caused by chloramphenicol in mice.

Several further reports on chloramphenicol mediated haematotoxicity have been made available after the last evaluation carried out by JECFA (FAO/WHO, 2004a).

Turton and co-workers (2006) carried out an investigation in which female B6C3F1 mice received 2 500 or 3 500 mg/kg b.w. per day chloramphenicol succinate by gavage for five days in order to characterise chloramphenicol myelotoxicity at high dose levels. Anaemia with reticulocytopenia was observed in conjunction with leucopenia at the end of the treatment period with both dose regimen. At the latest at day 21 post treatment, parameters reversed to normal. Neither peripheral blood cytopenia nor hepatocellular/acellular bone marrow was observed. Overall, this study confirms previous findings on the pattern of chloramphenicol-induced toxicity in rodents.

Saba and co-workers (2002) treated groups of five rabbits for 22 days either by rinsing their eyes with a 0.5 % chloramphenicol solution thrice a day or by oral application of suspensions containing 500 mg chloramphenicol. The authors report that the changes in blood parameters (reduced erythrocytes, packed cell volume, mean corpuscular haemoglobin and neutrophils) observed in the group treated orally were statistically significant while ocular application of chloramphenicol yielded no effects. The results from this study should be considered critically since neither for the ocular applications nor for the oral treatments have dose levels on a body weight basis been reported. Although the alterations in

blood parameters observed were statistically significant, they were very moderate in absolute numbers and, taking into account the low animal numbers, probably of disputable biological significance.

Oyeyemi and Adeniji (2009) assessed the impact of oral administration of 25 mg/kg b.w. per day chloramphenicol for 20 and 25 days on semen and blood parameters in two groups of five Wistar rats. Next to altered sperm parameters, reduced packed cell volume and increased lymphocyte counts were seen while red and white blood cell and neutrophil counts remained unchanged. Overall, the study suffers from very poor reporting and design and the inconsistent haematological findings have not been discussed.

Chen et al. (2013) treated male BALB/c mice with 2.0 Gy ^{60}Co γ-irradiation followed by i.p. injection of 40 mg/kg b.w. per day cyclophosphamide and 50 mg/kg b.w. per day chloramphenicol for three days. Reduced numbers of white and red blood cells and platelets were observed. In bone marrow, haematogenous cells were reduced while the number of non-haematogenic cells was increased, accompanied by interstitial oedema. The spleen was atrophied and haematopoietic focus and megakaryocytes decreased and enhanced interleukin 6 (IL-6) levels were observed in bone marrow cells. The authors suggest that IL-6 secretion is induced by aplastic anaemia and that IL-6 interferes with the stability of the haematopoietic environment. Based on the results, the authors claim successful establishment of an aplastic anaemia animal model. This conclusion can be questioned because the reported effects are not severe enough to be designated as aplastic anaemia.

Overall, the new evidence made available after the last extensive review by the JECFA (FAO/WHO, 2004a) corroborates the view that a dose-dependent mild reversible anaemia (at oral doses of 825 mg/kg b.w. per day or above) can be induced by chloramphenicol in laboratory animals whereas severe non-reversible aplastic anaemia has not been observed.

7.2.5. Developmental and reproductive toxicity

7.2.5.1. Studies on spermatogenesis

Total or incomplete inhibition of spermatogonial divisions with perturbed meiosis was found in rats treated with 30 mg/kg b.w. per day of chloramphenicol succinate for eight days (route of administration was not mentioned) (Timmermans, 1974).

Male albino Wistar rats (n = 5 in each group) were treated orally with 25 mg/kg b.w. per day of chloramphenicol (chemical form not specified) for 20 or 25 days (Oyeyemi and Adeniji, 2009). Sperm viability and motility, the number of spermatozoa and the sperm concentration were significantly decreased in treated groups. Chloramphenicol treatment also induced abnormal spermatozoa, with curved to looped tails.

Oyagbemi et al. (2010) investigated the testicular response after exposure to chloramphenicol (chemical form not specified) and a multivitamin–haematinics complex (containing ferrous gluconate, vitamins B_1, B_2, B_6 and B_{12}) in rats. Male Wistar rats (n = 6 in each group) received 28 mg/kg b.w. of chloramphenicol in capsules (probably sodium succinate), only the multivitamin–haematinics complex (0.08 mL/kg b.w.) or chloramphenicol and the multivitamin–haematinics complex together, every six hours during 10 days (112 mg/kg b.w. per day). A group of rats was treated with saline as control. All animals were sacrificed 24 hours after the last treatment. Testicular ALP activity was significantly increased but no significant changes in other biochemical parameters (AST, ALT and several parameters for oxidative stress (superoxide dismutase (SOD), catalase (CAT), glutathione S-transferases, 5´nucleotidase) in testes tissue were found in the chloramphenicol-treated group. Sperm analysis revealed no changes in percentage of morphologically abnormal sperms, but sperm motility and epididymal sperm number was decreased. Histopathology showed that chloramphenicol treatment alone resulted in mild disruption of the basement membrane of seminiferous tubules.

7.2.5.2. Embryotoxicity and teratogenicity

***In vivo* studies**

Fritz and Hess (1971) studied if chloramphenicol was embryotoxic or teratogenic in rats, mice and rabbits. High doses were used in all species. Except for decreased weight gain in mice treated with 2 000 to 3 000 mg/kg b.w. per day, the authors reported that no maternotoxicity was observed.

Pregnant female Sprague–Dawley rats were treated with chloramphenicol (chemical form not specified, but given in a suspension with 2 % carboxymethyl cellulose) by gavage in doses of 500, 1 000, 1 500 or 2 000 mg/kg b.w. per day at different early organogenetic stages of gestation (Fritz and Hess, 1971). Compared with historical controls (n = 553), the incidence of embryonic/fetal deaths (22.6 % in the controls) was 63 %, at the dose of 500 mg/kg b.w. per day given from day 5 to 15, 38.5 % at the dose of 1 000 mg/kg b.w. per day given from day 7 to 12 or 24.7 % at the dose of 1 500 mg/kg b.w. per day given from day 0 to 6 of gestation. The highest dose (2 000 mg/kg b.w. per day) did not produce embryonic/fetal deaths significantly different from the controls, when given daily from day 15 to 17 of gestation or as a single dose on day 5, 6 or 7 of gestation. Single doses of 2 000 mg/kg b.w. on day 8, 9 or 10 of gestation resulted in approximately 45 % embryonic/fetal deaths. Doses of 2 000 mg/kg b.w. per day during the most sensitive period of gestation, days 9–11, 6–8 or 7–9 caused 100, 75 or 74.3 % embryonic/fetal deaths, respectively. The average weight of fetuses was significantly decreased compared with controls in the group treated with 1 000 mg/kg b.w. per day and in all groups treated with 2 000 mg/kg b.w. per day, except the one treated on day five of pregnancy. Malformations were found at the three highest doses and in the control group, but not in the lowest dose group (500 mg/kg b.w. per day). Anomalies such as umbilical hernia or omphalocele were found when rats were treated during days 6–8 (8/22 live fetuses) or day 8 (5/46 live fetuses) with 2 000 mg/kg b.w. per day. Furthermore, delayed ossification of sternebrae and vertebrae were found after treatment with 1 000 mg/kg b.w. per day during days 7 to 12. Absence of ossification of the phalangeal nuclei of the forelegs and hindlegs and of the fifth sternebrae in fetuses treated with 2 000 mg/kg b.w. per day during days 11 to 13. In the lowest dose group (500 mg/kg b.w. per day given during day 5–15 of gestation) no malformations were found, but embryonic resorptions and the number of dead fetuses were significantly higher than in the controls.

Pregnant Charles River CD1 mice were treated by gavage with chloramphenicol (chemical form not specified, but given in a suspension with 2 % carboxymethyl cellulose) on days 5 to 15 with 500 mg/kg b.w. per day, day 6 to 12 with 1 000 mg/kg b.w. per day or day 8 to 10 with 2 000 mg/kg b.w. per day during gestation (Fritz and Hess, 1971). The results were compared with historical control data (n = 307) from the previous four-year period. The incidence of embryonic/fetal deaths was 24.4 %, 31 %, 71 % and 100 % at a dose of 0, 500, 1 000 or 2 000 mg/kg b.w. per day, respectively. Fused sternebrae and absence of ossification of the phalangeal nuclei of the forelegs and hindlegs as well as missing ossification of the fifth sternebrae in fetuses from dams treated with 1 000 mg/kg b.w. per day during days 6–12 were found. The only effects found in the lowest dose (500 mg/kg b.w. per day) were a slight, non-significant, increase in embryonic or fetal deaths and a significantly lower fetal weight, compared with controls.

Pregnant rabbits (n = 5–8, mixed breed) were treated by gavage, with chloramphenicol (chemical form not specified, but given in a suspension with 2 % carboxymethyl cellulose) in doses of 500 mg/kg b.w. per day on days 6 to 15 or 1 000 mg/kg b.w. per day on days 6 to 9 and on days 8 to 11 of gestation (Fritz and Hess, 1971). The results were compared with historical control data (192 rabbits) from the previous four-year period. Embryonic/fetal deaths were significantly increased with 25 % and 58 %, in dose groups treated with 1 000 mg/kg b.w. per day on days 6–9 or 8–11, respectively. Malformations were found in both dose groups. Delayed ossification and absence of ossification of the phalangeal nuclei of the forelegs, were found in the lowest dose group treated from days 6 to 15 of gestation. The same effects and also absence of ossification of the phalangeal nuclei of the forelegs and hindlegs as well as unevenly ossified vertebrae, were found in the group treated with 1 000 mg/kg b.w. per day on

days 6–9 of gestation. When dams were treated during day 8–11 of gestation with 1 000 mg/kg b.w. per day, the only effect found was missing ossification of the fifth sternebrae.

Sprague–Dawley rats were fed a diet containing 2 or 3 % chloramphenicol (chemical form not specified) corresponding to 1 000 or 1 500 mg/kg b.w. per day, respectively, during day 0 to 20 of gestation (Mackler et al., 1975). Fetal weight, placental weight and number of live fetuses were greatly reduced compared with controls. The number of resorptions (% of total implants) was dose dependently increased in the groups treated with chloramphenicol, 31.4 % and 57.0 % compared with 4.7 % in the control group. Chloramphenicol was also given on certain days of the gestation, 1 500 mg/kg b.w. per day (n = 3–6) during days 0–2 to days 0–8, 750 mg/kg b.w. per day (n = 3–11) during days 0–9 to days 0–12. Fetal weight decreased with increasing days of treatment. Up to day six of gestation no effect of the number of implantations was seen, but it increased with increasing days of gestation. Resorptions were highest after treatment for zero to five and zero to eight days, with absorptions of whole litters. In the groups treated during 0–20 days of gestation, increased incidences of wavy ribs (7 %), fused ribs (7 %) and fetuses with oedema (71 %) were found in the groups treated with 1 500 mg/kg b.w. per day of chloramphenicol. In the group treated with 1 000 mg/kg b.w. per day an incidence of 12 % oedema was found compared with 0 % in the control group. No information on maternotoxicity was mentioned and the study was poorly reported.

Newborn rats (Sprague–Dawley, albino) were treated with i.p. chloramphenicol sodium succinate to investigate the effect on mitochondrial enzymes (Hallman, 1971). Newborn rats (n = 3 to 6) were injected with different dosing schedules, three or four times a day for four to eight days, with increasing doses of chloramphenicol from 50 mg/kg b.w to 300 mg/kg b.w. per day. Chloramphenicol significantly retarded the growth of the rats as well as the weights of liver, kidney, heart and brain, after four to eight days of treatment. Cytochrome (cyt) aa3 and cyt b in liver, kidney, heart and brain and cyt c1 in brain was decreased compared with control animals. Only the activity of succinate dehydrogenase was significantly decreased in heart mitochondria from chloramphenicol treated rats. The respiratory chain, measured as ADP:O ratio, was not affected by chloramphenicol treatment.

Embryotoxicity was induced in rats when chloramphenicol (chemical form not specified) was i.p. injected on day 3 or 4 of gestation (Giavini et al., 1979). Mated female rats (Sprague–Dawley, Charles River) were treated either with 250 mg/kg b.w. of chloramphenicol, 300 µg/kg b.w. of actinomycin D as the positive control group or only saline as a control group. In contrast to actinomycin D, chloramphenicol did not reduce the average number of blastocysts recovered per pregnant female, on day 3 or 4 of gestation. However, treatment with chloramphenicol on day 3, but not on day 4, resulted in a significant reduction of blastomeres in the rat blastocysts, leading to embryotoxicity. Although no histopathological examination was done and only one dose was used, this study suggests that chloramphenicol is embryotoxic in rats.

***In vitro* studies**

Chloramphenicol was tested in an *in vitro* assay for teratogens using cultures of rat embryo midbrain (CNS) and mouse limb bud cells (Flint and Orton, 1984). Chloramphenicol inhibited differentiation of CNS at an IC_{50} of 230 µg/mL and of limb bud cells at an IC_{50} of 160 µg/mL. The maximum concentration, having no inhibitory effect for chloramphenicol, was 60 µg/mL. Chloramphenicol caused a statistically significant reduction in $^{35}SO_4^{2-}$ incorporation when tested in the mouse limb bud cell spot culture assay (Guntakatta et al., 1984).

Chicken eggs with embryos were injected with chloramphenicol (chemical form not specified) at doses of 0.5 or 1.0 mg/egg at 0, 24, 26 and 48 hours of incubation or at two- to four-hour intervals from 0 to 70 hours of incubation (Blackwood, 1962). Chloramphenicol inhibited the differentiation of the splanchnopleure, causing malformations in the head, heart and ventral trunk regions. The percentage of affected embryos in the low-dose group was 36 to 57 % at 16 to 19 hours' incubation and in the high-dose group 23–47 % and 36 to 67 % at 16 to 19 and at 32–48 hours' incubation,

respectively. This means that chloramphenicol affect the development of chicken embryos at an early stage of differentiation. No values for the control groups were included.

The effect of two different isomers, i.e. D and L-threo-chloramphenicol on the early development of chick embryos was investigated by Billett et al. (1965). Chicken eggs with embryos were obtained from farm bred White Leghorn hens. The embryos were explanted and incubated until they reached a 14- or 20-somite stage. Exposure of the embryos to 200 or 300 µg/mL D-threo-chloramphenicol for 22–24 hours caused several abnormalities not found in the controls. The two major effects were defects on the closure of the neural tubes and inhibition of haemoglobin formation. Embryos exposed to the L-isomer at doses of 300 or 600 µg/mL developed more or less normal, although neuronal tube defects and haemoglobin formation was affected in a few cases. The CONTAM Panel noted that this is the only toxicity study describing effects of two isomers of chloramphenicol.

Zebrafish embryos were exposed to chloramphenicol (chemical form not specified) at doses of 0.125, 0.250 and 0.500 mg/mL in petri dishes with aquarial water (Anderson and Battle, 1967). For each of the seven development stages, eggs (n = 100) were exposed for 12, 24 and 36 hours and thereafter transferred to aquarial water until 48 hours of development. Most severe abnormalities were found when eggs were exposed during cleavage and blastulation (up to two hours), but smaller effects were found during later development stages, and during early and late gastrula and optic cup formation. Abnormalities found in treated groups, were constriction of the germ ring during gastrulation of embryos, flexed tails, spina bifida, effects on pronephric ducts and heart beat disturbances. From these results it can be concluded that chloramphenicol is both embryotoxic and teratogenic in zebrafish eggs.

7.2.5.3. Multigeneration studies

No multigeneration reproduction studies were identified.

7.2.5.4. Concluding comments

In studies using only one dose level, the lowest oral dose tested (25 mg/kg b.w. per day) caused testes degeneration and effects on sperm quality in male rats. Therefore, no NOAEL can be identified for these effects in the studies available.

Embryotoxicity was observed in rats treated orally with chloramphenicol at doses from 500 to 2 000 mg/kg b.w. per day, in mice treated with 1 000 or 2 000 mg/kg b.w. per day and in rabbits treated with 1 000 mg/kg b.w. per day during organogenesis. At these doses, and also at the dose of 500 mg/kg b.w. in rabbits, inhibition of development and growth of fetuses was found. Furthermore, disturbed ossification was found in all three species at doses of 1000 mg/kg b.w. per day or higher, and in rabbits also at 500 mg/kg b.w. per day. Defects in ventral closure in the rat and fusion of sternebrae in both mice and rats were also found. These effects were dose-dependent, but the stage of gestation during treatment was even more important. In these studies the lowest tested dose of 500 mg/kg b.w. per day caused adverse effects in rabbits, rats and mice. Therefore, no NOAEL could be identified from the studies available. The CONTAM Panel also considers that these studies do not provide a reliable basis for establishing a LOAEL.

Intraperitoneal administration of 250 mg/kg b.w. chloramphenicol, on day 3 or 4 of gestation, caused embryotoxicity in rats.

In vitro studies on rat embryo midbrain (CNS) and limb bud cell cultures showed that chloramphenicol inhibited differentiation and development of the embryos. Furthermore, chloramphenicol was shown to inhibit the development of chicken embryos at an early stage of differentiation. The D-isomer (presumably D-threo (1R,2R) and D-erythro (1S,2R)) of chloramphenicol caused more severe defects on the closure of the neural tubes and inhibition of haemoglobin formation than the L-isomer (presumably L-threo (1S,2S) and L-erythro (1R,2S)) in chicken embryos. Chloramphenicol was also embryotoxic and teratogenic in zebrafish eggs.

7.2.6. Neurotoxicity

The effect of chloramphenicol on behaviour was studied in rats and mice.

Rats (n = 15) were treated s.c. during day 7 to 21 of pregnancy with 50 mg/kg b.w. per day chloramphenicol succinate, or newborn pups were treated s.c. during the first three days of life with 50 or 100 mg/kg b.w. per day (Bertolini and Poggioli, 1981). No maternotoxicity, fetotoxicity or malformations of pups was found. Learning ability tested by avoidance learning in pups at 60 days of age was dose-dependently reduced compared with controls. The effect was stronger when pups were treated postnatally.

Pregnant mice (five groups of eight mice) were treated orally for seven days with doses of 25, 50, 100 or 200 mg chloramphenicol/kg b.w. per day at the third trimester (Al Hachin and Al-Baker, 1974). After birth the dams were caged individually together with their offspring. Pups of the age of 30 days, tested in the conditioned avoidance response test for seven days, showed a dose-dependent significantly reduced avoidance response. When tested at the age of 38 days, mice showed a dose-dependent increased brain seizure threshold at doses of 50 mg/kg b.w. per day and higher. When mice were six weeks old they showed a significant non-dose-related decrease in performance in the open field tests, with lowest performance in the lowest dose group and highest at 50 mg/kg b.w. per day and then declining in the two highest dose groups.

A few studies investigating the effect of chloramphenicol on apoptosis, energy supply to the brain and sleeping disturbances have been identified.

The effect of chloramphenicol on both naturally occurring cell death and degeneration induced by surgical deafferentation of the superior colliculus in the rat was investigated (Guimarães and Linden, 2000). Neonatal rats (Lister hooded) were treated on postnatal day 2 with chloramphenicol (50 mg/kg b.w.) by systemic injection, one injection before surgery and the other injection after brain surgery or to rats without any brain surgery. Chloramphenicol increased deafferentation-induced cell death and increased the naturally occurring cell death. The authors concluded that chloramphenicol has a general, pro-apoptotic effect in the developing brain.

The effect of chloramphenicol on sleeping pattern was investigated in cats and rats. Cats (n = 7) were surgically prepared and thereafter treated with single oral doses of 0, 80, 165, 250 or 330 mg/kg b.w. of chloramphenicol (chemical form not specified) (Petitjean et al., 1975). It was found that the paradoxical sleep was depressed dose-dependently for 10, 20 and 27 hours. The lowest dose tested (80 mg/kg b.w. per day) did not cause alterations in sleep compared with controls and the highest dose also decreased the amount of slow-wave sleep.

Rats were surgically prepared and injected i.p. with 400 mg/kg b.w. of chloramphenicol succinate or chloramphenicol (chemical form not specified) (Moulin-Sallanon-et al., 2005; Chahboune et al., 2008). Chloramphenicol caused a significantly increased waking time (approximately 65 %) a moderately decrease in slow-wave sleep and a marked loss (60 %) in paradoxical sleep compared with control rats. A decreased availability in energy substrate for the brain and a depression in neuronal activity shown by electroencephalogram, was suggested to be the reason for the observed sleep deficit. The CONTAM Panel noted that these two studies were not performed according to the generally accepted safety assessment protocols and that rats were not treated orally. Therefore, the studies have limited value for the risk assessment of chloramphenicol.

