

**FINAL**

**Report on Carcinogens  
Background Document for**

**Chloramphenicol**

**December 13 - 14, 2000**

**Meeting of the  
NTP Board of Scientific Counselors  
Report on Carcinogens Subcommittee**

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## Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

### U.S. Department of Health and Human Services National Toxicology Program

#### **Known to be Human Carcinogens:**

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

#### **Reasonably Anticipated to be Human Carcinogens:**

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen*, or *reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.



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## Summary Statement

### Chloramphenicol

CASRN 56-75-7

### Carcinogenicity

Chloramphenicol is *reasonably anticipated to be a human carcinogen*, based on limited evidence of carcinogenicity from human cancer studies. Numerous case reports have shown leukemia to occur following chloramphenicol-induced aplastic anemia (IARC 1990). Three case reports have documented the occurrence of leukemia following chloramphenicol therapy in the absence of intervening aplastic anemia (IARC 1990). In a case-control study in China, Shu *et al.* (1987, 1988) reported elevated risks of childhood leukemia, which increased significantly with the number of days chloramphenicol was taken. Zahm *et al.* (1989) reported that chloramphenicol use was associated with an increased risk of soft-tissue sarcoma. Two case-control studies (Laporte *et al.* 1998, Issaragrisil *et al.* 1997) found high but nonsignificant elevations of risk of aplastic anemia associated with the use of chloramphenicol in the six months before onset of aplastic anemia. Two case-control studies (Zheng *et al.* 1993, Doody *et al.* 1996) found no association of chloramphenicol use with risk of adult leukemia. Taken together, the many case reports implicating chloramphenicol as a cause of aplastic anemia, the evidence of a link between aplastic anemia and leukemia, and the increased risk of leukemia found in some case-control studies support the conclusion that an increased cancer risk is associated with chloramphenicol exposure. Children may be a particularly susceptible subgroup.

Chloramphenicol was reported in an abstract to increase the incidence of lymphoma in two strains of mice and liver tumors in one strain (Sanguineti *et al.* 1983). However, because this study was incompletely reported, the findings are considered insufficient to establish a definitive link between chloramphenicol exposure and cancer in experimental animals.

### Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

When given in combination with busulfan, chloramphenicol significantly increased the incidence of lymphoma in male mice relative to the rates observed in the groups receiving busulfan or chloramphenicol alone (Robin *et al.* 1981).

Chloramphenicol blocks protein synthesis in bacteria by binding to the 50S subunit of the 70S ribosome. Ribosomes in the mitochondria of mammalian cells also are affected accounting for the sensitivity of proliferating tissues, such as the hematopoietic system, to the toxicity of chloramphenicol. Anemias, occasionally including aplastic anemia, are a recognized hazard associated with chloramphenicol use by humans.

Several studies (Isildar *et al.* 1988 a,b, Jimenez *et al.* 1990, Kitamura *et al.* 1997) show that the dehydrochloramphenicol metabolite produced by intestinal bacteria may be responsible for DNA damage and carcinogenicity. This metabolite can undergo nitroreduction in the bone marrow, where it causes DNA single-strand breaks. Mitochondrial abnormalities induced by chloramphenicol are similar to those observed in preleukemia, suggesting that mitochondrial DNA is involved in the pathogenesis of leukemia.

The available genotoxicity data show predominantly negative results in bacterial systems and mixed results in mammalian systems. The most consistently positive results were observed for cytogenetic effects in mammalian cells, including DNA single-strand breaks and increases in the frequencies of sister chromatid exchanges and chromosomal aberrations.

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# 1 Introduction

Chloramphenicol was isolated from *Streptomyces venezuelae* in 1947. Chloramphenicol was found to be effective against typhus in 1948 and became the first antibiotic to undergo large-scale production. By 1950, the medical community was aware that the drug could cause serious and potentially fatal aplastic anemia, and it quickly fell into disfavor. Chloramphenicol currently is used in the United States only to combat serious infections where other antibiotics are either ineffective or contraindicated.