In conclusion, chloramphenicol caused reduced learning ability in both rats (50 mg/kg b.w. per day s.c.) and mice (25 to 200 mg/kg b.w. per day orally). Chloramphenicol injected systemically was also shown to have a proapoptotic effect on the developing brain in rats. Sleeping patterns were disturbed in cats treated with oral doses of chloramphenicol of 165 mg/kg b.w. or higher. The same effects on sleeping pattern found in cats were also found in rats injected i.p. with chloramphenicol at a dose of

400 mg/kg b.w. In conclusion, chloramphenicol has a neurotoxic effect, which was also found in the acute toxicity studies.

7.2.7. Genotoxicity

A considerable number of published data on several relevant genotoxicity endpoints, mainly chromosomal aberration, sister chromatid exchange (SCE) and DNA damage and repair, has been retrieved on chloramphenicol and/or its metabolites through consultation of available review papers and previous assessments (Rosenkranz, 1988; IARC, 1990; Technology Planning and Management Corporation, 2000; FAO/WHO, 2004a) and a direct literature search especially of additional data published since 2003.

7.2.7.1. Genotoxic effects of chloramphenicol in prokaryotic and in lower eukaryotic systems

Most of the data available from tests carried out in bacterial systems did not show any convincing evidence of genotoxic effects induced by chloramphenicol, both with and without metabolic activation. Negative results were reported for the tests with *Escherichia coli* (Hemmerly and Demerec, 1955; Morgan et al., 1967; Mullinix and Rosenkranz, 1971; Rosenkranz et al., 1971; Slater et al., 1971; Shimizu and Rosenberg, 1973; Brem et al., 1974; Dworsky, 1974; Longnecker et al., 1974; Simmon et al., 1977; Nestmann et al., 1979; Boyle and Simpson, 1980; Kubinski et al., 1981; Leifer et al., 1981; Mamber et al., 1986); *Salmonella enterica* subsp. *enterica* serovar Typhimurium (Ben Gurion, 1978; Russell et al., 1980; Nader et al., 1981; Mortelmans et al., 1986); *Bacillus subtilis* (Ohtsuki and Ishida, 1975; Karube et al., 1981; Sekizawa and Shibamoto, 1982); *Staphylococcus aureus* for induction of SOS functions (Manthey et al., 1975) and *Proteus mirabilis* (Adler et al., 1976). Negative results were reported for the induction of SOS response in the SOS-umu assay and SOS-Lux assays (Baumstark-Khan et al., 2001; Toolaram et al., 2012). In both assays chloramphenicol exhibited a concentration-dependent increase in cytotoxicity, but no indication of DNA damage induction.

However, some controversial outcomes suggesting the possibility of DNA damage induced by chloramphenicol in *E. coli* and in *S.* Typhimurium have been published by McCann et al. (1976), Jackson et al. (1977), Mitchell et al. (1980) and Suter and Jaeger (1982). McCann et al. (1976) and Jackson et al. (1977) found that the toxicity of chloramphenicol interfered with attempts to examine the mutagenicity of this drug in *S.* Typhimurium. In *E. coli B/r* and *S.* Typhimurium strains, Jackson et al. (1977) tested mutagenicity of two chloramphenicol isomers: D-threo-chloramphenicol known to inhibit protein synthesis and L-threo-chloramphenicol that does not inhibit protein synthesis. The L-threo-chloramphenicol that was not toxic to bacteria induced reverse mutations in *S.* Typhimurium TA100 and TA1535 without metabolic activation, while the mutagenicity of D-threo-chloramphenicol was masked by its toxicity. Both isomers also induced DNA strand breaks in bacteria, however the D-threo-isomer was much less effective. Mitchell et al. (1980) reported that chloramphenicol induced forward mutation to L-azetidine-2-carboxylic acid resistance in *E. coli* WP2 and was weakly active in reversion of frameshift mutation in *S.* Typhimurium TA98, but these responses were closely correlated with the toxic effects. Suter and Jaeger (1982) tested chloramphenicol in different DNA-repair deficient bacterial strains and reported chloramphenicol to be inactive in *B. subtilis* (strains H17/M45 and HLL3g/HJ-15) and active at variable extents in different strains of *E. coli* (AB1157/JC5547, AB1157/JC2921, AB1157/JC2926 and AB1157/JC5519). It should be noted that bacterial genotoxicity assays are generally not appropriate for testing antibiotics because the high bacterial toxicity of antibiotics, which is not related to DNA damage, masks detection of genotoxic effects. Hence, the significance of the above-mentioned sporadic positive results, suggestive of a DNA-damaging effect of chloramphenicol in prokaryotic systems, remains doubtful. They might be due to an indirect mechanism associated with the generally low viability of the different tested prokaryotic strains caused by chloramphenicol cytotoxicity. In addition, the use of high concentrations of test substances characterised by cytotoxicity is generally considered to be a potential source of false positive results in *in vitro* genotoxicity testing (EFSA, 2005). In the genotoxicity test system with lower eukaryotes, chloramphenicol did not show any activity in inducing sex-linked lethal mutations in *Drosophila melanogaster* (Clark, 1963; Nasrat et al., 1977). Chloramphenicol was not mutagenic in

Saccharomyces cerevisiae diploid strains with and without metabolic activation (Carnevali et al., 1971; Mitchell et al., 1980) but positive in a specific cold-sensitive *Saccharomyces cerevisiae* haploid strain 1121, highly mutable into a cytoplasmic petite mutation that can also be induced by growth at the non-permissive temperature of 18 °C (Weislogel and Butow, 1970; Williamson et al., 1971).

7.2.7.2. Genotoxic effects of chloramphenicol in plants and algae

Chloramphenicol (up to 0.9 mM) induced reduction from 2n to n of the chromosome number in the cells of the first division cycle in the root tips of *Hordum vulgare* (Yoshida et al., 1972); morphological observations and no changes of relative DNA content per cell indicated that such reduction was due to a tight alignment of chromosomes in pairs up to mitotic prometaphase (Yoshida and Yamaguchi, 1973). Chromosomal aberrations were also induced by chloramphenicol (15.5 mM), alone or in combination with monofunctional alkylating agents, in *Vicia faba* seeds (Prasad, 1977). Concentrations of chloramphenicol between 0.5 and 1 mM induced a variety of chromosomal abnormalities in the green alga *Spirogyra azygospora;* genotoxic effects common with the other antibiotics tested (i.e. oxytetracycline and gentamicin) included mitotic delay, binucleate and anucleate cells, diagonal to transverse orientation of daughter nuclei, vacuolisation of nuclei and nucleoli and chromosome breakage and degeneration of nuclei, whereas more specific effects included frequent chromosome breakage, occasional chromatid breaks, chromosomal fusions, rare chromosomal exchanges and anaphase bridges between two daughter chromosomes (Vedajanani and Sarma, 1978).

Chloramphenicol was unable to induce recessive lethal mutation in *Arabidopsis* seeds (Muller, 1965) and micronuclei in pollen tetrads of *Tradescantia paludosa* (Ma et al., 1984).

7.2.7.3. Genotoxic effects of chloramphenicol and its metabolites in mammalian *in vitro* systems

Two studies showed that chloramphenicol is an *in vitro* mammalian cell mutagen. At non-cytotoxic concentrations it induced forward mutations at the thymidine kinase locus in mouse lymphoma cells L5178Y with and without metabolic activation (Mitchell et al., 1988; Myhr and Caspary, 1988; Table 7 number 1). In another study with Chinese hamster fibroblasts V79, chloramphenicol induced a dose-related increase in the frequency of thioguanine resistant (TG^r) mutants, however only without metabolic activation (Martelli et al., 1991; Table 7 number 2). In the latter study it was also demonstrated that chloramphenicol induced unscheduled DNA-repair synthesis in human and rat hepatocytes, but not DNA fragmentation.

Chloramphenicol was demonstrated to induce chromosomal aberrations *in vitro* in human blood peripheral lymphocytes and leucocytes (Mitus and Coleman, 1970; Goh, 1979; Sbrana et al., 1991; Table 7 numbers 4, 5 and 6). Chloramphenicol also induced chromosomal aberrations in bovine and porcine lymphocytes although the responses were weak and not dose-dependent (Quéinnec et al., 1975; Babilé et al., 1978; Table 7 number 8). On the other hand, chloramphenicol did not induce chromosomal aberrations in human fibroblasts, in Syrian hamster embryo cells and in another study with human blood peripheral lymphocytes (Jensen, 1972; Byarugaba et al., 1975; Hagiwara et al., 2006; Table 7 numbers 3, 7 and 9).

Chloramphenicol induced an increase in SCE frequency in bovine lymphocytes and fibroblasts (Arruga et al., 1992; Catalan et al., 1993; Table 7 numbers 11 and 12). Weak induction of SCE was reported also in V79 cells and human peripheral blood lymphocytes (Sbrana et al., 1991; Table 7 number 5) while in another study with human peripheral blood lymphocytes the result was negative (Pant et al., 1976; Table 7 number 10).

It should be noted that, in many reported chromosomal aberration and SCE studies, the data on the cytotoxicity and effect of chloramphenicol on the mitotic index are not presented. It is well known that, for detection of chromosomal aberrations as well as SCE in metaphases, the cells should undergo cell division during or after exposure. In two studies it was shown that chloramphenicol causes delay in the cell cycle (Arruga et al., 1992; Catalan et al., 1993; Table 7 numbers 11 and 12), which may explain the inconsistent results for chromosomal aberration and SCE testing of chloramphenicol.

Table 7: Genotoxic effects of chloramphenicol in specific mammalian *in vitro* systems (modified from Technology Planning and Management Corporation, 2000)

Test system	Endpoint	Tested dose range	Exposure conditions	Results	Reference
1. Mouse lymphoma cells	Mutation at the TK locus of L5178Y cells	3.2 – 15.5 mM	4 h	Cytotoxic at concentrations >10 mM (cloning efficiency and relative growth). Mutation induction (with or without metabolic activation) at non cytotoxic concentrations (about 10 mM).	Mitchell et al. (1988), Myhr and Caspary (1988)
2. Chinese hamster V79 cells, isolated primary rat and human hepatocytes	Mutations (TG resistance) in V79; DNA fragmentation (alkaline elution) in V79 and rat hepatocytes; UDS in rat and human hepatocytes	0.5- 4 mM	Mutations to TG resistance: 1 h without metabolic activation; 20 h with metabolic activation (co-cultivation with rat hepatocytes) DNA fragmentation: V79 1 h, rat and human hepatocytes 20 h UDS: 20 h exposure	Cytotoxic at concentrations > 2.0 mM (cloning efficiency and trypan blue exclusion). Dose-related increase in the number of TG resistant mutants in V79 without metabolic activation (significant at 2mM); Negative with metabolic activation. Dose-related increase in UDS in rat and human hepatocytes (significant at 1 and 2 mM). No increase in DNA fragmentation.	Martelli et al. (1991)
3. Human peripheral blood lymphocytes	Chromosomal aberrations	0.12 and 1.55 mM	48 h old HPBL cultures exposed to CAP for 6 and 24 h	Negative; toxicity not tested; mitotic index not indicated; no metabolic activation	Jensen (1972)
4. Human peripheral blood lymphocytes	Chromosomal aberrations	0.25 mM	Added at different phases of cell cycle: 0 h (G0), 24 h (G1) 68 h (late S), 71 h (G2). Harvested at 72 h of culturing.	Positive: the highest incidence of chromosomal aberration when CAP was added at G0 and G1 phase. Toxicity not tested; mitotic index not indicated, no metabolic activation.	Goh (1979)
5. Human peripheral blood lymphocytes, Chinese hamster V79 cells	Chromosomal aberrations; SCE	HPBL: 7.4 – 14.9 mM V79: 3-37 mM	48 h old HPBL cultures exposed to CAP for 24 h, harvested at 72 and 96 h; V79 cultures exposed to CAP for 16 h.	Significant dose-dependent increase in chromosomal aberration at 7.4 –10 mM associated with dose-dependent decrease in mitotic index (± 30 % at 7.4 mM to > 60 % at 10 mM). Only weak increase in SCE over the background level in HPBL and V79 cells. No metabolic activation used.	Sbrana et al. (1991)

Table 7: Genotoxic effects of chloramphenicol in specific mammalian *in vitro* systems (modified from Technology Planning and Management Corporation, 2000) (continued)

Test system	Endpoint	Tested dose range	Exposure conditions	Results	Reference
6. Human peripheral blood leucocytes	Chromosomal aberrations	0.03-0.12 mM	72 h old human peripheral blood leucocytes exposed to CAP for 6 h	Significant dose-dependent increase of chromosomal aberrations at all tested concentrations. Toxicity not tested; mitotic index not indicated; no metabolic activation.	Mitus and Coleman (1970)
7. Human fibroblasts	Chromosomal aberrations	1.9 mM	40 h	No increase in the number of chromosomal aberrations. The tested dose was half maximum limiting concentration for CAP determined by cell counting 24 h after the exposure to CAP (3.8 mM). No metabolic activation.	Byarugaba et al. (1975)
8. Bovine and porcine lymphocytes	Chromosomal aberrations	0.15 µM – 1.5 mM	72 h	Bovine lymphocytes: statistically significant increase in chromosomal aberration frequency only at 1.5 µM. Porcine lymphocytes: statistically significant increase in chromosomal aberration frequency only at the highest tested concentration 1.5 mM. The result is based on the analysis of only ± 50 metaphases per experimental point. Due to anti-mitotic effect in bovine lymphocytes the highest concentration could not be evaluated.	Quéinnec et al. (1975), Babilé et al. (1978)
9. Syrian hamster embryo cells	Chromosomal aberrations	0.09–3.0 mM	24 h	No induction of chromosomal aberrations (with or without metabolic activation). The tested doses were not cytotoxic as determined with the colony forming efficiency under the same exposure conditions.	Hagiwara et al. (2006)
10. Human peripheral blood lymphocytes	SCE	0.62 mM	72 h old leucocyte cultures were exposed for 24 h	No increase in SCE (one dose only); toxicity not tested; no metabolic activation. Chromosomal aberrations were also determined, but no data on the background level in untreated control is given.	Pant et al. (1976)

Table 7: Genotoxic effects of chloramphenicol in specific mammalian *in vitro* systems (modified from Technology Planning and Management Corporation, 2000) (continued)

Test system	Endpoint	Tested dose range	Exposure conditions	Results	Reference
11. Bovine lymphocytes	SCE	0.015–0.12 mM	72 h old bovine lymphocyte cultures were exposed for 24 h	Weakly positive, with the highest effect at the lowest dose (0.015 mM); the observed delay in cell cycle with increasing dose may explain the lack of the dose response in SCE induction. No metabolic activation was used.	Catalan et al. (1993)
12. Bovine fibroblasts	SCE	0.015–0.19 mM	48 and 60 h	Positive at all doses but no dose–response; no cytotoxicity measured, but slower growth at the highest dose. No metabolic activation was used.	Arruga et al. (1992)

h: hour/hours; HPBL: human peripheral blood lymphocytes; SCE: sister chromatid exchange; TK: thymidine kinase; TG: thioguanine; UDS: unscheduled DNA synthesis.

As discussed in Section 7.1, chloramphenicol can be metabolised into a number of metabolites, like the glucuronide, chloramphenicol base, an alcoholic derivative, dehydro-chloramphenicol, dehydro-chloramphenicol base, referred to as NPAP and amino-chloramphenicol, some of them thought to be formed by enterobacteria in the large bowel (Smith and Worrel, 1950). Another potential metabolite is nitroso-chloramphenicol, but this metabolite is very reactive and as such difficult to detect in tissues and blood of treated humans or animals (Isildar et al. 1988b). The ability of these metabolites to induce DNA damage in human cells was tested in human peripheral blood lymphocytes and in a lymphoma cell line (Raji) (See Table 8). The alkaline elution assay was used in all these studies. It should be noted that this test determines the extent of DNA fragmentation and this may not only point to DNA double or single strand breaks due to genotoxic activity but also to necrosis and apoptosis, an effect that has also been observed in cells treated with chloramphenicol (Section 7.3.2).

Yunis et al. (1987), showed with human lymphocytes and Raji lymphoblastoma cells that on a molar basis nitroso-chloramphenicol was significantly more effective in the alkaline elution assay (positive results at doses of 0.05–0.1 mM) than chloramphenicol (weakly positive at 2 mM). Thiamphenicol, a chloramphenicol structural analogue lacking the p-NO_2 group (see Figure 4 in Section 7.3.1), was without effect. Addition of N-acetyl-cysteine reduced the effect of the nitroso-derivative almost completely, probably due to binding of the reactive intermediate.

Four chloramphenicol metabolites known to be produced by intestinal bacteria were examined with respect to their capacity to induce DNA damage in cells in culture by Isildar et al. (1988b). The induction of DNA single-strand breaks in Raji cells, activated human lymphocytes, and human bone marrow cells was assayed by the alkaline elution technique. Dehydro-chloramphenicol showed a clear positive effect in all three cell systems at concentrations of 0.1 mM. Nitroso-chloramphenicol showed an even larger effect in all cells. Dehydro-chloramphenicol base showed a weak response, but only in Raji cells, whereas chloramphenicol itself, as well as arylamine chloramphenicol and p-nitrobenzaldehyde were negative. It was shown that dehydro-chloramphenicol can be further reduced to the amino-metabolite by all cell-types, contrary to chloramphenicol itself. This indicates that dehydro-chloramphenicol, being a relatively stable metabolite, can play an important role in the effects on the bone marrow.

The ability of chloramphenicol and six of its metabolites to induce DNA damage in a human bone marrow cell-line (RiBM cells) was compared with that measured in human peripheral blood lymphocytes in order to estimate the relative sensitivity of the two types of cells (Robbana-Barnat et al., 1997). Alkaline elution was used to detect DNA-damage, and the incorporation of radiolabelled thymidine for DNA-synthesis inhibition. Most compounds caused a dose-related inhibition of DNA synthesis in both cell types, but nitroso-chloramphenicol, dehydro-chloramphenicol and dehydro-chloramphenicol base were clearly more potent than chloramphenicol itself, chloramphenicol base, the alcohol or the glucuronide. Nitroso-chloramphenicol and dehydro-chloramphenicol caused a clear positive effect in the alkaline elution test with both cell types at 0.1 to 0.2 mM, concentrations that caused complete inhibition of DNA-synthesis. Chloramphenicol and the other metabolites were negative in RiBM cells, but human lymphocytes showed a positive response with dehydro-chloramphenicol base, contrary to RiBM cells. Overall, the results indicate that RiBM cells were much less susceptible to the genotoxic effect of chloramphenicol metabolites than human lymphocytes.

The same data on human lymphocytes were presented by Lafarge-Frayssinet et al. (1994), who compared the response with Raji lymphoma cells. The latter cell-line showed a very similar response to human lymphocytes with nitroso- and dehydro-chloramphenicol showing a clear positive response in the alkaline elution test and dehydro-chloramphenicol base a weak response.

Table 8: Genotoxic effects of chloramphenicol and its metabolites in specific mammalian *in vitro* systems, as measured by alkaline elution

Test system	Endpoint	Results	Reference
1. Human peripheral blood lymphocytes	DNA damage (single-strand breaks)	Positive for three CAP metabolites, i.e. dehydro-CAP, dehydro-CAP base and nitroso-CAP, at concentrations in the order of 0.1mM or greater. No effects for CAP or three other metabolites	Yunis et al. (1987), Isildar et al. (1988b), Lafarge-Frayssinet et al. (1994), Robbana-Barnat et al. (1997)
2. Human Raji lymphoma cells	DNA damage (single-strand breaks)	Positive for three CAP metabolites, i.e. dehydro-CAP, dehydro-CAP base and nitroso-CAP, at concentrations of 0.2mM and above. No effects for CAP or three other metabolites	Yunis et al. (1987), Isildar et al. (1988b), Lafarge-Frayssinet et al. (1994)
3. Human bone marrow cells	DNA damage (single-strand breaks)	Positive for nitroso-CAP and dehydro-CAP at concentrations of 0.1mM or greater. No effects for CAP or three other metabolites	Isildar et al. (1988b), Robbana-Barnat et al. (1997)

CAP: chloramphenicol.

7.2.7.4. Genotoxic effects of chloramphenicol in mammals *in vivo*

Dominant lethal mutation studies of chloramphenicol in mice gave negative results (Epstein et al., 1972; Sràm, 1972; Table 9 numbers 1 and 2). However, the *in vivo* investigations in mice and rats clearly showed the clastogenic activity of chloramphenicol in inducing bone marrow chromosomal aberrations in these animal species following i.m. or i.p. injection or oral administration (Jensen, 1972; Manna and Bardhan, 1972; Zaied, 1996; Table 9 numbers 4, 5 and 10 in Table 9) as well as in bone marrow of newborn pups from exposed pregnant rats (Zaied, 1996; Table 9 number 11, Table 9). Moreover, chromosomal aberrations increased substantially also in germinal cells of mice injected i.m. or i.p. with chloramphenicol (Sràm and Kocisova, 1974; Manna and Roy, 1979; Table 9 numbers 3 and 6) and in liver cells of developing embryos and 7-day old litters of exposed male mice mated with unexposed females (Manna and Roy, 1979; Table 9 number 6). In the only *in vivo* micronucleus study in rats, a negative result was observed (Martelli et al., 1991; Table 9 number 9).

Table 9: Genotoxic effects of chloramphenicol in mammals *in vivo* (modified from Technology Planning and Management Corporation, 2000)

Test system	Route of administration	Dose and exposure duration	Endpoint	Results	Reference
1.ICR/Ha Swiss Mice	i.p. injection	Single dose 333 and 666 mg/kg/b.w (concentrations adjusted to LD_5 and LD_{25})	Dominant lethal mutation	Negative	Epstein et al. (1972)
2.Mice	i.p. injection	Single dose 500 mg/kg b.w.	Dominant lethal mutation	Negative	Sràm (1972),
3.Mice	i.p. injection	Single dose 500 mg/kg b.w.	Abnormal spermatocytes (structural and numerical chromosomal aberrations)	Significantly increased number of abnormal spermatocytes.	Sràm and Kocisova (1974)
4.Rats	i.m. injection	1 000 mg/kg b.w. daily for three consecutive days (72, 48 and 24 h before sacrifice)	Chromosomal aberrations in bone marrow cells	3/250 cells contained structural chromosomal aberration; none was detected in the control group. Considered negative by the authors.	Jensen (1972)
5.Mice	i.p. injection	Single dose 50 mg/kg b.w., sampling at 12 intervals	Chromosomal aberrations and effect on mitotic index in bone marrow cells	Increased frequency of chromosomal aberrations, with the highest number after 12 h exposure. No significant decrease of mitotic index.	Manna and Bardhan (1972)
6.Swiss albino mice	i.m. injection	Male mouse received 50 mg/kg b.w. per day during 1 week mating with 4 females	Chromosomal aberrations in liver cells of developing embryos at 12^{th},16^{th}, and 18^{th} day of gestation and 7 days old litters.	Increased frequencies of chromosomal aberrations were observed in developing embryos (15/ 20) and in 7 day old litters (3/4).	Manna and Roy (1979)
7.Swiss albino mice	i.m. injection	Single dose 50 mg/kg b.w. sacrificed after 2 and 24 h	Chromosomal aberrations in spermatogonia and spermatocytes	Structural and numerical chromosomal aberrations were observed in both types of cells. Polyploidy was more frequent in spermatogonial metaphases.	Roy and Manna (1981)

Table 9: Genotoxic effects of chloramphenicol in mammals *in vivo* (modified from Technology Planning and Management Corporation, 2000) (continued)

Test system	Route of administration	Dose and exposure duration	Endpoint	Results	Reference
8. CDI male mice	i.p. injection	Single dose 50 and 100 mg/kg b.w.; sacrificed after 6, 12, 18 and 24 h.	Chromosomal aberrations in bone marrow cells	Early decrease of mitotic indices. No significant difference between control and treated groups except after 6 h exposure to 50 mg/kg b.w.	Sbrana et al. (1991)
9.Male Sprague–Dawley rats	Oral (gavage)	Single dose 1250 mg/kg b.w (half LD_{50}), sacrificed after 48 h	Micronuclei in hepatocytes and polychromatic erythrocytes	Negative for both tissues. The ratio of normochromatic to polychromatic erythrocytes was in treated animals in the range of control group.	Martelli et al. (1991)
10.Male rats	Oral (gavage)	Single dose 50 and 100 mg/kg b.w. sacrificed after 6, 12, 18, 24 and 30 h.	Chromosomal aberrations in bone marrow cells	Positive at 100 mg/kg b.w. 12 and 18 h post treatment.	Zaied (1996)
11.Newborn pups of treated pregnant rats	Oral (gavage)	50 and 100 mg/kg b.w. was administered to pregnant females for 10 successive days from 8^{th} – 18^{th} day of gestation.	Chromosomal aberrations in bone marrow cells of newborn pups	Positive at 100 mg/kg b.w. per day	Zaied (1996)
12.Calves	i.m. injection	10 mg/kg b.w. per day for 4 consecutive days (minimal therapeutic dose). Blood collected 4 days and 3 weeks after the last dosage.	Chromosomal aberrations and SCE in blood cultures	Significant increase of the frequency of chromosomal aberrations after 4 days and 3 weeks and SCE after 4 days. , significant decrease of mitotic index.	Othman et al. (2005)

b.w.: body weight; i.m:, intramuscular; i.p.: intraperitoneal; SCE: sister chromatid exchange.

Only one study on genotoxic effects in farm animals was identified. Eight female calves (2–4 months old) were injected with chloramphenicol sodium citrate (10 mg/kg b.w.) i.m. once a day for four successive days. Three blood samples were collected from each animal, the first one just before injection (as control), the second at four days after stopping injections and the last one at three weeks after the last treatment (Othman et al., 2005). Four days after the last injection of chloramphenicol, the numbers of cells with breaks and gaps or deletions and fragments were increased. The number of total aberrant cells with structural chromosomal aberrations was also increased. Furthermore, after three weeks from the last day of injection, the numbers of cells with breaks and gaps were increased as well as the number of total aberrant cells with structural chromosomal aberrations. All of the above mentioned increases were statistically significant. However, the increase in the number of cells with deletions and fragments in the three week group was not statistically significant. A statistically significant increase in the frequency of sister chromatid exchanges occurred only after four days from the last injection. A statistically significant decrease in the mitotic index was observed in cultures after four days and three weeks from the last injection.

Finally, it should be noted that some investigations also indicate the ability of chloramphenicol to interact with and modulate activity of other genotoxic agents. Ehling (1971) showed that pretreatment of male mice with chloramphenicol (1.5 g/kg b.w. i.p) prior to irradiation increased the frequency of dominant lethal mutations, while Sram (1972) reported that chloramphenicol in combination with tris(1-aziridinyl) phosphi-neoxide (TEPA) induce higher frequency of dominant lethal mutations than TEPA alone.

7.2.7.5. Concluding comments

While being largely non-genotoxic in bacterial and lower eukaryotic test systems, chloramphenicol was shown to induce chromosomal damage and DNA fragmentation in a variety of rodent and human cell lines *in vitro*, although also negative results were obtained. In mammalian cells (V79 and mouse lymphoma L5178Y cells) chlorampenicol induced forward mutations. Moreover, a few metabolites of chloramphenicol, namely nitroso-chloramphenicol and dehydro-chloramphenicol, were shown to be much more active than chloramphenicol itself in the alkaline elution assay (indicative of DNA-strand breaks) in various human cells. In particular, dehydro-chloramphenicol may play an important role in some of the adverse effects due to its relative stability in blood and potential absorption and further activation though nitro-reduction by various tissues.

Chloramphenicol has been shown to be genotoxic *in vivo* in mice, rats and calves. The investigations in mice and rats clearly showed the clastogenic activity of chloramphenicol in inducing chromosomal aberrations in bone marrow following i.m. or i.p. injection or oral administration. Chromosomal aberrations were also increased in germinal cells of mice injected with chloramphenicol as well as in calves' blood cells. In addition, chromosomal aberrations were observed in somatic cells of developing foetuses and newborns from male mice exposed to chloramphenicol and in bone marrow of pups of treated pregnant rats, indicating transplacental exposure. However, chloramphenicol did not induce dominant lethal mutations in mice.

Therefore, in agreement with the conclusions of the IARC (1990) and the JECFA (FAO/WHO, 2004a), the CONTAM Panel concluded that the genotoxic activity of chloramphenicol in mammalian cells *in vitro* concerns mainly chromosomal aberrations as well as mutations, whereas *in vivo* it induces chromosomal aberrations. The genotoxic activity of chloramphenicol is likely to be dependent on the metabolic competence of the exposed organism(s) in view of the higher potency of certain metabolites (see also Section 7.1).

7.2.8. Carcinogenicity

7.2.8.1. Oral administration in mice

Groups of 50 male and 50 female BALB/c and C57B1/6N mice, six weeks old, were administered chloramphenicol in drinking water for 104 weeks. Lymphomas as well as other types of tumours were

observed. This study was reported only in an abstract by Sanguineti et al. (1983). Due to pulmonary infection in rats, no statistical analysis was possible and no full reporting of the study has been published (personal communication Sanguineti M, 2014). Therefore, the CONTAM Panel concluded that the study could not be used for the risk assessment.

In a limited study in which the carcinogenicity of nitrofurans, nitroimidazoles, nitrobenzenes and some related compounds in Sprague–Dawley rats was reported (Cohen et al., 1973), chloramphenicol was administered at only one dose of 0.05 % in the diet (equivalent to about 25 mg/kg b.w.) for 66 weeks (with follow-up until 75 weeks). Tumours (eight fibroadenomas in the breast and one hepatoma) were found in 9 out of 36 animals (25 %). Tumour incidences in the control were similar (12 fibroadenomas and six adenocarcinomas in the breast and one myxosarcoma in the ovary in 18 out of 71 animals (25 %). The authors suggested that the negative results for chloramphenicol may be due to its metabolism. The CONTAM Panel concluded that this study could not be used for the risk assessment.

7.2.8.2. Intraperitoneal injection in mice

A group of 45 six- to eight-week-old BALB/c AF1 male mice were pre-treated with four i.p. injections of 0.25 mL of acetone in distilled water, then given 0.25 mL (2.5 mg) of chloramphenicol (unspecified purity) in 0.9 % saline solution once a day, five days per week, for five weeks. A control group of 45 male BALB/c × AF1 mice received four i.p. injections of 0.25 mL of acetone in distilled water, followed by saline solution only. All surviving mice were sacrificed on day 350 of the study. Tumour incidence was not significantly increased in the treated mice (Robin et al., 1981).

Two groups of 45 male BALB/c AF1 mice, six to eight weeks old, were given four i.p. injections of 0.5 mg of busulfan (1,4-butanediol dimethanesulphonate) in 0.25 mL acetone, one injection every two weeks. Two other groups of 45 male BALB/c AF1 mice received injections of acetone diluted with distilled water. After a 20-week rest period, one of the groups previously given busulfan and one of the groups previously given acetone diluted with water were administered 2.5 mg (0.25 mL) of chloramphenicol (purity unspecified), five days per week for five weeks. A control group of 45 male BALB/c AF1 mice received four i.p. injections of 0.25 mL of acetone in distilled water, followed by saline solution only. All surviving mice were sacrificed on day 350 of the study and microscopically examined. The incidence of lymphoma was higher in the busulfan–chloramphenicol group (13/37, $p = 0.02$) than in the busulfan-only group (4/35) and in animals treated with chloramphenicol alone was 2/41. No lymphomas were found in the 41 surviving controls (Robin et al., 1981).

Data obtained with C57BL/61 mice injected into the tail H1299 cells (non-small-cell lung cancer), show that treatment of H1299 cells for 24 hours with chloramphenicol (100 µg/mL) led to a remarkable increase in metastatic colony formation on lung surface, possibly mediated through a chloramphenicol-induced matrix metalloproteinase (MMP)-13 expression (Li et al., 2010).

No conclusion can be drawn regarding the potential carcinogenicity of chloramphenicol because of the lack of appropriate and well-documented long-term studies.

7.3. Modes of action

Chloramphenicol is a wide-spectrum antimicrobial drug targeting protein synthesis in bacteria. It is effective against a wide range of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. At clinically achievable concentrations it is bactericidal against *Haemophilus influenzae, Streptococcus pneumoniae* and *Neisseria meningitidis* (Rahal and Simberkoff, 1979).

Chloramphenicol inhibits protein synthesis by binding to the 50S subunit of the 70S ribosome at the peptidyl transferase centre A. This leads to inhibition of the peptidyl transferase activity and prevention of protein chain elongation (Wolfe and Hahn, 1965; Greenwood and Whitley, 2002; Anderson et al., 2012). The binding of chloramphenicol to the peptidyl transferase centre A is highly specific and reversible, illustrating its bacteriostatic nature. It has been suggested that the binding of

chloramphenicol to this site is as a result of its similarity in structure to an aminoacylated nucleoside (Hansen et al., 2003).

Relatively few studies were performed to compare the antimicrobial effects or other effects of the four stereoisomers of chloramphenicol, the active form D-threo (1R,2R), dextramycin or L-threo (1S,2S), L-erythro (1R,2S) and D-erythro (1S, 2R). Maxwell and Nickel (1954) showed that D-threo (1R,2R) has clear antibacterial effects towards *E. coli*, contrary to L-threo (1S,2S) and D-erythro (1S,2R) which showed no effects. However, the L-erythro isomer (1R, 2S) showed some antibacterial effect (1–2 % of the D-threo isomer). The same was true for inhibition of protein synthesis in *E. coli*. The effect of L-erythro was not due to impurities of D-threo. Hahn et al. (1954) showed that L-erythro was able to inhibit the synthesis of polypeptides, composed of D-amino acids, by *B. subtilis*, in contrast to D-threo which only caused a delay in the synthesis. D-Threo is known to inhibit the synthesis of peptides from L-amino acids, the ones occurring naturally. The other two isomers were inactive. Contrary to *E. coli*, only D-threo, so also not L-threo, was able to inhibit the growth of *B. subtilis*. Rendi and Ochoa (1962) showed the inhibition of amino acid incorporation into proteins in cell-free extracts from *E. coli* by D-threo. However, also L-erythro and L-threo showed some inhibition, although to a lesser extent.

Schlender et al. (1972) investigated effects on plant growth and showed that in coleoptiles from *Avena sativa* and *Triticum vulgare*, all four isomers were equally active in inhibiting protein synthesis. They were also equally potent in inhibiting the synthesis of α-amylase by *Hordeum vulgare*. However, concentrations required are much higher than in bacteria and the mechanism of action might be quite different, potentially acting through effects on the mitochondria.

These studies show that the antimicrobial effects of the naturally occurring D-threo are much stronger than those of the other stereoisomers but that effects in plants and animals may not differ between the various forms.

The similarity between prokaryotic and eukaryotic (mitochondrial) 70S ribosomes might be the reason for the observed adverse effects caused by chloramphenicol treatment. The most serious adverse effect is bone marrow toxicity, which may occur in two distinct forms: bone marrow suppression, which is a direct toxic effect and usually reversible (Yunis and Bloomberg, 1964; Ambekar et al., 2000; Shukla et al., 2011), and aplastic anaemia, which is idiosyncratic, rare, unpredictable, unrelated to dose and generally fatal (Yunis and Bloomberg, 1964; Yunis, 1978; Turton et al., 2002).

7.3.1. Bone marrow suppression and aplastic anaemia

Chloramphenicol can cross most cell membranes, but side effects are found in the bone marrow. This might be because maturing haematopoietic cells are completely dependent upon transferritin for iron intake (Ponka, 1997; Leiter et al., 1999), and these cells are exquisitely sensitive to hypoferritinisation. Chloramphenicol is capable of diminishing the mitochondrion-based transferritin receptor expression, resulting in ferritin depletion in mitochondria. Consequently, ferritin-free mitochondria are metabolically dysfunctional, and affected erythrocytes manifest this phenomenon via hypochromic–microcytic anaemia during the dose-dependent anaemia associated with chloramphenicol (Barnhill et al., 2012).

Inhibition of growth of human bone marrow cells in culture by chloramphenicol at concentrations above about 10 µg/mL was observed by Morley et al. (1974). Additionally, using cultures of human bone marrow cells, Burgio et al. (1974) observed inhibition of haem synthesis at concentrations of 10 µg/mL and higher. At concentrations in the order of 10 µg/mL they found stimulation of DNA synthesis, but inhibition occurred at higher concentrations (> 100 µg/mL). Although this inhibition occurred at concentrations higher than therapeutic doses, a possible link between inhibition of DNA synthesis and the induction of aplastic anaemia was suggested by Burgio et al. (1974) because it had been shown by Yunis and Bloomberg (1964) that in bone marrow of patients treated with chloramphenicol, inhibition of DNA synthesis can be observed. According to the authors this might be suggestive for a hypersensitivity phenomenon. These observations confirm the inhibition of nucleic

acid synthesis by chloramphenicol in bone marrow cells at a concentration of 50 µg/mL, as previously described by Yunis and Harrington (1960).

Yunis et al. (1980b) compared the effects of chloramphenicol and nitroso-chloramphenicol on DNA synthesis in human bone marrow cells, on CFU-C growth of human myeloid committed (CFU-C) stem cells, on cell viability in human bone marrow, and on mouse CFU-S (pluripotential hemapoietic stem cells) viability *in vitro*. Chloramphenicol at concentrations > 300 µM only caused reversible inhibition of DNA synthesis and CFU-growth, without affecting marrow cell viability. In contrast, 50 µM nitroso-chloramphenicol inhibited DNA synthesis and caused irreversible inhibition of CFU-C growth and cell death as well as irreversible damage in mouse CFU-S. In the same study it was found that in a rapidly growing human lymphoid cell line nitroso-chloramphenicol caused accumulation of cells in the G2M pre-mitotic phase and increased cell death in the arrested population (Yunis et al., 1980b).

Jimenez et al. (1987) studied the effect of chloramphenicol and its metabolites dehydro-chloramphenicol and NPAP and nitroso-chloramphenicol on the growth and DNA synthesis of human bone marrow cells. At a concentration of 5 µM dehydro-chloramphenicol caused a total and irreversible inhibition of myeloid colony (CFU-GM) growth, indicating that it is 10- to 20-fold more cytotoxic than chloramphenicol. At this concentration nitroso-chloramphenicol caused about 40 % inhibition and NPAP was less toxic, causing 69 % inhibition at a higher concentration (10 µM). At a concentration of 100 µM dehydro-chloramphenicol inhibited DNA synthesis by 80 %. Because of its high cytotoxicity and its stability the authors suggest that dehydro-chloramphenicol may play a significant role in chloramphenicol-induced haematotoxicity.

Nara et al. (1982a) studied the effects of chloramphenicol on the haematopoietic inductive microenvironment and found that chloramphenicol at concentrations of 10, 50 or 100 µg/mL suppressed the growth of human bone marrow granuloid-commited progenitor cells (CFUc) and the colony forming activity in human fibroblasts *in vitro*.

Reduction of the nitro group, present in chloramphenicol, but absent in the less toxic derivatives florfenicol and thiamphenicol (Figure 4), appears to be essential for the occurrence of aplastic anaemia (Skolimowski et al., 1983). Nitroreduction of chloramphenicol and production of chloramphenicol hydroxylamine has been demonstrated by Ascherl et al. (1985) with rat liver microsomes, and Abou-Khalil et al. (1985) demonstrated nitroreduction of chloramphenicol by mitochondrial p-dinitroreductase. Teo et al. (1986) investigated an alternative pathway in the nitroreduction of chloramphenicol and found that incubation of rat liver microsomes resulted in the increased formation of superoxide anion radicals, as detected by the reduction of succinylated ferricytochrome c. Based on this, it was postulated that the oxidative metabolism of amino-chloramphenicol to chloramphenicol hydroxylamine and nitroso-chloramphenicol and subsequent reduction of these metabolites produced a redox cycle resulting in the formation of superoxide anion radicals. According to the authors the subsequent formation of hydroxyl radicals could account for the DNA-damaging effect of chloramphenicol.