Chloramphenicol was listed in the First Annual Report on Carcinogens in 1980 as a human carcinogen (NTP 1980). This listing was based on human study case reports suggesting that aplastic anemia due to chloramphenicol use was associated with subsequent development of leukemia. Chloramphenicol was removed from the Second Annual Report on Carcinogens in 1981 based on a re-evaluation of the International Agency for Research on Cancer (IARC) assessment of this drug. The IARC had concluded that the data on carcinogenicity of chloramphenicol in humans were inadequate in terms of strength of evidence and that there were no data on experimental animals (IARC 1976). The IARC reviewed chloramphenicol again in 1987 and in 1990 and concluded that there was *limited evidence of carcinogenicity in humans* and *inadequate evidence of carcinogenicity in experimental animals*. In making the overall evaluation, the IARC noted that chloramphenicol induces aplastic anemia and that this condition is related to the occurrence of leukemia. The IARC's overall evaluation is that chloramphenicol *is probably carcinogenic to humans* (Group 2A) (IARC 1987, 1990).

Chloramphenicol was nominated for listing in the Report on Carcinogens (RoC) by the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP) RoC Review Group (RG1) based on the IARC listing of chloramphenicol as *probably carcinogenic to humans* (Group 2A).

## 1.1 Chemical identification

Chloramphenicol (C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>, mol wt 323.1322, CASRN 56-75-7) also is known by the following names:

ak-chlor	farmicetina
alficetyn	fenicol
amphicol	globenicol
anacetin	intermycetine
aquamycetin	intramycetin
austracol	intramyctin
c.a.f.	juvamycetin
chemiceticol	kamaver
chlomycol	kemicetine
chloramex	klorita
chloramfilin	leukomycin
chloramphenicol crystalline	levomicetina
chloramsaar	levomycetin
chlorasol	loromicetina



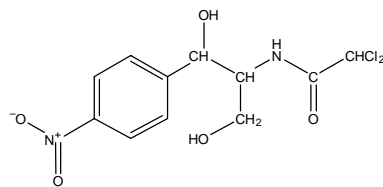
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chloricol	mycinol
chlormycetin r	myscel
chlorocaps	novomycetin
chlorocid	opclor
chloromycetin	ophthochlor
chloronitrin	pantovernil
chloroptic	paraxin
chlorsig	quemicitina
cidocetine	ronfenil
ciplamycetin	septicol
cloramfen	sintomicetina
cloramficin	sno phenicol
cloramical	stanomycetine
cloramicol	synthomycetine
clorocyn	tea-cetin
cloromissan	tevcozin
cyphenicol	tifomycine
duphenicol	treomicetina
embacetin	unimycetin
enicol	verticol
enteromycetin	victeon
D-(-)- <i>threo</i> -1-( <i>p</i> -nitrophenyl)-2-dichloroacetamido-1,3-propanediol	
2,2-dichloro- <i>N</i> -[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]acetamide	
D- <i>threo</i> - <i>N</i> -dichloroacetyl-1- <i>p</i> -nitrophenyl-2-amino-1,3-propane-diol	
D(-)- <i>threo</i> -2-dichloroacetamido-1- <i>p</i> -nitrophenyl-propanediol	
D- <i>threo</i> - <i>N</i> -(1,1'-dihydroxy-1- <i>p</i> -nitrophenylisopropyl)dichloroacetamide	
acetamide, 2,2-dichloro- <i>N</i> -[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-, [ <i>R</i> - ( <i>R</i> *, <i>R</i> *)]-	
D-(-)- <i>threo</i> -2,2-dichloro- <i>N</i> -[ $\beta$ -hydroxy-alpha-(hydroxy-methyl)- <i>p</i> - nitrophenethyl]acetamide	

Chloramphenicol's RTECS code is AB6825000, and its shipping code is UN 1851.

## 1.2 Physical-chemical properties

Chloramphenicol exists as a white to grayish-white or yellowish-white fine crystalline powder, needles, or elongated plates, with a melting point of 150.5 to 151.5°C. It sublimates in high vacuum and is sensitive to light. The nitro group is readily reduced to the amine group. Of the four possible stereoisomers, only the  $\alpha$ R,  $\beta$ R (or *D-threo*) form is active (IARC 1990). The structure of chloramphenicol is illustrated in Figure 1-1, and its physical and chemical properties are listed in Table 1-1.