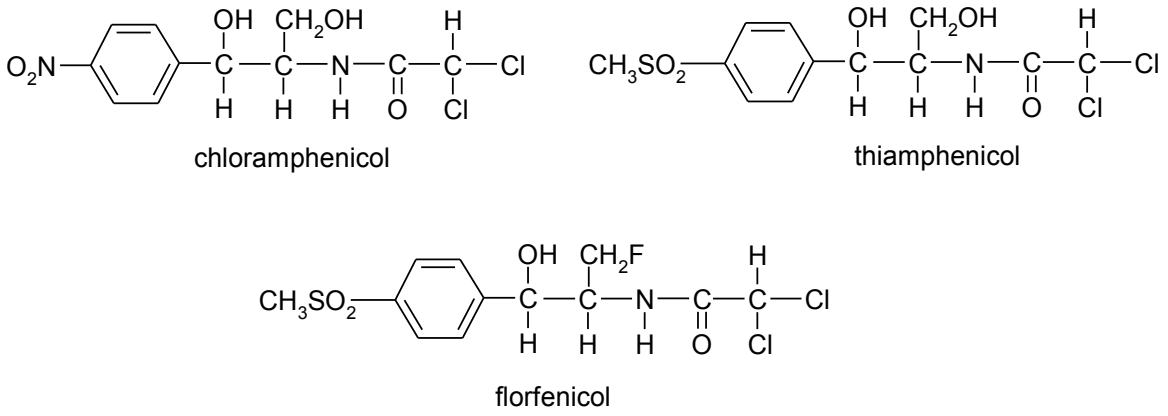

Figure 4: Chemical structures of chloramphenicol, thiamphenicol and florfenicol

Holt and Bajoria (1999) incubated human fetal and neonatal liver tissue extracts with chloramphenicol in the low nM range and observed the formation of chloramphenicolamine, both under aerobic and anaerobic conditions. They could not demonstrate nitroso-chloramphenicol, but they speculated that transient nitroso-chloramphenicol formed in the bone marrow might inhibit DNA synthesis and may lead to apoptosis. Whether nitric oxide (NO), also capable of causing apoptosis (Bonfoco et al., 1995), is involved in this effect is unclear, although Holt and Bajoria (1999) found some indications for its role in chloramphenicol toxicity, because perfusion of cannulated human placenta by chloramphenicol (200 mg added to the maternal perfusate, concentration not given) caused a decrease in fetal blood pressure. However, the final conclusion of Holt and Bajoria (1999) was that there is no hard evidence that chloramphenicol is a direct or indirect NO donor.

It appears that ancillary factors such as underlying mitochondrial dysfunction (Leiter et al., 1999), genetic polymorphisms that accentuate chloramphenicol binding to mitochondrial rRNA (Metha et al., 1989; Kim et al., 2008), or a genetic predisposition (Nagao and Mauer, 1969) that enhances the ability of the bone marrow to nitroreduce chloramphenicol into its myelotoxic derivative are needed to cause aplastic anaemia (Barnhill et al., 2012).

Benestad (1974), analysing data on aplastic anaemia, suggested that the aplasia caused by chloramphenicol or one of its metabolites may be the result of stem cell damage of an autoimmune or idiosyncratic nature. For the latter case it was proposed that a hereditary defect in stem cells make them more susceptible to damage by chemicals such as chloramphenicol.

Yunis and Salem (1980), reviewing earlier *in vitro* and *in vivo* studies on the mitochondrial damage caused by chloramphenicol, reported that mitochondrial protein synthesis was exquisitely sensitive to chloramphenicol. Since this effect is not tissue specific the vulnerability of erythroid cells in the bone marrow for chloramphenicol requires an additional mechanism. They indicated that ferrochelatase, which is associated with the inner mitochondrial membrane, could be suppressed by chloramphenicol, leading to a block of the haem synthesis and thus to effects in bone marrow. Inhibition of ferrochelatase activity was also found in bone marrow of dogs after oral administration of 100 mg/kg b.w. per day for three weeks (Manyan and Yunis, 1970; Manyan et al., 1972). It was concluded (Yunis and Salem, 1980; Yunis et al., 1980a) that reversible bone marrow suppression and sideroblastic anaemia induced by chloramphenicol, results from inhibition of mitochondrial protein synthesis.

In isolated perfused hearts of newborn pigs Werner et al. (1985) found that chloramphenicol concentrations of 25 to 100 µg/mL in the recirculating buffer caused acute reduction of cardiac pressure and output. Studies with isolated heart mitochondria of these pigs, showed that chloramphenicol at concentrations in the range of 25–200 µg/mL inhibited state 3 oxidation of succinate and glutamate. This suggests that oxidation of both fatty acids and carbohydrates might be

affected by chloramphenicol and that these effects might be the basis for the observed acute myocardial effects observed in the isolated hearts.

Because highly reactive intermediates may be involved in the aplastic anaemia induced by chloramphenicol Murray et al. (1982) investigated the role of nitroso-chloramphenicol in the degradation of isolated DNA. They found that a mixture containing 100 µM nitroso-chloramphenicol in the presence of $CuCl_2$ and NADH was able to completely degrade *E. coli* DNA in a period of 30 minutes. The damage to DNA was in the form of single-stranded scissions. Oxygen was necessary for the nitroso-chloramphenicol -mediated DNA damage, and a role for H_2O_2 was indicated, because CAT inhibited the degradation. The importance of the nitroso moiety of nitroso-chloramphenicol was evidenced by the lack of DNA damage seen when nitroso-chloramphenicol was replaced by chloramphenicol.

Paez et al. (2008) found that chloramphenicol at concentrations of 2–16 µg/mL increased the production of reactive oxygen species (ROS) in isolated human neutrophils *in vitro*. The activity of SOD, CAT and diaphorase was increased at these concentrations. At higher levels (32 µg/mL) the production of ROS was decreased, as was the activity of SOD and diaphorase. The authors suggested that ROS and antioxidant enzymes should be investigated to detect patients with different responses to chloramphenicol, considering that the haematological effects might be the consequence of oxidative damage.

7.3.2. Effects on apoptosis

Holt et al. (1997) reported apoptosis by chloramphenicol in dividing cells from a monkey-kidney cell line and in haematopoietic progenitor cells from neonatal cord blood. Incubation for 24 or 48 hours at concentrations ranging from 0.2 to 2 mM chloramphenicol caused severe morphological changes in monkey-kidney cells, and up to 1 mM chloramphenicol a progressive increase in the number of apoptotic cells was seen. At concentrations above 1 mM, the number of apoptotic cells fell, most probably because the cells would have been disintegrated to such an extent that they would not have been counted as apoptotic. Erythroid progenitor cells appeared to be more susceptible to chloramphenicol because apoptosis and a reduction of 50 % in growth was observed already at a concentration of 5 µM chloramphenicol. According to the authors this concentration corresponds with a plasma concentration of 1.6 mg/mL, which would be considered less than a therapeutic level. The observed effects could be ameliorated by co-culture with antioxidants (mercaptoethylamine or vitamin C), suggesting the involvement of free oxygen radicals in the chloramphenicol-induced apoptosis.

Kang et al. (2005) also found that chloramphenicol can induce apoptotic cell death. In chronic myelogenous leukaemia K562 cells *in vitro* chloramphenicol inhibited growth in a dose dependent manner, at concentrations ranging from 10 to 80 µg/mL after six days of incubation. It was shown that the activity of caspase-3 was increased, suggesting that the cells underwent apoptosis through a caspase-dependent pathway. The viability of the cells increased after removal of chloramphenicol, indicating that the inhibition of the growth of the K562 cells was reversible. In addition, observed reduced expression of cyclin D1 and E2F-1 proteins suggested that chloramphenicol affects cell cycle regulatory molecules at a translational and/or transcriptional level, which may result in inhibition of cell growth.

Because chloramphenicol is widely used for topical application in ophthalmology and dermatology, Popadic et al. (2006) investigated the effect of chloramphenicol on keratinocytes *in vitro*. Chloramphenicol significantly inhibited proliferation and induced apoptosis of cultivated human keratinocytes. The chloramphenicol-induced keratinocyte apoptosis was associated with activation of caspases and increased production of ROS. The pro-apoptotic action of chloramphenicol was antagonised by the antioxidant agent N-acetylcysteine, the protein synthesis inhibitor cycloheximide, and by PD98059, a selective inhibitor of extracellular signal-regulated kinase (ERK) activation. It can be concluded that chloramphenicol inhibits keratinocyte proliferation through induction of oxidative stress and ERK-mediated caspase-dependent apoptosis (Popadic et al., 2006).

7.3.3. Drug interactions

It has been mentioned above (see Sections 7.1.1, 7.1.3, 7.1.5 and 7.1.7) that inhibition by chloramphenicol of CYP-enzymes may lead to unwanted interactions in humans and animal species with concomitantly administered drugs being CYP substrates. In addition, Pai et al. (2006) reported a number of examples of chloramphenicol increasing the serum concentration of drugs with a narrow therapeutic range, such as the immunosuppressants cyclosporine and tacrolimus, the sulphonylurea type hypoglycaemics, and the anticoagulant warfarin, which could lead to life-threatening situations.

It has also been reported that haematotoxic effects and anaemia can be influenced by the combination of chloramphenicol with other therapeutic compounds. Saidi et al. (1961), for instance, reported that chloramphenicol can oppose the treatment of anaemia with iron or vitamin B_{12} (cyanocobalamin). In four anaemic patients receiving vitamin B_{12} for pernicious anaemia and given 52 to 60 mg chloramphenicol/kg b.w. per day for three to seven days, the expected rise in reticulocytes was delayed, until chloramphenicol administration was withdrawn. In two patients treated with iron dextran for iron-deficiency anaemia, a similar decreased reticulocyte response was observed following simultaneous chloramphenicol administration.

Farber and Brody (1981) reported a case of a 61-year-old male patient receiving therapeutic i.v. doses of cimetidine (1.2 g per day from postoperative day 3 to day 19) also being given chloramphenicol (i.v. 1 g every six hours, from postoperative day 15 to 19). On day 20 chloramphenicol treatment was stopped, but cimetidine was continued. Two weeks later the patient died, with diagnosed aplastic bone marrow (treatment period not mentioned), having developed fatal aplastic anaemia. This deterioration was more rapid than previously described cases of chloramphenicol related aplastic anaemia, and the authors suspect an interactive effect (additive or synergistic) of the two bone marrow-suppressive agents, chloramphenicol and cimetidine.

In addition, West et al. (1988) reported a case of rapid development of fatal aplastic anaemia following combined administration of chloramphenicol and cimetidine. A 54-year-old patient was admitted to the hospital because of purulent drainage from a previous surgical wound. He was treated with i.v. cimetidine (300 mg every 12 hours from day 9 to day 20 of admission to the hospital) and chloramphenicol (750 mg every six hours from day 9 to day 16). A bone marrow biopsy on day 21 disclosed marked marrow hypoplasia. The patient died on day 27. The rapid onset of aplastic anaemia again suggested an additive or synergistic interaction between cimetidine and chloramphenicol.

The CONTAM Panel noted, however, that the above-mentioned interactions happen at therapeutic levels and, therefore, are not directly relevant for the risk assessment of residues of chloramphenicol in food and feed.

7.3.4. Concluding comments

Chloramphenicol can easily cross most cell membranes but toxic effects are predominantly found in the bone marrow. It has been suggested that reversible bone marrow suppression induced by chloramphenicol results from inhibition of mitochondrial protein synthesis. Since this effect is not tissue specific, the vulnerability of erythroid cells in the bone marrow to chloramphenicol requires an additional mechanism. Maturing haematopoietic cells are completely dependent upon ferritin for iron intake and exquisitely sensitive to ferritin depletion. Therefore, chloramphenicol-induced ferritin depletion of inhibition of ferrochelatase, which is associated with the inner mitochondrial membrane, has been indicated as the possible cause of reversible bone marrow suppression and anaemia resulting from the inhibition of protein synthesis.

It appears, however, that ancillary factors are needed to result in chloramphenicol-induced aplastic anaemia. Nitroreduction to nitroso-chloramphenicol and the production of ROS leading to DNA damage seem to be crucial factors in the induction of aplastic anaemia. In addition, genetic predisposition enhancing the ability of the bone marrow to nitroreduce chloramphenicol into its

myelotoxic derivative might play an important role. But the mechanism for chloramphenicol-induced aplastic anaemia in humans has not yet been elucidated.

7.4. Adverse effects in livestock, fish, horses and companion animals

7.4.1. Ruminants

Most of the available papers deal with the parenteral dosing of the antibiotic due to its rapid inactivation (NO_2 reduction) brought about by the ruminal biota. One of the few studies involving the oral route of administration was carried out in female beef cattle (weight range 208–219 kg) administered with 25 mg chloramphenicol palmitate/kg b.w. per day for four consecutive days (Gassner and Wuethrich, 1994). As measured by HPLC combined with UV/DAD and electrochemical detectors, the appearance of the haematotoxic metabolite dehydro-chloramphenicol at levels of 3 to 7 ng/mL as early as three hours after the first administration was documented in serum samples. However, a quantitative correlation between the circulating levels and the extent of bone marrow suppression (not investigated in this study) could not be established.

An experiment was performed in newborn Holstein bull calves to evaluate the acute response to high dosages of chloramphenicol administered via the i.v. route and to determine the effects of the presence of a sustained high serum level of the antibiotic. Four calves administered with 50 or 100 mg chloramphenicol/kg b.w. showed a dramatic decrease of blood pressure (from 140/105 to 30/25) which was not dependent on the infusion rate. The most consistent adverse effects occurring after repeated chloramphenicol administration (100 mg/kg b.w. per day) were gastrointestinal disturbances (diarrhoea), weakness, and depression. Death ensued in three of five treated calves after 9, 17, and 18 treatments, respectively. Reduction in packed cell volume and haemoglobin values occurred in one calf only (Burrows et al., 1988). Decreases in weight gain and in serum cortisol levels were reported in male calves (weight range 95 to 120 kg) daily dosed with 60 mg/kg b.w. by the i.m. route for 42 days; both parameters were not modified in calves receiving 20 mg/kg b.w. per day (formerly used therapeutic dosage) according to the same protocol (Mitema and Musewe, 1984). Chromosomal aberrations have been detected in calves. Eight female calves (2–4 months old) were i.m. injected daily with chloramphenicol sodium citrate (10 mg/kg b.w.) once a day for four successive days and blood was collected at the end of the treatment and after three weeks from the last injection. A statistically significant increase (> 100 %) in chromosomal breaks and gaps as well as in the total number of chromosomal aberrations occurred in blood cultures of treated calves at both time points. In addition, lymphopenia was observed in blood samples collected 21 days after chloramphenicol withdrawal (Othman et al., 2005)

Chloramphenicol has resulted in hypersensitivity reactions in cattle. A four-year-old Holstein–Friesian crossbred cow (weight not reported) suffering from mastitis was given 1 g chloramphenicol succinate by slow i.v. injection; immediately after dosing, it showed trembling, collapse, tachycardia, and depression but recovered after appropriate anti-shock therapy (Bhat et al., 1995).

7.4.2. Pigs

In a pig farm, nervous symptoms, meteorismus and death occurred after an overdose of chloramphenicol. This was experimentally reproduced at a dose of 1 g/kg b.w. The authors reported a similarity with the so-called grey baby syndrome in paediatrics (Vajda and Békéssy, 1981; only English abstract available).

7.4.3. Poultry

As a follow-up of problems with egg production on a turkey farm, Friedman et al. (1998) performed a study to investigate potential combined effects of chloramphenicol and monensin. Treatment of turkeys with monensin via the feed at levels of 42 mg/kg for 15 days had no effects on egg production. When in addition chloramphenicol was supplied via drinking water at a concentration of 500 mg/L (corresponding approximately to 33–50 mg/kg b.w. per day, assuming a drinking water consumption

of 2–3 times the feed intake (EFSA FEEDAP Panel, 2010), a feed intake of 400 g per day and a body weight of 12 kg for turkeys (EFSA FEEDAP Panel, 2012)) for eight days, there was a transient 15 % drop in the egg production, both in the presence and absence of monensin. In a second phase of the study, the level of monensin was increased to 70 mg/kg feed, applied for seven days in combination with 500 mg/L chloramphenicol in drinking water for four days. On days 4, 5, 6 and 7, 1, 4, 5 and 4, respectively, of the 22 animals died and egg production stopped at day 5 and did not recover after withdrawal of monensin during the remaining 11 days. Treatment with chloramphenicol alone (500 mg/L for four days) resulted in a 9 % drop in egg production, whereas treatment with monensin alone at 85 mg/kg feed for seven days had no effect. In the combined treatment, also the level of creatine phosphokinase in plasma was strongly increased. This study shows a combined effect of chloramphenicol and the higher dose of monensin.

In a study by Rigdon et al. (1954), White Peking ducks were given 250 mg chloramphenicol capsules (maximum total dose 54 g) and two of 100 ducks died. Analysis of peripheral blood and haematopoietic organs showed indications of anaemia although there is insufficient detail in the paper to assess this further.

7.4.4. Fish

Shalaby et al. (2006) studied the effects of oral exposure to chloramphenicol on physiological parameters, growth performance, survival rate, and bacteriological characteristics in Nile tilapia (*Oreochromis niloticus*). Fish (7 g) were assigned to four groups with triplicates of each; 0, 15, 30 or 45 mg chloramphenicol/kg diet administered at a rate of 6 % body weight daily for 90 days. Results showed that the final weight and specific growth rate of *O. niloticus* increased significantly with increasing levels of chloramphenicol. The highest growth performance was found in the group fed 30 mg chloramphenicol/kg diet, this group also had the lowest feed conversion ratio. There were significant differences in the protein efficiency ratio with all treatments, except with 45 mg chloramphenicol/kg diet. No changes in the hepatosomatic index or survival rate were observed. Ash of whole fish showed significantly higher values in the group fed 15 mg chloramphenicol/kg diet while the lowest value was observed in the control group. Blood parameters, erythrocyte count and haemoglobin content in fish fed on diets containing chloramphenicol were significantly higher than in controls. Significantly higher haematocrit values were seen in fish fed with 30 and 45 mg chloramphenicol/kg diet, whereas there were no effects on mean corpuscular volume or mean corpuscular haemoglobin concentration. Plasma glucose increased significantly with increasing levels of chloramphenicol. Total lipids were significantly reduced in fish fed the 30 mg chloramphenicol/kg diet, while total plasma protein content was significantly higher in fish fed on diets containing 30 and 45 mg chloramphenicol/kg diet. AST and ALT activities in plasma decreased significantly with increasing levels of chloramphenicol. All chloramphenicol levels decreased total bacteria and coliforms in water, muscles and intestine compared with the control group. Treated groups had lower mortality rate than the control group during a challenge test.

Kasagala and Pathiratne (2008) evaluated the effects of different concentrations of chloramphenicol (0, 2, 5 or 10 mg/L for 10 days) on haematological parameters and phagocytic activity in the blood of koi carp, *Cyprinus carpio*. Results showed that treatment of fish with 10 mg chloramphenicol/L for 10 days significantly depressed haematocrit, erythrocyte counts and mean corpuscular volume, leading to anaemia. The absolute neutrophil counts and thrombocytes in the blood of fish exposed to 5 mg chloramphenicol/L or 10 mg chloramphenicol/L for 10 days were significantly higher than that of fish exposed to 2 mg chloramphenicol/L and controls. There was no significant difference in absolute monocyte counts in the blood among exposed fish and controls. The absolute counts of lymphocytes in fish exposed to 10 mg chloramphenicol/L were significantly higher than in fish exposed to 2 mg/L and controls. The phagocytic index of the fish exposed to 10 mg/L for 10 days was significantly increased compared with the controls.

The effects of chloramphenicol on haematopoiesis in the European eel (*Anguilla anguilla*) was reported by Kreutzmann (1977). Feral eels were adapted to aquarium conditions and assigned to a

control group, or treatment group involving an i.p. injection of 20 mg/kg body weight at the start of the experiment. Blood was extracted after 24 hours, 72 hours, 7 days and 14 days. Increased vacuolation of the plasma, modifications of the nucleus, decreased number of erythroblasts and disturbance in fat metabolism of the erythrocytes was observed in the fish exposed to chloramphenicol. Deleterious effects were observed in leucocytes and thrombocytes, in particular heterophile granulocytopenia, thrombocytopenia accompanied by monocytosis and a relative lymphocytosis. Changes were reversible and returned to resemble control blood seven days after administration of chloramphenicol.