Source: ChemFinder (2000)

**Figure 1-1. Structure of chloramphenicol**

**Table 1-1. Physical and chemical properties of chloramphenicol**

Property	Information	Reference
Molecular weight	323.1322	Budavari <i>et al.</i> 1996, ChemFinder 2000
Color	white to grayish-white or yellowish-white	Budavari <i>et al.</i> 1996, CRC 1998, ChemFinder 2000
Taste	bitter burning	HSDB 1995
Physical state	crystals, crystalline powder, needles, or elongated plates	Budavari <i>et al.</i> 1996, CRC 1998, ChemFinder 2000
Melting point (°C)	150.5–151.5	Budavari <i>et al.</i> 1996, CRC 1998, HSDB 1995
pH	neutral to litmus	HSDB 1995
Vapor pressure (mm Hg)	$1.73 \times 10^{-12}$	HSDB 1995
Half-life in humans	1.6–4.6 h	HSDB 1995
Solubility		
Water at 25°C	slightly soluble, 2.5 mg/mL	ChemFinder 2000, HSDB 1995
Propylene glycol	150.8 mg/mL	HSDB 1995
50% Acetamide	5%	HSDB 1995
Chloroform	soluble	HSDB 1995
Methanol	very soluble	HSDB 1995
Ethanol	very soluble	HSDB 1995
Butanol	very soluble	HSDB 1995
Ethyl acetate	very soluble	HSDB 1995
Acetone	very soluble	HSDB 1995
Ether	soluble	HSDB 1995
Benzene	insoluble	HSDB 1995
Petroleum ether	insoluble	HSDB 1995
Vegetable oils	insoluble	HSDB 1995

### 1.3 Identification of metabolites

Chloramphenicol is eliminated primarily following biotransformation. In humans, as much as 90% of administered chloramphenicol is eliminated in urine as the chloramphenicol glucuronide conjugate. In other species (e.g., dog and rat), urinary elimination is dominant, but larger amounts are eliminated in bile as aromatic amines. In humans, as much as 10% of the administered dose may be eliminated unchanged in the bile. The direct conjugation to form glucuronide is at the primary rather than the secondary alcoholic group (Testa and Jenner 1976, cited in HSDB 1995).

Chloramphenicol yields D-*threo*-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol and chloramphenicol- $\beta$ -D-glucuronide metabolites in humans and in rats (Goodwin 1976, cited in HSDB 1995). Chloramphenicol 3-glucuronide was the major metabolite produced by isolated rat hepatocytes, along with a minor metabolite (Siliciano *et al.* 1978, cited in HSDB 1995). Oxamic acid, alcohol, base acetylarylamine, and arylamine metabolites have been found as secondary metabolites in rats treated with chloramphenicol (IARC 1990). The structures for some of the metabolites of chloramphenicol are presented in Section 6.

## 2 Human Exposure

### 2.1 Use

Chloramphenicol is an antimicrobial agent with restricted use, because it causes blood dyscrasia. It is used to combat serious infections where other antibiotics are either ineffective or contraindicated. It can be used against gram-positive cocci and bacilli and gram-negative aerobic and anaerobic bacteria (DFC 2000). Chloramphenicol has been used since the 1950s to combat a wide range of microbial infections, including typhoid fever, meningitis, and certain infections of the central nervous system (IARC 1990). It currently is used in eye ointments to treat superficial ocular infections involving the conjunctiva or cornea, in topical ointments to treat the external ear or skin, in various tablets for oral administration, and in intravenous (i.v.) suspensions to treat internal infections (PDR 2000). Chloramphenicol also has been used in veterinary medicine as a highly effective and well-tolerated broad-spectrum antibiotic. Because of its tendency to cause blood dyscrasia in humans, its use in food-producing animals is now prohibited. Chloramphenicol still is used in cats, dogs, and horses to treat both systemic and local infections (MVM 1998).

An average adult dose of chloramphenicol is 25 to 100 mg/kg body weight (b.w.) per day, divided into four oral or i.v. doses. Dosing usually continues for two to five days or until the infection is cleared. Follow-up at therapeutic levels of chloramphenicol is suggested for many infections, ranging from 48 hours for eye infections to eight to 10 days for typhoid fever. Chloramphenicol also is used in ophthalmic preparations, including ointments, solutions, and drops. Pediatric doses must be lower, to avoid gray baby syndrome. Gray baby syndrome is characterized by cardiovascular collapse in infants, apparently due to an accumulation of active, unconjugated chloramphenicol in the serum, resulting from its decreased glucuronide conjugation in the liver (DFC 2000). Children, especially neonates and young infants, metabolize chloramphenicol at a much slower rate than adults. Initial doses are 25 mg/kg b.w. every 24 hours for infants under one week of age, 25 mg/kg every 12 hours for infants aged one to four weeks, and 50 mg/kg every six hours for children weighing under about 25 kg (Sills and Boenning 1999). No published information was found on current prescriptions in the United States.

### 2.2 Production

Chloramphenicol is produced naturally by *Streptomyces venezuelae*. It is now produced by chemical synthesis followed by a step to isolate stereoisomers. A fermentation process has been described that does not require separation of stereoisomers (IARC 1990).

The first commercial production of chloramphenicol in the United States was reported in 1948. U.S. production of chloramphenicol was estimated to be greater than 908 kg (2,002 lb) in 1977 and 1979. U.S. imports for these years were estimated at 8,150 kg (17,970 lb) and 8,200 kg (18,080 lb), respectively (HSDB 1995). Current production levels for either veterinary and human use were not found in the literature.

### 2.3 Analysis

Chloramphenicol can be detected in blood serum, plasma, or cerebrospinal fluid by high-pressure liquid chromatography (HPLC). HPLC or enzyme immunoassay may be used to determine chloramphenicol levels in blood. Chloramphenicol can be measured in pharmaceutical preparations for humans and animals with microbiological, turbidimetric, and spectrophotometric assays. Thin-layer chromatography and densitometry are used in the analysis of prescription drugs. Chloramphenicol levels in meat, milk, and eggs have been determined with thin-layer HPLC and radioimmunoassay (HSDB 1995).

### 2.4 Environmental occurrence

Chloramphenicol may be released to the environment and may be found in various waste streams because of its use as a medicinal and research antimicrobial agent. Chloramphenicol also may be isolated from *Streptomyces venezuelae* in the soil (HSDB 1995).

### 2.5 Environmental fate

Chloramphenicol may be present in the environment because of releases into various waste streams. If released into the atmosphere, chloramphenicol will exist primarily in the particulate phase. Removal of atmospheric chloramphenicol would occur mainly through dry deposition. The atmospheric half-life of chloramphenicol is 12 hours, as it will react with photochemically produced hydroxyl radicals. If released to water, chloramphenicol will be essentially nonvolatile. Adsorption to sediment or bioconcentration in aquatic organisms are not expected to be important processes. If released to soil, chloramphenicol is expected to have high soil mobility. Volatilization of chloramphenicol is not expected from either dry or wet soils (HSDB 1995).

Various biodegradation studies indicate that chloramphenicol may biodegrade in soil and water. Chloramphenicol degraded when adapted activated sludge was used as the inoculum. It also was degraded by intestinal bacteria via amidolysis; 18 metabolites were observed, with 2-amino-1-(*p*-nitrophenyl)-1,3-propanediol and its *p*-aminophenyl reduction byproduct as the major metabolites (HSDB 1995).

### 2.6 Environmental exposure

Exposure to chloramphenicol may occur through inhalation, dermal contact, ingestion, or contact with contaminated water or soil (HSDB 1995).

Because of potentially harmful effects to humans, chloramphenicol was banned by the Food and Drug Administration (FDA) in 1997 from use in food-producing animals (FDA 1997). No data on levels of chloramphenicol in food products were found in the literature.

### 2.7 Occupational exposure

Occupational exposure during the manufacture of chloramphenicol may occur through inhalation, dermal contact, or ingestion (HSDB 1995). Medical and veterinary personnel who use drugs containing chloramphenicol may be exposed (MVM 1998, DFC 2000). No exposure data were found in the literature.