Nwani et al. (2014) examined the effects of aquatic exposure of chloramphenicol, on behaviour and haematological parameters of *Clarias gariepinus*. Fish were assigned to one of three (2.5, 5.0 and 10.0 mg/L) concentrations of chloramphenicol or a control. Abnormal behavioural changes (erratic swimming, circling movement and hyperactivity) were observed in fish exposed to chloramphenicol. Blood erythrocytes were sampled on days 1, 5, 10 and 15 post exposure to evaluate haematological parameters. Results showed concentration- and time-dependent significant increase in packed cell volume after day 5 of exposure ($p < 0.05$). Haemoglobin values also significantly decreased from day 5, whereas values for mean cellular volume significantly decreased throughout the experimental period ($p < 0.05$). Lymphocytes were the most dominant leucocyte species in the peripheral blood of *C. gariepinus* exposed to chloramphenicol. There was a significant increase in the percentage of lymphocytes in the WBC counts of chloramphenicol-treated fish during the exposure period, whereas no significant differences were observed in monocytes, eosinophils and basophils among fish in the treatment groups and the control.

7.4.5. Horses

No studies in horses were identified.

7.4.6. Companion animals

Conner and Gupta (1973) studied kinetics and bone marrow effects in cats after repeated ocular ointment treatment with 1 % chloramphenicol, three times per day for 21 days. While plasma and notably urine levels indicated systemic exposure, no changes in bone marrow or haematology were noted.

Penny et al. (1967) reported that in cats ($n = 4$), treated for 21 days i.m. with 50 mg chloramphenicol/kg b.w.per day, depression and loss of appetite after seven days occurred, followed by haematological and bone marrow effects (including vacuolation). Both white and red blood cell systems were affected and affected cats developed diarrhoea and were euthanised *in extremis*.

Watson and co-workers (Watson and Middleton, 1978; Watson, 1980) reported that an oral dose of 120 mg/kg b.w. per day within one week caused depression, dehydration, loss of appetite and weight in cats. After one week, bone marrow analysis showed reduction in erythroid and lymphoid lineages. After two weeks, reduction of neutrophils, lymphocytes, reticulocytes and platelets in the peripheral blood was reported. At lower doses (25–60 mg/kg b.w. per day for three weeks), these changes also occurred but were less severe.

Baig et al. (1994); studied the effects (notably haematological) after an oral dose of chloramphenicol palmitate at 300 mg/kg b.w. per day for 14 days in dogs. A reduction in red and white blood cell parameters and reticulocytes, as well as blood glucose and serum protein were seen, but bilirubin, ALT and AST were increased. Bone marrow revealed a reduction in erythroid cells and consequently a shift in erythroid–myeloid ratio. It was noted that a coinciding reduction in appetite, and consequently malnutrition, might have been a contributing/complicating factor. This latter finding is further supported in experiments by Penny et al. (1973), in which 50 mg/kg b.w. per day produced no bone marrow changes; in contrast, a dose of 150 mg/kg b.w. per day was associated with loss of appetite.

Watson (1977, 1991) reported toxic effects in dogs after oral doses of 75–300 mg/kg b.w. per day for 2–3 weeks. At the lower dose (up to 100 mg/kg b.w. per day), food intake and weight gain increased, while at the higher dose (175 mg/kg b.w. per day) animals became depressed and ate less (dysphagia) and at 225 mg/kg b.w. per day and above, bone marrow depression (both erythroid and granulocytes) became apparent while in peripheral blood only reticulocytopenia was found.

7.4.7. Concluding comments

Despite the former widespread use as a veterinary drug, limited information is available concerning adverse effects in livestock, especially after oral treatment. Some effects were described in calves treated i.m. or i.v. with doses of 20–100 mg/kg b.w., including chromosome aberrations in lymphocytes from treated animals. In cats and dogs, prolonged treatment with doses higher than 50 mg/kg b.w. resulted in effects in the bone marrow/blood system.

7.5. Observations in humans

7.5.1. Human pharmacological and toxicological data

The use of chloramphenicol in antibacterial therapies has resulted in many case studies reporting adverse effects in humans. Most frequently reported is haematotoxicity, but other adverse effects, including neurotoxicity, cardiotoxicity, hepatotoxicity and allergy, have also been reported. An overview of different case studies is given below; however, the CONTAM Panel noted that this is not a complete overview of the case studies that could be available.

7.5.1.1. Clinical trials

In a number of clinical trials in which the efficacy of chloramphenicol in the treatment of typhoid fever was investigated against several other antibiotics, adverse effects have been reported. Mild and transient epigastric pain were reported by Morelli et al. (1992) and Cristiano et al. (1995) in 6 and 5, respectively, of 30 patients suffering from typhoid fever that received an oral dose of 2 g chloramphenicol per person per day for a period of 15 days. Applying the same dose regimen, Carcelen et al. (1989) reported moderate sideroblastic anaemia in one and moderate neutropenia in another one of 33 patients after seven days of treatment. Both effects resolved spontaneously within a week. Leukopenia, neutropenia and anaemia were also reported by Tanaka-Kido et al. (1990) in 3, 6, and 5, respectively, of 18 typhoid fever patients treated with 100 mg chloramphenicol/kg b.w. per day for about two weeks. Arnold et al. (1993) reported gastrointestinal hemorrhages and perforation and pneumonia in 2 of 61 patients receiving a dose of 50 mg chloramphenicol/kg b.w. per day for a period of two weeks. According to the authors, these effects were not related to the treatment, because they are common complications related to typhoid fever. In these studies no other, more serious, adverse effects have been reported.

7.5.1.2. Case studies

Haematotoxicity

Since the introduction of chloramphenicol as a broad-spectrum antibacterial agent in 1948, reports of bone marrow toxicity are reported. Chloramphenicol induces two types of bone marrow toxicity (Cohen and Huang, 1973; Awwaad et al., 1975; Lery et al., 1978; Silver and Zuckerman, 1980; Smyth and Pallet, 1988; Flegg et al., 1992; Wareham and Wilson, 2002). One type, which occurs during treatment, is dose dependent and characterised by anaemia, with or without leucopenia or thrombocytopenia, and a normocellular bone marrow. It has been indicated that this adverse effect occurs at dose levels > 4 g/day and is usually reversible when the drug is discontinued. It may be caused by an inhibitory effect of chloramphenicol on mitochondria (Wareham and Wilson, 2002). The other type of bone marrow toxicity shows a later onset, is not dose related and is characterised by an aplastic bone marrow, pancytopenia and an often fatal outcome. Even in the first year of its clinical use, chloramphenicol-induced aplastic anaemia was described (Lery et al., 1978).

Case studies following clinical chloramphenicol administration

In the literature many case studies following clinical use of chloramphenicol are described. The CONTAM Panel noted that in several of these studies the authors did not specify the route of chloramphenicol administration. However, considering that therapeutic use for the treatment of systemic infections will generally be through i.v. or oral administration and that the bioavailability of chloramphenicol upon oral administration is high, as a first approximation dose levels when reported without definition of the route of administration could be considered to be fully bioavailable. In the case of (unspecified) i.v. administration this would represent a worst-case approach for oral risk assessment.

Some reports have suggested a more frequent occurrence of aplastic anaemia upon oral than i.v. dosing, but this has been related to the far greater use of oral dosing regimens (Smyth and Pallet, 1988).

A fatal case of aplastic anaemia in a six-year-old girl following two days of chloramphenicol therapy was described by Cone and Abelson (1952). The patient received four doses of 250 mg chloramphenicol during two consecutive days. Two months after treatment she had developed severe bone marrow aplasia with absence of megakaryocytes.

Lasky et al. (1953) reported a patient receiving 6 g of chloramphenicol daily for about six weeks (route of administration not specified) developing a severe anaemia, which gradually disappeared upon discontinuation of the chloramphenicol therapy. The patient also developed bilateral optic neuritis and cutaneous manifestations (acne-like lesions).

Brunton and Shapiro (1962) described three fatal cases were the patient was treated during short repeated courses of about one or two weeks with a dose of about 1 g chloramphenicol per day. In all cases severe bone marrow hypoplasia was seen, sometimes accompanied by lack of megakaryocytes and mature granulocytes. Another patient, a 51-year-old male, receiving three courses with a similar dose of chloramphenicol (about 1 g per day) developed the same symptoms, but recovered after termination of chloramphenicol treatment.

Cohen and Greger (1967) described a female patient who died at the age of 47 of acute myeloid leukaemia following seven years of chloramphenicol-induced aplastic anaemia. The patient had been treated with chloramphenicol on several occasions, receiving a total dose of approximately 40 g during a three-month period (route of administration not specified).

Awwaad et al. (1975) reported a six-year-old girl who received chloramphenicol for 10 days at a dose level of 25 mg/kg b.w. per day (route of administration not described), showing vacuolisation of leucocytes in bone marrow aplasia. After six months, she returned with purpura, hepatosplenomegaly and enlargement of the axillary nodes, and a bone aspirate showed acute myeloblastic leukaemia.

Daum et al. (1979) reported a case of fatal aplastic anaemia developing in a 23-year-old male several years after treatment with chloramphenicol for 10 days, starting at 500 mg, followed by 250 mg orally four times per day. Upon a new hospitalisation, 19 years after the first treatment, the patient was again treated with chloramphenicol (750 mg every six hours by i.v. at 61.8 mg/kg b.w. per day). The patient developed progressive anaemia on the twelfth day of administration, and bone marrow aspiration showed that all erythroid precursors were absent and that there was hypoplasia of all other bone marrow elements. Chloramphenicol treatment was discontinued but the patient did not recover.

Alavi (1983) reported a 27-year-old patient who developed aplastic anaemia three months after i.v. administration of chloramphenicol at a total dose of 30 g over 12 days, and who died of the disease four years later.

Wiest et al. (2012) described a 12-year-old patient developing manifestations of chloramphenicol toxicity. He had been given a 6–8 week course of i.v. metronidazole, 550 g every eight hours

(33 mg/kg b.w. per day) and chloramphenicol, 1 g every six hours (88 mg/kg b.w. per day). The patient was sent home on i.v. metronidazole, 500 g every eight hours and i.v. chloramphenicol, 1 g every six hours, and divalproex 2 g orally at bedtime. At day 36 of chloramphenicol treatment, he was hospitalised again where his previous regimen of i.v. chloramphenicol, metronidazole and oral divalproex was continued. Over the next weeks the patient developed symptoms such as anaemia, thrombocytopenia, reticulocytopenia and severe metabolic acidosis and eventually a presumptive diagnosis of chloramphenicol toxicity was considered. Chloramphenicol administration was discontinued and hemodialysis was initiated for severe metabolic acidosis. The patient recovered but had severe visual field deficits (see also Section 7.5.1.2).

Case studies upon topical ophthalmic administration

Bone marrow hyperplasia in a 36-year-old male following use of chloramphenicol-containing eye drops (0.5 % in aqueous solution) for about two days per months over a 23-month period was described by Rosenthal and Blackman (1965). The patient's family history suggested an inherited and undefined enzyme deficiency as a factor in the marrow hypersensitivity.

Carpenter (1975) described a case of bone marrow aplasia in a 37-year-old male who used eye drops once daily over a period of two months and was exposed to a total of 32.5 mg chloramphenicol and 650 mg sulphacetamide.

Abrams et al. (1980) reported the development of severe aplastic anaemia with fatal outcome upon use of chloramphenicol eye ointment in a 33-year-old man treated several months with a chloramphenicol-polymyxon B sulphate eye salve (dose not reported). The authors assumed that the severe aplastic anaemia resulted either from absorption of chloramphenicol through the conjuctival membranes or from drainage down the lachrymal duct with eventual gastrointestinal absorption. They stipulated that is was remarkable that a small amount of chloramphenicol could have such a devastating effect, and mentioned that the lack of dose–response relationships has been noted before by Cone and Abelson (1952) and that this supports the hypothesis for an individual, possibly genetic predisposition to this toxicity. It was also noted that the longer the interval between the last dose of chloramphenicol and the first sign of haematological abnormality, the greater the mortality, and that nearly all patients with intervals longer than two months died.

Red cell aplasia was reported by Fernandez de Sevilla et al. (1990) in a 85-year-old man, admitted to hospital because of fatigue and progressive dyspnoea. There was no significant past medical history and he had not taken any drugs, except chloramphenicol eye drops for the previous two months. A bone marrow aspirate and biopsy showed pure red cell aplasia. The bone marrow depression did not recover following withdrawal of chloramphenicol and treatment with 6-methylprednisolone.

Fraunfelder et al. (1993) reported on 23 cases of patients with blood dyscrasias related to ophthalmic administration of chloramphenicol. Eleven of these cases were fatal. Total doses ranged from 0.03 to 1.64 g for periods ranging from 18 to 1 460 days. The authors concluded that onset and severity did not relate to the total dose administered.

In relation to the topical use of chloramphenicol and the occurrence of aplastic anaemia, systemic absorption following topical administration was studied by Trope et al. (1979) and Walker et al. (1998). Trope at al. (1979) failed to detect chloramphenicol in the urine of five children under nine years of age treated with 0.5 % chloramphenicol eye drops every second hour for five to seven days until a dose between 40 and 52 mg was administered. The LOD of the method used was 1 mg/L. Walker et al. (1998) studied two groups of patients (new patients and postoperative patients) receiving chloramphenicol 0.5 % eye drops four times daily in one eye for one or two weeks. The chloramphenicol dose of the first group ranged from 2.9 to 18.1 mg, and of the second group from 3.6 to 32.1 mg. Blood was collected at the last day of treatment and analysed for chloramphenicol by HPLC using an RP-C_{18} column. In none of the samples could chloramphenicol be detected, with an LOD of 1 mg/L. The CONTAM Panel noted, however, that the LODs might not have been low

enough to detect low systemic levels of chloramphenicol, possibly present following topical ocular administration.

Reviews of case studies

Weiss et al. (1960) investigated the use of chloramphenicol in the newborn infant focussing on the possible role for the variations in the metabolic disposition of chloramphenicol in the newborn infant. The authors suggested a safe and effective dose schedule stating that a tentative maximum dose of 50 mg/kg b.w. per day for full-term newborn infants up to the age of one month was recommended and of 25 mg/kg b.w. per day for premature infants that can be extended into the first week of life of the full-term infant.

Wallerstein et al. (1969) studied the reports of 225 000 deaths that occurred in the State of California in the period 1 January 1963 to 30 June 1964 with a special focus on fatalities related to aplastic anaemia. It appeared that the risk of developing fatal aplastic anaemia was about 13 times higher among individuals treated orally with chloramphenicol than in the general population. Based on an average dose of 4.5 g per person, the risk of death related to aplastic anaemia is about 1 in 40 800 orally treated patients, and for an average dose of 7.5 g per person the death risk is about 1 in 24 500. The authors also mentioned that clinical treatment was usually short and that the interval between treatment and the development of anaemia was usually brief.

Keiser and Buchegger (1973) studied the haematological side effects of chloramphenicol in 44 patients with aplastic anaemia after treatment with chloramphenicol at total dose levels that varied from 3 to 315 g. In these patients white blood cell, haemoglobin and platelet counts were slightly decreased and these effects were reversible. Survival time of most patients was limited, and after three months 21 of the 44 patients were deceased resulting in a mean survival time of 2.8 months.

Lery et al. (1978) reviewed chloramphenicol-induced blood disorders. They reported that the estimates for the frequency of aplastic anaemia varied from 1 in 60 000 to 1 in 600 000 treatments, but that more realistic estimates report values between 1 in 6 000 and 1 in 25 000 treatments, and that a reasonable incidence would be 1 in 11 500 to 1 in 40 000 treatments. As a result, the probability for aplastic anaemia in treated patients is 10 to 13 times higher than in the non-treated population. Lethal outcome occurs in 60 to 80 % of cases of aplastic anaemia with the chances of survival being very low when aplastic anaemia occurs six months after treatment. The review also indicates that anaemic aplasia is not linked to total dose of treatment.

Krishna et al. (1981) provided a survey of literature data on aplastic anaemia in humans caused by several compounds including chloramphenicol. It was indicated that the main drawbacks of studying aplastic anaemia in humans induced by drugs are the low incidence in which this toxicity occurs, with an incidence varying somewhere between 1 in 20 000 and 1 in 100 000, and the fact that the toxicity appears to be independent of the dose. The authors also indicate that the mechanism by which chloramphenicol causes aplastic anaemia is not clearly understood, mainly because of a lack of suitable animal models.

Nahata (1987) studied the possible correlation between serum chloramphenicol concentrations and haematological adverse effects in 45 patients. It was concluded that chloramphenicol toxicity may not be predictable from serum concentrations.

Nahata (1989) studied 45 patients (newborn up to 12 years of age) who received 50–100 mg/kg b.w. per day chloramphenicol sodium succinate i.v. over 2–49 days for the treatment of CNS infections. Chloramphenicol therapy was effective in all patients. Anaemia was present in 10, leukopenia in four, neutropenia in four, and eosinophilia in 16 patients. These adverse effects occurred between 3 and 34 days after the initiation of therapy. Chloramphenicol therapy had to be discontinued in three patients, who had very low neutrophil counts. All adverse effects were reversible. Demographic factors, daily dose, duration of therapy, steady-state peak and trough serum concentrations, area under

the serum concentration–time curve normalised for dose and the elimination half-life were not correlated with the occurrence of the chloramphenicol-induced adverse effects. The mean cumulative dose of chloramphenicol succinate ranged from 1.2 to 1.8 g/kg b.w. in patients with adverse effects and 0.9–1.1 g/kg b.w. in patients without adverse effects (not statistically significantly different).

In a short paper, Doona and Walsh (1995) stated that, although the number of documented cases of aplastic anaemia associated with topical use of chloramphenicol are few, patients should not be subjected to this potential risk. In a reaction on this paper, Mulla et al. (1995) stated that there is little evidence to implicate topical chloramphenicol administration as a cause of aplastic anaemia. They highlighted that in some case studies there were other possible explanations for the observed aplastic anaemia, such as exposure to other medicines, e.g. sulphacetamide (see Carpenter, 1975) or abnormal liver function. Therefore, they concluded that there is insufficient evidence linking topical chloramphenicol administration with aplastic anaemia.

Additionally McGhee and Anastas (1996) reacted to the paper of Doona and Walsh (1995) and stated that based on previously reported literature "*a theoretical but as yet not conclusively proved risk of chloramphenicol induced idiosyncratic aplastic anaemia exists with topical ophthalmic therapy, with the absolute, but highly improbable, maximum risk of death (equalling that of systemic therapy) being 1 in 50 000 to 90 000*." The authors mentioned that statements condemning topical chloramphenicol need to be tempered "*with its proved safety, tolerance, cost, and efficacy while acknowledging an extremely remote risk of the very serious adverse effect of drug induced aplastic anaemia*", and stated that the only known factor to be associated with vulnerability in the case of topical chloramphenicol is family history.

Iwata and Akita (1997) presented a review of adverse effects of antibiotics and concluded that the bone marrow toxicity of chloramphenicol usually occurs during the medication given for five to seven days at dose levels of 75 mg/kg b.w. per day or higher. These effects are reversible and patients recover upon discontinuation of treatment. Irreversible aplastic anaemia may occur at an incidence of 1 in 25 000 to 1 in 40 000 regardless of the dose, and usually develops from several weeks to several months after treatment. The origin of these incidences is not clear from the paper, but most probably the authors refer to Wallerstein et al. (1969). This paper is cited above. The authors also suggest that, to prevent grey baby syndrome, doses in newborns should be restricted to a maximum of 25 mg/kg b.w. per day and to keep the blood concentration below 22 µg/mL.

Lancaster et al. (1998) reported a study on the risk of serious haematological toxicity from chloramphenicol eye drops, based on information of a British general practice database. A total of more than 440 543 patients were identified that received in total 674 148 chloramphenicol prescriptions. Only three patients developed serious haematological toxicity and one mild, transient leucopenia. The authors concluded that even in the unlikely event that all three serious cases were caused by chloramphenicol, the risk of serious haematological toxicity attributable to chloramphenicol eye drops is small.

Ismail et al. (1998) studied data from 168 children treated with chloramphenicol administered either orally or as a one-hour i.v. infusion at doses between 25 and 100 mg/kg b.w. per day in four doses. Doses were chosen such that plasma levels were maintained between 10 and 15 µg/mL and amounted to 40.5 mg/kg b.w. for neonates and 75.5 mg/kg b.w. for older children. Side effects were restricted to mild reversible haematological abnormalities observed in 11 % of the children in which plasma levels were high.

Sah et al. (1999) reported that out of eighteen patients diagnosed with aplastic anaemia, 16 cases were idiopathic, and one case was associated with chloramphenicol toxicity, and one case with hepatitis B infection. The authors concluded that this high prevalence (12.8 %) of aplastic anaemia among their patients may not reflect the actual prevalence in the local community.

Găman et al. (2009) established the correlation between aetiology, pathophysiology, bone marrow histology and negative prognosis for 16 patients with acquired aplastic anaemia. An unfavourable evolution correlated with ethiology and pathophysiology. They indicated that most of the aplastic anaemia cases associated with medical drugs use (including chloramphenicol and others) were idiosyncratic.

Malik et al. (2009) reported a hospital-based descriptive study of 100 patients with aplastic anaemia. Chloramphenicol was found to be the most common causative drug. Mortality was 35 %.

Mathew (2004) presents a review on effects of maternal antibiotics on breast feeding infants. Chloramphenicol is assigned to the group of drugs that are not recommended for breast feeding mothers because effects are not known.

Isenberg (2003) presented a review on the risks from the topical use of chloramphenicol. They mentioned that in a study in the UK spanning a period of 10 years only 11 suspected cases of blood dyscrasia, all non-fatal, were found related to topical administration of chloramphenicol in more than 200 million uses (McGhee and Anastas, 1996). They also quoted the Spanish study of Laporte et al. (1998) indicating that the incidence of aplastic anaemia among users of ocular chloramphenicol was 0.36 per one million weeks of treatment. The authors concluded that these reports strongly suggest a decreased concern for the toxicity of chloramphenicol.

Fraunfelder and Fraunfelder (2013) recently re-evaluated the risk from the use of topical ocular chloramphenicol eye drops, based on results from epidemiologic population based studies, case reports from literature and spontaneous reporting databases for the period 1993 to 2013. They quoted an incidence of one in a million treatment courses, but they also stated that this incidence is based on a very small number of case reports, and that it is "*probably impossible to prove causation between topical ocular chloramphenicol and blood dyscrasia, primarily because an extremely rare reaction such as this cannot be practically studied*". In addition, there are apparently genetically susceptible individuals to this idiosyncratic reaction. It is the authors'opinion that causation between topical ocular chloramphenicol use and blood dyscrasia cannot be proven beyond the statement that it is "possible". Thereby they modified their previous designation (Fraunfelder and Fraunfelder, 2007) that this association, according to WHO criteria, was "probable".

Neurotoxicity

Neurotoxicity of chloramphenicol was reported upon its use for antibacterial therapy especially in the 1960s and 1970s, when young children with cystic fibrosis received prolonged oral therapy. Case studies were described where patients developed blurred vision, decreased visual acuity, constructed visual fields and peripheral neuritis following oral chloramphenicol therapy with doses of in the range of 1–2 g per day for periods of 7 to 36 weeks (Cole et al., 1957; Huang et al., 1966; Cogan et al., 1973) and 6 g per day for a period of 42 days (Lasky et al., 1953).

Wallenstein and Snyder (1952) reported a case study of a 24-year-old woman receiving a total dose of 471 g of chloramphenicol over a period of 171 days to treat ulcerative colitis. She developed bilateral loss of vision due to optic neuritis, peripheral neuritis of the lower extremities, and an associated relative leukopenia. The adverse effects were reversible upon termination of treatment.

Charache et al. (1977) described a case of peripheral and optic neuritis in a 17-year-old girl treated against *Salmonella* osteomyelitis with an oral dose of chloramphenicol for a long period: 3 g per day for one month and after an interval of seven months a second course with a dose of 500 mg every six hours for a period of three months. In addition to these effects she complained about numbness, pain in her feet and decreased visual acuity. According to the authors the pathophysiological mechanisms related to these findings were unclear.

Three young patients suffering from cystic fibrosis of the pancreas and treated with total oral doses of chloramphenicol ranging from 86 to 166 g for a period of three to five months developed optic neuropathy, with blurred vision, loss of visual acuity, visual field defects and colour vision disturbances (Godel et al., 1980). After termination of treatment with the drug, the visual functions partly recovered.

Malbrel et al. (1977) reported a case of optic atrophy in a two-year-old child treated with penicillin and chloramphenicol (600 mg per day) for meningitis, resulting in a total dose of 10.8 g chloramphenicol. The recovery of vision was rapid after cessation of treatment.

Ramilo et al. (1988) described a case of a 12-year-old male patient admitted to the hospital for evaluation of paraesthesias and visual impairment after prolonged treatment with penicillin and chloramphenicol. Four months before admission he was treated in another hospital for multiple cerebral abcesses, i.v. with penicillin (three million units every four hours) and 1 g of chloramphenicol (every six hours) for 36 days. After a three weeks interval with no medication, the patient was recommenced on the same antibiotic regimen for 14 days, followed by a 55 days oral treatment with chloramphenicol at a dose achieving a blood level of 20 µg/mL. Following this treatment the patient developed paraesthesias of his feet and decreased visual acuity. The authors reported that side effects observed were compatible with the diagnosis of chloramphenicol neurotoxicity, including abnormalities in physical examination, visual impairment and magnetic resonance imaging (MRI) lesions suggesting demyelination and treated the patient with vitamin B_6 and B_{12} leading to complete resolution of eye symptoms after three weeks and disappearance of paresthesias after six weeks.

Wiest et al. (2012) described a 12-year-old patient with complications of long-term chloramphenicol administration. This case study is also described in Section 7.5.1.1. In addition to haematotoxic effects, the patient developed other manifestations of chloramphenicol toxicity such as neutropenia, visual field changes, and peripheral neuropathy. Chloramphenicol administration was discontinued, and hemodialysis was initiated for severe metabolic acidosis. The patient recovered with severe visual field deficits.

Cardiotoxicity

Cardiovascular collapse is a complication of chloramphenicol therapy in neonates and infants and one of the symptoms of the so-called Grey Baby Syndrome (Biancaniello et al., 1981 and references therein; Fripp et al., 1983; Suarez and Ow, 1992).

Biancaniello et al. (1981) reported a case study in which a 6.5-month-old infant developed acute left ventricular cardiac dysfunction following a single i.v. treatment with ampicillin (100 mg/kg b.w.) and chloramphenicol (50 mg/kg b.w.) followed by ampicillin (200 mg/kg b.w. per day) and chloramphenicol (100 mg/kg b.w. per day) for four days. Effects were reversible upon cessation of the treatment.

Fripp et al. (1983) reported cardiovascular collapse in a three-month-old infant with meningitis given chloramphenicol at 50 mg/kg b.w per day for three days. The child died and autopsy revealed that both left and right ventricles were dilated. Myocardial histology revealed extensive intracellular vacuolisation but no evidence of acute myocarditis.

Suarez and Ow (1992) reported severe cardiac dysfunction in a nine-month-old infant treated with nafcillin (150 mg/kg b.w. per day) (changed to vancomycin at 45 mg/kg b.w. per day on the second day) and chloramphenicol (75 mg/kg b.w. per day) for five days. The initial chloramphenicol level cleared within five days after cessation of the therapy and the cardiomegaly and impaired cardiac function gradually improved to normal within seven days after cessation of the therapy.

In a review on drug toxicity in the neonate, McIntyre and Choonara (2004), referring to the study by Weiss et al. (1960) above, indicated that a reduction in the total daily dose of chloramphenicol from 100 to 50 mg/kg b.w. prevented the development of the so-called grey baby syndrome.

Hepatotoxicity

Casale et al. (1982) reported that a 15-year-old boy who was given chloramphenicol (a total dose of 20 gram given i.v. over 20 days) and gentamicin developed a rash, fever, hepatitis and pancytopenia, which resolved immediately upon termination of the chloramphenicol treatment. The paper also provides an overview of 22 other patients treated with chloramphenicol for periods of between 7 and 60 days at total dose levels between 6 and 62 g developing a hepatitis–pancytopenia syndrome while not showing recovery.

Brown (1982) reported a case of chloramphenicol toxicity in a 16-year-old girl treated with penicillin and chloramphenicol (1 g i.v. every six hours, at 95 mg/kg b.w. per day) for one day followed by chloramphenicol at 1 g orally every six hours on the second day and 3.75 g per day i.v. from the third day onwards, because adverse effects starting to develop on the second day. The effects were characterised by peripheral vascular insufficiency, metabolic acidosis and encephalopathy, similar to the syndrome described in neonates. The authors indicated that the effects were due to systemic toxicity due to toxic blood levels of chloramphenicol, perhaps reflecting hepatic injury and moderate compromise of renal function. Chloramphenicol treatment was discontinued on day five and the patient recovered.

A rare case of acute hepatitis associated with topical administration of chloramphenicol was reported by Doshi and Sarkar (2009) in a 37-year-old male patient treated for conjunctivitis with 0.5 % chloramphenicol eye drops (one drop in each eye every two hours for 24 hours followed by one drop every six hours for four days). He had not used over the counter antihistamines or analgesics, or chloramphenicol, previously, and tests for viruses that might induce liver dysfunction were negative. Ten months after stopping the chloramphenicol treatment his liver function test had returned to normal.

Allergy

Forck (1971) reported that in 1 088 patients with a suspected antibiotic allergy, 399 tested positive in skin tests (epi- or intracutane or scratch test). In 103 (26.2 %) of these 399 allergic patients a chloramphenicol allergy was observed. For 84 cases, a previous treatment with chloramphenicol could be confirmed.

Research by van Joost et al. (1986) reported a high incidence of chloramphenicol sensitisation in eight patients with periocular or periauricuar dermatitis. In six of these, a relationship with previous chloramphenicol treatment was established.

Concluding comments

The therapeutic use of chloramphenicol has been reported to result in various adverse effects with haematotoxicity being most frequent and severe. Reversible anaemia with or without leucopenia or thrombocytopenia, may be caused by an inhibitory effect of chloramphenicol on mitochondria and has been indicated to occur at dose levels above 4 g per day (Wareham and Wilson, 2002) which would equal 57 mg/kg b.w. per day for a 70 kg person. Incidences of reversible haematotoxic effects have also been reported at lower dose levels including, for example, 25 mg/kg b.w. for 10 days (Awwaad et al., 1975). The other type of bone marrow toxicity is aplastic anaemia with an often fatal outcome. Occurrence of this adverse effect has been reported to be idiosyncratic and not dose-related. Development of aplastic anaemia has been reported to occur at total doses from 3 to 315 g (Keiser and Buchegger, 1973), usually given over a few days up to several weeks. For a 70-kg person, assuming a 10 days exposure period, these doses would amount to about 4 to 410 mg/kg b.w. per day. Other case studies reported development of aplastic anaemia upon dose regimens of 1 g per day for two days on a

six-year-old child (using an estimated body weight of 20 kg this corresponds to approximately 50 mg/kg b.w. per day (Cone and Abelson, 1952)), 1 g per day for one or two weeks, corresponding to 14 mg/kg b.w. per day for a 70 kg adult (Brunton and Shapiro, 1962), 62 mg/kg b.w. for 10 plus 12 days (Daum et al., 1979), 30 gram over 12 days, which corresponds to about 36 mg/kg b.w. per day for a 70-kg person (Alavi et al., 1983), or 40 g in three months which is about 6 mg/kg b.w. per day for a 70-kg person (Cohen and Greger, 1967).

The CONTAM Panel noted that the dose levels causing aplastic anaemia are lower than the dose levels generally inducing adverse effects in animal studies. Therefore, the dose levels from the human case studies should be taken into account when selecting a reference point for the risk assessment of possible non-neoplastic effects resulting from exposure to chloramphenicol.

7.5.2. Epidemiological data on chloramphenicol

The epidemiological evidence for chloramphenicol-induced aplastic anaemia has been reviewed by JECFA (FAO/WHO, 1995, 2004a) and by the Technology Planning and Management Corporation for the National Toxicology Program of the US Department of Health and Human Services in which also epidemiological studies on chloramphenicol-induced leukaemia were discussed (Technology Planning and Management Corporation, 2000).

7.5.2.1. Aplastic anaemia

Smick et al. (1964) have carried out a retrospective study to investigate possible associations of aplastic anaemia with use of certain drugs. Prior to the study a statistical analysis was carried out comparing deaths from aplastic anaemia with sales of chloramphenicol in California, the United States and Canada in the years between 1949 and 1961 (total numbers of deaths from blood dyscrasias not provided). While no correlation could be found for Canada, statistically significant positive associations were found for California and the U.S.A. For the main study a total of 138 fatal cases of blood dyscrasias occurring between January 1957 and June 1962 were analysed, of which 86 met the diagnostic criteria for aplastic anaemia. A total of 30 fatal cases had a history of chloramphenicol use. Of these, 25 were deaths caused by aplastic anaemia. Exposure to chloramphenicol was more frequent in aplastic anaemia cases than was exposure to any other of the drugs investigated (mainly antibiotics). The study authors suggest a correlation between chloramphenicol use and aplastic anaemia; however, this is based on the prior analysis of chloramphenicol sales and aplastic anaemia incidence, rather than on the main study from which no statistical analyses have been provided.

Mizuno et al. (1982) have carried out a time series analysis in which they compared death rates from aplastic anaemia occurring between 1958 and 1978 with chloramphenicol production in Japan. They report that chloramphenicol produced per capita was unrelated to aplastic anaemia fatality in younger age groups while the trend curves (between aplastic anaemia deaths and chloramphenicol) tended to be parallel in the advanced age groups. The authors hypothesise that although the results would indicate that chloramphenicol is especially hazardous to the aged population, such a correlation should be seen also in the entire population. They conclude that the very low frequency of the disease and the reduced prescription rates for the drug may preclude statistical establishment of any drug-disease relationship.

Clausen (1986) reports that a total of 39 cases of aplastic anaemia in children aged 0–14 years had been registered in Denmark between 1967 and 1982, corresponding to an incidence rate of 2.2 cases per million per year. He attributed two of these cases to chloramphenicol exposure. It is neither reported how exposures have been determined nor on what basis these two cases have been attributed to chloramphenicol use as no statistical analysis has been provided.

A case–control study was carried out between 1980 and 1995 in the metropolitan area of Barcelona to investigate a possible association between the ocular use of chloramphenicol and aplastic anaemia (Laporte et al., 1998). A total of 145 observed cases of aplastic anaemia was combined with 1 226 matched controls for analysis. Three cases were exposed to chloramphenicol via eye drops of which two were also exposed to other medications previously associated with aplastic anaemia leading

to an increased odds ratio. In the subsequent case–control analysis, in which also confounding factors were taken into account, it was shown that this association was not statistically significant. The authors concluded that the risk, if there is any, of developing aplastic anaemia from ophthalmic use of chloramphenicol is very small.

Issaragrisil (1999) reports a case–control study started in 1989 carried out in the city of Bangkok and the rural regions of Songkla and Khonkaen in Thailand. Incidences of aplastic anaemia cases per million people per year were 3.9 (Bangkok), 3.0 (Songkla) and 5.0 (Khonkaen). A strong inverse relationship with occurrence of aplastic anaemia has been observed with high socioeconomic status while no correlation was seen with viral infections. Increased incidences were seen in subpopulations exposed to pesticides and solvents and to a lesser extent a history of treatment with certain drugs (i.e. thiazide diuretics, sulphonamide and mebendazole) leading to excess risk ranging from 9 to 12 cases per million per year for these pharmaceuticals. Although leading to an increased risk ratio, use of chloramphenicol failed to increase incidences of aplastic anaemia statistically. The author claims the present study on aplastic anaemia to be the largest ever conducted. However, neither absolute numbers of (sub-) populations nor the absolute number of aplastic anaemia cases are reported. A precise timeline for the observation period was not provided. Methods of assessment for occupational and drug exposure were also not provided.

Issaragrisil et al. (2006) report updated results from the case–control study on aplastic anaemia in the three regions in Thailand, described above, that was extended until 2002. In total, 541 aplastic anaemia patients together with 2 261 controls were investigated. The study was carried out in two phases: 1989–1999 and 1995–2002. In phase 1, potential cases were identified on the basis of haematological parameters suggesting a condition of aplastic anaemia and matched with appropriate controls. Information about socioeconomic status, medical history, drug, pesticide and chemical use was obtained in interviews. The results obtained from this first phase of the study are reported in Issaragrisil (1999). In phase 2, data collection from phase 1 was expanded to receive further details on these exposures. The inverse association of aplastic anaemia with high income could not be confirmed in the extended study. Risk increased significantly with the use of organophosphates, DDT and carbamates as pesticides and with exposures to animals and use of drinking water stemming from wells and rainwater in one rural region. Significantly elevated risks were observed with benzene and other solvents and could also be confirmed for the use of sulphonamides, thiazides and mebendazole. Only a few individuals reported exposure to chloramphenicol. Overall these exposures were not associated with an increased risk of developing aplastic anaemia, thereby confirming the results published in the earlier report (Issaragrisil, 1999) and suggesting, according to the authors, that any risks for developing aplastic anaemia in chloramphenicol users might have been overestimated previously.

A case–control study was carried out in the state of Parana (Brazil) to estimate the incidence of and to identify risk factors for aplastic anaemia (Maluf et al., 2002). From observing established cases between 1997 and 1999, an overall evidence of aplastic anaemia of 2.4 per million per year was established. A total of 117 aplastic anaemia cases and 104 matched controls were analysed to establish risk factors for acquiring aplastic anaemia. There was neither an association with the socioeconomic status nor with a history of viral infections (i.e. hepatitis and dengue). No significantly increased risks were seen with reported exposure to benzene/kerosene/gasoline based solvents while exposure to unspecified thinners and acetone based solvents were associated with diagnosis of aplastic anaemia. No association was seen with exposure to veterinary, household or agricultural pesticides. No positive associations could be established with use of pharmaceuticals including chloramphenicol. The authors note, however, that only few cases and controls were exposed to this substance making establishment of any correlation difficult.

In a multinational (Brazil, Argentina, Mexico) case–control study, Maluf et al. (2009) studied risk factors for agranulocytosis and aplastic anaemia. A total of 224 aplastic anaemia cases established according to defined criteria were matched with four corresponding controls. Relevant information such as demographic data, previous diseases, medication use, contact with animals, exposure to

different chemicals and radiation was obtained by standardised interviews. Statistically significant increased odd ratios were found for exposure to some pesticides and to benzene. Use of azithromycin was positively associated with aplastic anaemia while chloramphenicol exposure failed to increase such a risk significantly. This essentially corroborates similar negative findings from this group of researchers reported in an earlier study (Maluf et al., 2002). However, the authors note that it is very difficult to find out about possible associations, for instance because of the low overall incidence of aplastic anaemia, the low prevalence of chloramphenicol use and consequent exposure.

The CONTAM Panel noted that the overall incidence of aplastic anaemia was estimated in several studies, in which no assessment was performed on the association between aplastic anaemia and exposure to specific drugs (i.e. chloramphenicol).

Based on the results of a population-based study in French metropolitan areas, Mary et al. (1990) reported an overall incidence of 1.5 cases per million per year, and Kaufman et al. (2006) reported that the relative incidence of aplastic anaemia in a number of European countries ranged from 0.7 to 3 cases in 1 million. Montané et al. (2008) reported the results of a prospective multicentre study on the incidence of aplastic anaemia in the area of Barcelona. Based on information collected between 1980 and end of 2003, an overall incidence of 2.34 per million per year was reported.

These studies indicated that the overall incidence rate of aplastic anaemia is very low and appears to be in the region of about two cases per million per year in a Western population.

7.5.2.2. Leukaemia

A case–control study enrolling 309 children under 15 years diagnosed with leukaemia (172 cases of acute lymphocytic-, 94 of acute non-lymphocytic- and 43 of chronic/unspecified leukaemia, respectively) together with two matched controls for each case was conducted in Shanghai in order to study risk factors for the disease (Shu et al., 1987). All relevant information in that regard both from cases and controls was collected via a questionnaire, in most instances from the parents. Reduced risks were associated with uptake of vitamins and some medications (mainly antibiotics, such as penicillin and streptomycin) while an increased risk of all leukaemia types combined was seen with syntomycin (a racemic mixture containing 50 % laevorotatory and 50 % dextrorotatory chloramphenicol) use. This increase was also dose dependent for acute non-lymphocytic anaemia. Chloramphenicol caused a significant and also dose-dependent positive association with occurrence of both acute lymphocytic and non-lymphocytic leukaemia (the latter association being stronger).

Zheng et al. (1993) carried out a case–control study to elucidate the role of medical conditions, different medications and medical radiation in the development of leukaemia. A total of 486 cases of adult leukaemia (236 cases of acute non-lymphocytic leukaemia, 79 of chronic myeloid leukaemia, 81 with acute lymphocytic leukaemia and 21 with chronic lymphocytic leukaemia), diagnosed between 1987 and 1989 in the urban area of Shanghai were matched with 502 healthy controls. Information potentially relevant for disease aetiology was obtained via questionnaires. Significantly increased risks for specific forms of leukaemia were observed for cases having a history of tuberculosis, for several other chronic disorders and for the use of salicylates. The authors pointed out, however, that all these results were based on only a few cases. Notably, and in contrast to an earlier study where and increased risk was claimed for childhood leukaemia (Shu et al., 1987), chloramphenicol use was not associated with any form of adult leukaemia in this study.

A case–control study aimed at elucidating the impact of different medications on blood dyscrasias is reported by Doody et al. (1996). A total of 94 cases of non-Hodgkin's lymphoma, 159 of multiple myeloma and 257 of leukaemia diagnosed in the period between 1960 and 1979 in northwest California and in the period between 1958 and 1982 in northern California were compared with 695 matched controls. Information on drug use was obtained by evaluating prescriptions. Increased risks for non-Hodgkin's lymphoma were reported for the use of lidocaine, meprobamate and amphetamines. Use of mineral oil led to increased risk of multiple myeloma. It is not reported whether

these associations reached statistical significance. The use of chloramphenicol was associated with a decreased risk of leukaemia. However, that association was considered as being spurious by the authors.

Traversa (1998) studied the relationship between the use of a large number of different medications and occurrence of acute leukaemia. A case–control study was carried out in Rome with 10 matched controls for each of the 202 adult patients diagnosed with acute leukaemia (of these, 118 had a diagnosis of acute myeloid leukaemia, 69 of acute lymphocytic leukaemia and 15 of biphenotypic leukaemia). Information on drug use of the study population was obtained through the Italian National Health Service. Increased odd ratios were reported with the use of tricyclic antidepressants and contraceptives while the use of very high doses of non-steroidal anti-inflammatory drugs was associated with a decreased morbidity. High use of chloramphenicol was associated with a moderately increased odds ratio; however, it was not described whether or not this reached any statistical significance.

In order to elucidate a possible association between topical uses (as eye drops) of chloramphenicol and development of leukaemia in adults, Smith et al. (2000) carried out a case–control study with 797 established cases of acute leukaemia and 1 570 matched controls in the UK between 1991 and 1996. Drug use was established by perusing general practitioners' records. Overall 195 out of 797 cases and 384 out of 1 570 controls had a record of topical chloramphenicol use. A statistically significant increased risk of acute leukaemia associated with topical use of chloramphenicol was not observed, even after dividing the sample into subgroups, or even with cases receiving three or more prescriptions.

7.5.2.3. Other adverse effects

Zahm et al. (1989) investigated potential risk factors for soft-tissue sarcoma. In their study, 133 identified cases in Kansas between 1976 and 1982 were compared with 948 matched controls. Information on potential risk factors was obtained via telephone interviews with living patients and with next of kin for those who were deceased. The risk of development of soft-tissue sarcoma was significantly increased with the use of smokeless tobacco, while no such association was seen with medical radiation. A significantly increased risk of development of soft-tissue sarcoma was associated with the use of chloramphenicol. The authors suggest further evaluation of the role of such medications in the aetiology of soft-tissue sarcoma. However, it needs to be noted here that only four of the cases self-reported chloramphenicol treatment.

In order to evaluate the teratogenic potential of chloramphenicol, a case–control study was carried out in Hungary (Czeizel et al., 2000). The case group, consisting of 22 865 offspring with malformations (designated in subgroups of poly/syndactyly, cardiovascular congenital abnormalities, hypospadias, undescended testis, clubfoot, other congenital abnormalities and multiple congenital abnormalities) was compared with a population control of 35 151 births. In the case group, 52 mothers had oral chloramphenicol treatment during pregnancy compared with 51 mothers in the control group. A higher odds ratio upon treatment of mothers in the critical period for major malformations (2–3 months of pregnancy) was only found in the subgroup with undescended testis. However, that group consisted of only two cases. Overall, the authors conclude that chloramphenicol treatment during early stage of pregnancy is, if at all, only a minor risk of development of malformations in offspring.

7.5.2.4. Concluding comments

While in case studies (see Section 7.5.1) it has been clearly demonstrated that chloramphenicol exposure can cause aplastic anaemia, a clear link between chloramphenicol exposure and the development of aplastic anaemia could not be established in epidemiological studies. Notably, all the relevant studies evaluated were case–control studies, highly prone to recall bias (exposures were established in many instance on self- or next of kin reporting and going back many years in time) and other confounders, that cannot be easily excluded in such kind of studies. All studies are furthermore hampered by the limited number of individuals with a proven history of chloramphenicol use. In

addition, the idiosyncratic nature of the disease and its very low overall incidence rate, which appears to be in the region of about two cases per million per year in Western populations, render establishment of any statistically significant correlation difficult.

A positive association of chloramphenicol exposure with an increased risk of developing leukaemia was reported in one study but not observed in subsequent studies. A positive correlation of occurrence of soft tissue sarcoma with chloramphenicol treatment was suggested in one study; however, this association was established on the basis of only four exposed cases.

7.6. Considerations for derivation of a health based guidance value

In humans, chloramphenicol causes two major types of haematotoxic effects: (a) reversible and dose-related mild anaemia and reticulocytopenia, and (b) irreversible aplastic anaemia characterised by severe pancytopenia accompanied by a hypo- or even acellular bone marrow. No dose–response relationship has been established for aplastic anaemia, which is often fatal. Development of aplastic anaemia and mild anaemia are not related to each other (see also FAO/WHO, 2004a; Turton et al., 2006). While the symptoms and findings relating to mild anaemia have been reported in a series of *in vivo* studies upon application of chloramphenicol, aplastic anaemia has not been established in animal models (for review see FAO/WHO, 2004a).

Overall, the new evidence corroborates the conclusion by JECFA (FAO/WHO, 2004a) that mild reversible anaemia can be induced by chloramphenicol in animals, while severe non-reversible aplasia is seen only in humans.

Besides haematotoxic effects, chloramphenicol also caused cardiotoxic and neurotoxic effects. Chloramphenicol caused liver toxicity in rats and mice. In studies in rats the lowest tested dose of 25 mg/kg b.w. per day was hepatotoxic. Chloramphenicol also caused embryotoxicity and teratogenicity in laboratory animals orally administered chloramphenicol in doses ranging from 500–2 000 mg/kg b.w. per day. In addition, degeneration of the testes and effects on sperm quality were observed in three studies in rats applying only one oral dose level in the range from 25 to 112 mg chloramphenicol/kg b.w. per day administered over periods spanning from 8 to 25 days.

Chloramphenicol is genotoxic *in vivo*, inducing chromosomal aberrations, SCE and/or DNA damage in mice and rats following oral or parenteral administration. The CONTAM Panel noted that no conclusion can be drawn regarding the potential carcinogenicity of chloramphenicol because of the lack of appropriate and well-documented long-term studies.

The CONTAM Panel concluded that available animal and human data indicate that the derivation of a health-based guidance value for chloramphenicol is not appropriate. Instead it was concluded that a margin of exposure (MOE) approach should be used in the human health risk characterisation of chloramphenicol.

Based on the assessment of the toxic effects of chloramphenicol in animals and humans the CONTAM Panel decided that three serious effects, i.e. aplastic anaemia in humans and reproductive and liver toxicity in animals can be envisaged as providing a basis for reference points for the risk characterisation.

The clinical case studies addressing aplastic anaemia show that doses in a range from 4 to 410 mg/kg b.w. chloramphenicol per day administered over periods spanning from several days to months are associated with the development of aplastic anaemia (see Section 7.5.1.2).

The CONTAM Panel recognized that exposure to much lower doses, in the order of 0.5 mg per person per day, as used in topical ocular administration of chloramphenicol has also been associated with the development of aplastic anaemia. There is, however, controversy regarding this issue. The CONTAM Panel noted that the reported occurrence of aplastic anaemia following ocular use is very small, ranging from 1 in 1 million to 1 in 20 million patients (see Section 7.5.1.1, ‘Reviews of case studies’).

The CONTAM Panel noted that this latter figure is similar to the overall incidence rate of aplastic anaemia in the general population, which is reported to be in the region of about 2 cases per million per year (see Section 7.5.2.4). Furthermore, confounding factors such as co-exposure to other medicines, genetic predisposition, and other illnesses hampered a clear conclusion about the association between topical ocular use of chloramphenicol and aplastic anaemia. In addition, systemic absorption following topical administration could not be demonstrated in the only two available studies (see Section 7.5.1.2.1, 'Case studies upon topical ophthalmic administration'), although the CONTAM Panel noted that the LODs might not have been low enough to detect low systemic levels of chloramphenicol, possibly present following topical ocular administration.

Because of these uncertainties, the CONTAM Panel concluded that it could not use topical data in its assessment of the risk of aplastic anaemia caused by chloramphenicol, and selected the lowest dose of 4 mg chloramphenicol/kg b.w. chloramphenicol per day, as a reference point, from the case studies on systemic use from which an exposure could be estimated.

Based on the results of toxicological studies in laboratory animals, the lowest effect dose of 25 mg/kg b.w. per day causing testes degeneration, effects on sperm quality and hepatotoxicity was selected as a second reference point to assess the risk of possible reproductive/hepatotoxic effects of exposure to chloramphenicol.

8. Risk characterisation

8.1. Human health risk characterisation

Since a reliable dietary exposure assessment is not possible, the CONTAM Panel considered several exposure scenarios in comparison with the two different reference points that have been identified in Section 7.6: 4 mg/kg b.w. per day for the development of aplastic anaemia and 25 mg/kg b.w. per day for the reproductive/hepatotoxic effects of chloramphenicol. Owing to the lack of appropriate data, the CONTAM Panel cannot assess the risk of carcinogenicity.

In Section 6.1.2, four different exposure scenarios have been presented:

- scenario 1, in which all foods of animal origin are contaminated with chloramphenicol;
- scenario 2, which includes foods in which enzyme preparations, reported to be contaminated with chloramphenicol, may be used during food production;
- scenario 3, which includes grains and grain-based products in which chloramphenicol could occur naturally;
- scenario 4, the combination of scenarios 1, 2 and 3.

The CONTAM Panel emphasises that these scenarios represent the worst-case situations, in which all foods covered by each scenario are contaminated with chloramphenicol at the RPA, a highly unlikely situation.

The CONTAM Panel decided to use scenario 4 for the risk characterisation since this covers all potential dietary exposure. Considering that only limited occurrence data are available, the current RPA value of 0.3 µg/kg was used. Assuming that all foods covered by the scenarios were contaminated, it was concluded that the use of average consumption data is the most realistic method of representing a chronic dietary exposure scenario.

Based on the considered scenario, the median chronic dietary exposure across European countries and dietary surveys for the average consumer would be 15 and 3.1 ng/kg b.w. per day for toddlers (the highest exposed population group) and adults, respectively. The minimum and maximum chronic

dietary exposures across European countries and dietary surveys for the average consumer would be 11 and 17 ng/kg b.w. per day for toddlers, respectively, and 2.2 and 4.0 ng/kg b.w. per day for adults, respectively (see Table 4).

When comparing the median chronic dietary exposure across European countries and dietary surveys for the average consumer with the reference point for aplastic anaemia (4 mg/kg b.w. per day), the MOE is about 2.7×10^5 for toddlers and 1.3×10^6 for adults. For the minimum and maximum chronic dietary exposures across European countries and dietary surveys for the average consumer, the MOEs for toddlers are about 3.6×10^5 and 2.4×10^5, respectively, and for adults are about 1.8×10^6 and 1.0×10^6, respectively.

The CONTAM Panel noted that MOEs of 100 are often considered of low concern for thresholded effects. For substances that are both genotoxic and carcinogenic the Scientific Committee proposed that a MOE of 10 000 or higher, if based on the lower 95 % confidence limit for a benchmark response of 10 % extra risk ($BMDL_{10}$) from an animal carcinogenicity study, would be of low concern from a public health point of view (EFSA, 2005). Aplastic anaemia caused by chloramphenicol is an idiosyncratic adverse reaction only observed in humans and for which no dose-relationship has been established. For such idiosyncratic adverse reactions, no MOE has been proposed that would be of low concern for public health. Considering the calculated MOEs for aplastic anaemia ($\geq 2.3 \times 10^5$) based on worst-case exposure estimates and the relatively low frequency of occurrence of aplastic anaemia (1 in 20 000 to 1 in 40 000) following systemic treatment of patients with chloramphenicol (4 to 410 mg/kg b.w. per day), it is unlikely that exposure to food contaminated with chloramphenicol at or below 0.3 µg/kg represents a health concern.

When comparing the median chronic dietary exposure across European countries and dietary surveys for the average consumer with the reference point for reproductive/hepatotoxic effects (25 mg/kg b.w. per day), the MOE is about 1.7×10^6 for toddlers and 8.1×10^6 for adults. For the minimum and maximum chronic dietary exposures across European countries and dietary surveys for the average consumer, the MOEs for toddlers are about 2.3×10^6 and 1.5×10^6, respectively, and for adults are about 11×10^6 and 6.3×10^6, respectively. The CONTAM Panel noted that an MOE of 100 would apply for reproductive/hepatotoxic effects observed in animal studies, which would result from a thresholded mode of action. The calculated MOEs for reproductive/hepatotoxic effects of chloramphenicol ($\geq 1.5 \times 10^6$) are not based on a NOAEL or a benchmark dose lower confidence limit (BMDL) but on an effect level. However since the MOEs are of the order of 10^6, they are considered to be sufficiently large and not to indicate a health concern for reproductive/hepatotoxic effects of chloramphenicol.

For enzyme-based food supplements, containing the highest reported concentration of chloramphenicol (1 800 µg/kg), a worst-case dietary exposure of 12 ng/kg b.w. per day was estimated from a single serving per day (see Section 6.1.2.2). It should be noted that these supplements are usually taken for several weeks or months. When comparing the exposure estimate of 12 ng/kg b.w. per day with the reference point for aplastic anaemia of 4 mg/kg b.w., a MOE of 3.3×10^5 is calculated. Considering the calculated MOE based on worst-case exposure estimates and the relatively low frequency of occurrence of aplastic anaemia (1 in 20 000 to 1 in 40 000) following systemic treatment of patients with chloramphenicol (4 to 410 mg/kg b.w. per day), it is unlikely that exposure to this enzyme-based food supplement represents a health concern with respect to the risk of developing aplastic anaemia.

When the estimated dietary exposure is compared with the reference point for reproductive/hepatotoxic effects of 25 mg/kg b.w. per day, the MOE is about 2.1×10^6. The CONTAM Panel noted that this MOE is sufficiently large to conclude that exposure to such an enzyme-based food supplement is unlikely to be of health concern regarding reproductive/hepatotoxic effects of chloramphenicol.

Because dietary exposure from other enzyme-based food supplements reported to contain chloramphenicol has been estimated to be about two orders of magnitude lower (see Section 6.1.2.2), the MOEs will consequently be two orders of magnitude higher than the ones mentioned above. Therefore, the CONTAM Panel concluded that exposure of humans from enzyme-based food supplements that might contain chloramphenicol at the concentrations reported in the RASFF notifications is unlikely to be of health concern.

Conclusion

Overall, the CONTAM Panel concludes that the presence of chloramphenicol in food at or below a level of 0.3 µg/kg is unlikely to be a health concern for aplastic anaemia or reproductive/hepatotoxic effects. Owing to the lack of appropriate data, the CONTAM Panel cannot assess the risk of carcinogenicity.

8.2. Animal health risk assessment

Based on the limited occurrence data for chloramphenicol, farm animals may be exposed through the use of certain enzymes in feed, through straw or via the uptake of soil. Worst-case scenarios indicated that maximum dietary exposure through these routes would be below 1 µg/kg b.w. per day based on concentrations in compound feed of 5.9 µg/kg and in straw of 32 µg/kg (see Section 6.2). Some adverse effects were described in farm animals, but these were at doses in the mg/kg b.w. range and in most cases after injection rather than after oral treatment. Although most studies lacked dose–response information, it is unlikely that dietary exposures around 1 µg/kg b.w. per day would result in adverse effects.

In animals treated illicitly with chloramphenicol, the residue levels determined in food will vary depending on the time period since the last treatment. When animals are exposed via a natural source, such as grass or straw, it seems rather unlikely that this will result in levels above 0.3 µg/kg in foods of animal origin (see Section 7.1.8).

8.3. Appropriateness of the reference point for action for the protection of public and animal health

Exposure scenario 4 assumes that all foods in which chloramphenicol could be present (see Section 6.1.2) contain the concentration level of 0.3 µg/kg. The CONTAM Panel noted that the MOEs calculated for scenario 4 do not indicate a health concern for aplastic anaemia or reproductive/hepatotoxic effects (see Section 8.1). Therefore, the CONTAM Panel concludes that the RPA of 0.3 µg/kg for food of animal origin is adequate to protect public health with respect to aplastic anaemia and reproductive/hepatotoxic effects. It was also concluded that it is appropriate to apply the RPA for food of animal origin to food of non-animal origin. However, because of the lack of appropriate data, the CONTAM Panel cannot assess the risk of carcinogenicity.

A concentration of 0.3 µg/kg in feed leads to a substantially lower dietary exposure (ng/kg b.w. range) than the previously applied therapeutic doses in the mg/kg b.w. range, which showed only limited toxic effects. Therefore, the RPA for food of animal origin is considered an appropriate RPA to be applied to feed for the protection of animal health. Furthermore, a concentration of 0.3 µg/kg in feed is unlikely to result in concentrations in animal derived food above the RPA and as such is also protective for public health.

9. Uncertainty analysis

The CONTAM Panel concluded that the lack of occurrence data in food precludes a reliable human dietary exposure assessment and consequently a detailed evaluation of the inherent uncertainties. Instead, the CONTAM Panel calculated the hypothetical human chronic dietary exposure considering the RPA of 0.3 µg/kg as a hypothetical occurrence value for four different scenarios. These calculations can be considered as worst-case scenarios and they introduce considerable uncertainty in

the extent of actual human chronic dietary exposure. While in case studies it has been clearly demonstrated that chloramphenicol exposure can cause aplastic anaemia, such a relationship could not be established in epidemiological studies. The CONTAM Panel noted that the design of such studies, in particular retrospective studies, appears not to be appropriate to detect such kind of relationships due to the low incidence of aplastic anaemia and the idiosyncratic nature of the disease. Additional uncertainty is caused by the fact that no dose–response relationship has been established. Moreover, the evidence for an association between chloramphenicol and an increased risk of developing leukaemia is uncertain. The lack of reliable toxicity studies (particularly carcinogenicity studies) in experimental animals also adds to the uncertainty. Furthermore, the isomer composition of the compounds tested is mostly not known.

The lack of occurrence data in feed precludes a reliable animal dietary exposure assessment and consequently a detailed evaluation of the inherent uncertainties.

It is unclear whether formation of residues at the high doses (mg/kg b.w.) previously used in animals can be extrapolated to the formation of residues at low exposure levels (less than 1 µg/kg b.w.) owing to the natural occurrence of chloramphenicol. Moreover, there is uncertainty about potential occurrence of residues of genotoxic metabolites in animals.

Overall, the CONTAM Panel considered that the impact of the uncertainties on the risk assessment of human and animal exposure to chloramphenicol through the consumption of food and feed is substantial.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Chloramphenicol is a broad-spectrum antibiotic, originally obtained from the bacterium *Streptomyces venezuelae*, that has, in the past, been widely used to treat infections in both humans and animals.
- In veterinary medicine, chloramphenicol is not authorised for use in food-producing animals in the EU.

Methods of analysis

- Most of the sampling of food, and of related materials, for chloramphenicol testing in foods of animal origin is undertaken in the context of the national residue monitoring plans.
- Suitable screening methods measure chloramphenicol residues with sufficient sensitivity to satisfy the current regulatory requirements, at the minimum required performance limit (MRPL) of 0.3 µg/kg, and include immunoassay, biosensor and chromatographic techniques.
- Confirmatory methods, typically based on gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry, have been developed for determination of chloramphenicol in a wide range of sample types and have decision limits (or limits of detection) in the range of < 0.01 to 0.15 µg/kg and detection capability (or limits of quantification) values in the range of 0.01 to 0.3 µg/kg.

Occurrence/Exposure

- Chloramphenicol can be produced by bacteria in the soil, where it is rapidly degraded by other soil organisms. However, it can partly be absorbed by plants, thus explaining recent findings in herbs, grass and straw. Whether these levels might be responsible for positive findings in food-producing animals is unknown.

- Data on occurrence of chloramphenicol in food were extracted from the EC's database on residues of veterinary medicines reported on animal and animal products for the years 2002 to 2012. There were 306 targeted samples reported to be non-compliant for chloramphenicol. The animal species/food products in which chloramphenicol was reported were pigs, poultry, bovines, aquaculture, sheep/goats, rabbit, farmed game, honey and milk.
- Data were extracted from the Rapid Alert System for Food and Feed (RASFF) database for the years 2002 to 2013. There were 440 notification events reported for chloramphenicol; 402 for food and 38 for feed. Among these were 24 notification events reported for enzyme concentrates, enzyme preparations or target food containing enzyme preparations; 19 for food and five for feed, all of them occurring in 2013. Three of these 19 notification events for food concerned enzyme-based food supplements.
- The EFSA Panel on Contaminants in the Food Chain (CONTAM) concluded that data extracted from the EC's database and the RASFF database were too limited to carry out a reliable human dietary exposure assessment. Instead, the CONTAM Panel calculated the hypothetical human dietary exposure considering as an occurrence value the RPA of 0.3 µg/kg, for four scenarios. The CONTAM Panel emphasises that these scenarios represent the worst-case situations, in which all foods covered by each scenario are contaminated with chloramphenicol, a highly unlikely situation.
- The exposure scenario 4, in which specific food groups (foods of animal origin, foods in which enzyme preparations, reported to be contaminated with chloramphenicol, may be used during food production, and grains and grain-based products in which chloramphenicol could occur naturally) are considered to contain chloramphenicol at the concentration level of 0.3 µg/kg, covers all potential dietary exposure. The mean chronic dietary exposure across the different European countries and dietary surveys for this scenario, would range from 11 to 17 ng/kg b.w. per day for toddlers and from 2.2 to 4.0 ng/kg b.w. per day for adults.
- The daily dietary exposure to chloramphenicol from enzyme-based food supplements at the concentrations reported in RASFF notifications ranged between 0.1 and 12 ng/kg b.w. per day.
- Some decrease in chloramphenicol concentration has been reported during food processing, as well as the production of degradation products, but the toxic potential of these degradation products is unclear.
- Potential dietary exposure of livestock to chloramphenicol from feed enzymes, straw or soil was estimated to be below 1 µg/kg b.w. per day.

Hazard identification and characterisation

Toxicokinetics

- In humans, chloramphenicol is highly bioavailable upon oral exposure and may easily cross both placental and mammary barriers. Under normal conditions, the drug is extensively biotransformed and rapidly eliminated, mainly as glucuronide derivatives. However, conditions known to depress the glucuronidation rate may allow the drug to enter reductive and/or oxidative pathways yielding toxic/reactive metabolites, which have been implicated in the generation of blood dyscrasias and possibly genotoxicity.
- In ruminants, chloramphenicol is extensively metabolised in the rumen, resulting in poor absorption of the parent compound. Ruminal degradation products might be absorbed but have not been clearly identified.
- In pigs, the available data indicate that chloramphenicol is widely bioavailable by the oral route and is distributed in all edible tissues. According to a limited dataset, residues of the parent drug and its main metabolites (chloramphenicol base and chloramphenicol glucuronides) are slowly depleted and may be still detected in the µg/kg range several days after withdrawal of treatment.

- In avian species, chloramphenicol displays a limited oral bioavailability (35–45 %) and a remarkable first-pass effect. The parent drug and different metabolites have been detected in liver, muscles and eggs up to several days after termination of treatment. The presence of toxic metabolites in edible tissues of chickens has been reported in one study involving repeated administration but not in others where animals were exposed to a single dose.
- In horses, chloramphenicol is rapidly and extensively absorbed and widely distributed to tissues.
- In fish, metabolism of chloramphenicol is dependent on species and a variety of environmental factors, such as water temperature and water flow.
- Cats exhibit a longer elimination half-life of the drug compared to other domestic animal species investigated.
- Exposure of farm animals to radiolabelled chloramphenicol at doses formerly used therapeutically, typically around 50 mg/kg b.w., resulted in levels in meat, milk and eggs in the range of 1 to 100 mg/kg, expressed as chloramphenicol equivalents, during or shortly after the treatment. Linear extrapolation of these exposure levels to maximal intakes calculated for recent findings in feed enzymes, straw and soil indicate that levels in edible products will not exceed the current RPA.
- Various metabolites were identified in carry-over studies at doses of chloramphenicol formerly used therapeutically. There is uncertainty about potential occurrence of residues of genotoxic metabolites in various animal species, with one study reporting their occurrence in broilers, whereas unpublished studies submitted to FAO/WHO could not confirm their presence in meat and organs of pigs, calves and broilers.

Toxicity studies

- In mice, the oral median lethal dose (LD_{50}) was estimated to be 2 640 mg/kg b.w. and neurotoxic effects were observed after acute dosing at 1 250 mg/kg b.w. and higher. In dogs, neurotoxic effects were observed at 300 mg/kg b.w. (orally).
- Chloramphenicol caused liver toxicity in rats and mice. In studies in rats the lowest tested dose of 25 mg/kg b.w. per day was hepatotoxic. Consequently a no observed adverse effect level (NOAEL) for repeated-dose toxicity could not be identified from these studies.
- Chloramphenicol caused dose-dependent mild reversible anaemia in laboratory animals at oral doses of 825 mg/kg b.w. per day or above, while severe non-reversible aplastic anaemia has not been observed.
- Chloramphenicol at doses of 25–112 mg/kg b.w. per day caused testes degeneration and effects on sperm quality in rats.
- Embryotoxicity and teratogenicity were found in laboratory animals orally exposed to chloramphenicol doses in the range of 500–2 000 mg/kg b.w. per day.
- Chloramphenicol is neurotoxic in certain species, shown by reduced learning ability in rats (50 mg/kg b.w. per day s.c.) and mice (25 to 200 mg/kg b.w. per day orally) and disturbed sleeping pattern in rats (400 mg/kg b.w. i.p.) and cats (165 mg/kg b.w. or higher, orally).
- Chloramphenicol was largely inactive in prokaryotic and lower eukaryotic genotoxicity test systems. Chloramphenicol was mutagenic and clastogenic *in vitro* in different types of mammalian cells, although it was negative in some tests. Moreover, several metabolites were shown to be much more active than chloramphenicol itself in inducing DNA-strand breaks in human cells. The *in vitro* genotoxic activity of chloramphenicol may be dependent on the metabolic competence of the test system.

- *In vivo,* chloramphenicol induced chromosomal aberrations in bone marrow in mice and rats and in blood cells of calves, following administration via different routes. Oral gavage studies showed clastogenic effects in newborn rats exposed transplacentally.
- No conclusion can be drawn regarding the potential carcinogenicity of chloramphenicol because of the lack of appropriate and well-documented long-term studies.

Mode of action

- Although the mechanism for chloramphenicol-induced aplastic anaemia in humans has not been elucidated, nitroreduction to nitroso-chloramphenicol and the production of reactive oxygen species leading to DNA damage seem to be crucial factors in the induction of aplastic anaemia. Genetic predisposition enhancing the ability of the bone marrow to reduce chloramphenicol into its myelotoxic derivative also plays an important role.

Adverse effects in livestock, fish and companion animals

- Despite the former widespread use of chloramphenicol as a veterinary drug, limited information is available concerning adverse effects in livestock, especially after oral treatment. Some effects were described in calves treated i.m. or i.v. with doses of 20–100 mg/kg b.w., including chromosome aberrations in lymphocytes from treated animals.
- In cats and dogs, prolonged treatment with doses higher than 50 mg/kg b.w. resulted in effects in the bone marrow/blood system.

Human data

- Aplastic anaemia caused by chloramphenicol is an idiosyncratic adverse reaction only observed in humans and for which no dose-response relationship has been established.
- The therapeutic use of chloramphenicol in humans has been reported to result in various adverse effects, with haematotoxicity being most frequent and severe. Reversible anaemia, with or without leukopenia or thrombocytopenia, may be caused by an inhibitory effect of chloramphenicol on mitochondria.
- While in case studies it has been clearly demonstrated that chloramphenicol exposure can cause aplastic anaemia, a relationship could not be established in epidemiological studies. The CONTAM Panel noted that the design of such studies, in particular retrospective studies, appears not to be appropriate to detect such a relationship due to the low incidence of aplastic anaemia and the idiosyncratic nature of the disease. A positive association of chloramphenicol exposure with an increased risk of developing leukaemia was reported in one study but not observed in subsequent studies.

Considerations for derivation of a health-based guidance value

- Available animal and human data indicate that the derivation of a health-based guidance value for chloramphenicol is not appropriate.
- Three serious effects of chloramphenicol, i.e. aplastic anaemia in humans and reproductive and liver toxicity in animals, were envisaged as providing a basis for reference points for the risk characterisation.
- Clinical case studies addressing aplastic anaemia show that doses in a range from 4 to 410 mg chloramphenicol/kg b.w. per day administered over periods spanning from several days to months are associated with the development of aplastic anaemia. The lowest dose of 4 mg/kg b.w. chloramphenicol per day was selected, as a reference point, from the case studies on systemic use from which an exposure could be estimated.
- In rats, testes degeneration, effects on sperm quality and hepatotoxicity were observed at a dose of 25 mg chloramphenicol/kg b.w. per day. This effect dose of 25 mg/kg b.w. per day was selected as a reference point to assess the risk of possible reproductive/hepatotoxic effects of exposure to chloramphenicol.

Risk characterisation

Human health risk characterisation

- Considering exposure scenario 4, median chronic dietary exposure across European countries and dietary surveys for the average consumer results in a margin of exposure (MOE) for aplastic anaemia of approximately 2.7×10^5 for toddlers and 1.3×10^6 for adults and an MOE for reproductive/hepatotoxic effects of approximately 1.7×10^6 for toddlers and 8.1×10^6 for adults.
- Considering these large MOEs and the relatively low frequency of occurrence (1 in 20 000 to 40 000) of aplastic anaemia following systemic treatment of patients with chloramphenicol (4 to 410 mg/kg b.w.), it is unlikely that exposure to food contaminated with chloramphenicol at or below 0.3 µg/kg is a health concern with respect to aplastic anaemia or reproductive/hepatotoxic effects.
- Considering the consumption of enzyme-based food supplements contaminated with chloramphenicol at the highest observed level of 1 800 µg/kg, MOEs of 3.3×10^5 for aplastic anaemia and 2.1×10^6 for reproductive/hepatotoxic effects were calculated. Exposure to such an enzyme-based food supplement is unlikely to represent a health concern with respect to aplastic anaemia or reproductive/hepatotoxic effects.
- Owing to the lack of appropriate data, the CONTAM Panel cannot assess the risk of carcinogenicity.

Animal health risk characterisation

- Potential dietary exposure of livestock to chloramphenicol from feed enzymes, straw or soil was estimated to be below 1 µg/kg b.w. Some adverse effects were described in farm animals, but for dosages in the mg/kg b.w. range. It is unlikely that exposures around 1 µg/kg b.w. would result in adverse effects.

Appropriateness of the RPA for the protection of public and animal health

- The RPA of 0.3 µg/kg for chloramphenicol in food of animal origin is adequate to protect against potential adverse health effects of chloramphenicol with respect to aplastic anaemia or reproductive/hepatotoxic effects. Because of the lack of appropriate data, the CONTAM Panel cannot assess the risk of carcinogenicity.
- The RPA for food of animal origin is also appropriate to be applied to food of non-animal origin.
- The RPA for food of animal origin is also appropriate to be applied to feed.

RECOMMENDATIONS

- More information is needed on the stereoselectivity of the chemical synthesis systems used to produce chloramphenicol and the extent to which the potential presence of different enantiomers in the chloramphenicol preparation used may have influenced the observed adverse effects.
- There is a need for information on the carcinogenicity and the mechanisms underlying genotoxic effects of chloramphenicol.
- Further studies are required on the presence of chloramphenicol in soil (hot spots) and on the possible uptake by cereals and vegetables, including the formation of plant metabolites.
- The potential formation of reactive intermediates of chloramphenicol, which could result in residues in foods of animal origin, should be studied. Additional data are needed on the occurrence of toxic metabolites and the formation of bound residues in edible tissues of food-producing animals.

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Appendix A. Consumption

Table A1: Dietary surveys considered for the chronic dietary exposure assessment with the number of subjects in the different age classes

Code[a]	Country	Dietary survey[b]	Method	Days	Age (years)	Number of subjects[c]						
						Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
BE/1	Belgium	Diet National 2004	24-hour dietary recall	2	15–105				584	1 304	518	712
BE/2	Belgium	Regional Flanders	Food record	3	2–5		36[d]	625				
BG/1	Bulgaria	NUTRICHILD	24-hour recall	2	0.1–5	860	428	433				
CZ	Czech Republic	SISP04	24-hour recall	2	4–64			389	298	1 666		
DK	Denmark	Danish Dietary Survey	Food record	7	4–75			490	479	2 822	309	20[d]
DE/1	Germany	DONALD 2006-2008	Dietary record	3	1–10		261	660				
DE/2	Germany	National Nutrition Survey II	24-hour recall	2	14–80				1 011	10 419	2 006	490
IE	Ireland	NSFC	Food record	7	18–64					958		
EL	Greece	Regional Crete	Dietary record	3	4–6			839				
ES/1	Spain	AESAN	Food record	3	18–60					410		
ES/2	Spain	AESAN-FIAB	24-hour recall	2	17–60				86	981		
ES/3	Spain	NUT INK05	24-hour recall	2	4–18			399	651			
ES/4	Spain	enKid	24-hour recall	2	1–14		17[d]	156	209			
FR	France	INCA2	Food record	7	3–79			482	973	2 276	264	84
IT	Italy	INRAN-SCAI 2005–06	Food record	3	0.1–98	16[d]	3 [d]	193	247	2 313	290	228
CY	Cyprus	Childhealth	Dietary record	3	11–18				303			
LV	Latvia	EFSA_TEST	24-hour recall	2	7–66			189	470	1 306		
HU	Hungary	National Repr Surv	Food record	3	18–96					1 074	206	80
NL/1	Netherlands	DNFCS 2003	24-hour dietary recall	2	19–30					750		
NL/2	Netherlands	VCP kids	Food record	3	2–6		322	957				
FI/1	Finland	DIPP	Food record	3	1–6		497	933				
FI/2	Finland	FINDIET 2007	48-hour recall	2	25–74					1 575	463	
FI/3	Finland	STRIP	Food record	4	7–8			250				

Table A1: Dietary surveys considered for the chronic dietary exposure assessment with the number of subjects in the different age classes (continued)

Code[(a)]	Country	Dietary survey[(b)]	Method	Days	Age (years)	Number of subjects[(c)]						
						Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
SE/1	Sweden	RIKSMATEN 1997–98	Food record	7	18–74					1 210		
SE/2	Sweden	NFAn	24-hour recall	4	3–18			1 473	1 018			
UK	United Kingdom	NDNS	Food record	7	19–64					1 724		

(a): Abbreviations to be used consistently in all tables on exposure assessment.
(b): More information on the dietary surveys is given in the guidance of EFSA "Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment" (EFSA, 2011).
(c): Number of available subjects for chronic exposure assessment in each age class.
(d): 95th percentiles calculated over a number of observations fewer than 60. These require cautious interpretation, as the results may not be statistically robust (EFSA, 2011).

ABBREVIATIONS

ADI	acceptable daily intake
AFSSA	Agence française de sécurité sanitaire des aliments
AGP	α1-acid glycoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
AST	aspartate amino transferase
BfR	Federal Institute for Risk Assessment
BgVV	Federal Institute for Consumer Health Protection and Veterinary Medicine
BMDL	benchmark dose lower confidence limit
$BMDL_{10}$	the lower 95 % confidence limit for a benchmark response of 10 % extra risk
BSA	bovine serum albumin
b.w.	body weight
CAP	chloramphenicol
CAT	catalase
CCα	decision limit
CCβ	detection capability
CDV	canine distemper virus
CNS	central nervous systems
CONTAM Panel	EFSA Scientific Panel on Contaminants in the Food Chain
CRM	certified reference material
CVMP	Committee for Veterinary Medicinal Products
CYP	cytochrome P450
cyt	cytochrome
DAD	diode-array detection
EC	European Commission
ECD	electron capture detection
EFSA	European Food Safety Authority
EI	electron impact ionisation
ELISA	enzyme-linked immunosorbent assays
EMA	European Medicines Agency
EMEA	European Agency for the Evaluation of Medicinal Products
ERK	signal-regulated kinase
ESI	electrospray ionisation
EU	European Union
FAD	flavine adenine dinucleotide
FAO	Food and Agriculture Organisation
FAPAS	Food Analysis Performance Assessment Scheme
FDA	Food and Drug Administration
FSA	Food Standards Agency
FYROM	Former Yugoslav Republic of Macedonia
GC	gas chromatography
GC–ECD	gas chromatography-electron capture detection
GC–MS	gas chromatography–mass spectrometry
GC–MS/MS	gas chromatography–tandem mass spectrometry
GI	gastrointestinal
h	hour/hours
HPBL	human peripheral blood lymphocytes
HPLC	high-performance liquid chromatography
HPLC–UV/DAD	high-performance liquid chromatography–ultra violet/diode-array detection
HPLC–UV	high-performance liquid chromatography–ultra violet

HRMS	high-resolution mass spectrometry
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IAC	immunoaffinity chromatography
IARC	International Agency for Research on Cancer
IL	interleukin
IP	identification point
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC–IRMM	Joint Research Centre-Institute for Reference Materials and Measurements
LC	liquid chromatography
LC–MS	liquid chromatography–mass spectrometry
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LD_{50}	medium lethal dose
LDH	lactate dehydrogenase
LLE	liquid–liquid extraction
LOAEL	lowest observed adverse effect level
LOD	limit of detection
Log K_{ow}	octanol/water partition coefficient
LOQ	limit of quantification
MB	middle bound
MIP	molecularly imprinted polymer
MMP	matrix metalloproteinase
MOE	margin of exposure
MRL	maximum residue limit
MRPL	minimum required performance limit
MS	mass spectrometry
MSPD	matrix solid phase dispersion
NADPH	nicotinamide adenine dinucleotide phosphate
NCI–MS	negative chemical ionisation-mass spectrometry
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NPAP	nitrophenylaminopropanedione
NVWA	The Netherlands Food and Consumer Product Safety Authority (Nederlandse Voedsel-en Warenautoriteit)
p.o.	per os (orally)
RASFF	Rapid Alert System for Food and Feed
RIVM	National Institute for Public health and Environment (Rijksinstituut voor Volksgezondheid and Milieu)
ROS	reactive oxygen species
RPA	reference point for action
s.c.	subcutaneous
SCE	sister chromatid exchange
SFE	supercritical fluid extraction
SIM	selected ion monitoring
SOD	superoxide dismutase
SPE	solid phase extraction
SPR	surface plasmon resonance
SRBC	sheep red blood cells
SRM	selected reaction monitoring
TEPA	tris(1-aziridinyl) phosphi-neoxide
TG	thioguanine
TK	thymidine kinase
TLC	thin-layer chromatography
TR-FIA	time-resolved fluoroimmunoassay

TTC	Threshold of Toxicological Concern
UDS	unscheduled DNA synthesis
UGT	UDP-glucuronosyltransferase
UK	The United Kingdom
UV	ultraviolet
Vd	volume of distribution
VMP	veterinary medicinal product
WHO	World Health Organization