## 2.8 Biological indices of exposure

Chloramphenicol can be detected in blood serum, plasma, cerebrospinal fluid, and urine. It is rapidly absorbed from the gastrointestinal tract and is distributed extensively through the human body, regardless of route of administration. It has been found in the heart, lung, kidney, liver, spleen, pleural fluid, seminal fluid, ascitic fluid, and saliva. Upon metabolism, chloramphenicol yields *D-threo*-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol and chloramphenicol- $\beta$ -D-glucuronide. Around 90% of chloramphenicol is excreted in urine. The majority of the release is in the form of metabolites, including conjugated derivatives, while only 15% is excreted as the parent compound (HSDB 1995).

The half-life of chloramphenicol in adult humans ranges from 1.6 to 4.6 hours. Peak levels appear two to three hours after oral administration of chloramphenicol. In adults given eight 1-g doses, one every six hours, the average peak serum level was 11.2  $\mu$ g/ml one hour after the first dose and 18.4  $\mu$ g/ml after the fifth dose. Mean serum levels ranged from 8 to 14  $\mu$ g/ml over the 48-hour period (DFC 2000). In infants, chloramphenicol's half-life is much longer. The half-life ranged from 10 to > 48 hours in infants aged one to eight days and from five to 16 hours in infants aged 11 days to eight weeks.

## 2.9 Regulations

The American Industrial Hygiene Association recommends an eight-hour time-weighted average workplace environmental exposure level of 0.5 mg/m<sup>3</sup>. The FDA regulates manufacturers, packers, and distributors to ensure proper labeling, certification, and usage requirements for any drug containing chloramphenicol. The FDA also describes specifications and conditions of use for chloramphenicol tablets, capsules, suspensions, ointments, and solutions for dogs and cats, and requires that chloramphenicol not be used in any food-producing animals. FDA regulations are summarized in Table 2-1.

**Table 2-1. FDA regulations**

Regulatory action	Effect of regulation and other comments
21 CFR 201—PART 201—LABELING. Promulgated: 40 FR 13998 03/27/75. U.S. Codes: 21 U.S.C. 321, 331, 352-53, 355-58, 360, 360b, 360gg-360ss, 371, 374, 379e.	The regulations govern the proper labeling procedures for a drug and drug product. For drugs containing chloramphenicol and derivatives, no new drugs may be released for interstate commerce without proper labeling.
21 CFR 314—PART 314—SUBPART F – Administrative Procedures for Antibiotics. Promulgated: 50 FR 7493, 02/22/85. U.S. Codes: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 371, 374, 379e.	The FDA will not promulgate a regulation providing for the certification of any batch of any drug composed wholly or in part of any kind of chloramphenicol or any derivative thereof intended for human use, and no existing regulation will be continued in effect unless it is established by substantial evidence that the drug will have such characteristics of identity, strength, quality, and purity necessary to adequately ensure safety and efficacy of use.
21 CFR 430—PART 430—ANTIBIOTIC DRUGS; GENERAL. Promulgated: [39 FR 18925, 04/30/74.U.S. Codes: 21 U.S.C. 321, 351, 352, 353, 355, 357, 371; 42 U.S.C. 216, 241, 262.	This part identifies any and all antibiotic drugs that contain chloramphenicol and its derivatives.
21 CFR 455—PART 455—CERTAIN OTHER ANTIBIOTIC DRUGS. Promulgated: 39 FR 19166, 05/30/74. U.S. Codes: 21 U.S.C. 357.	This part identifies requirements for certification for chloramphenicol and various other chloramphenicol-related drugs, covering (1) standards of identity, strength, quality, and purity, (2) labeling, and (3) requests for certification, including requirements for samples.
21 CFR 460—PART 460—ANTIBIOTIC DRUGS INTENDED FOR USE IN LABORATORY DIAGNOSIS OF DISEASE. Promulgated: 39 FR 19181 05/30/74. U.S. Codes: 21 U.S.C. 357.	This part regulates the standards of identity, strength, quality, and purity for susceptibility discs, powders, and test panels. This includes labeling, packaging, tests, and methods of assays.
21 CFR 520—PART 520—ORAL DOSAGE FORM NEW ANIMAL DRUGS. Promulgated: 62 FR 35076, 06/30/97. U.S. Codes: 21 U.S.C. 360b.	This part regulates specifications and exact wording for labels of oral dosage forms of animal drugs. The drugs affected are chloramphenicol oral dosage forms, chloramphenicol tablets, chloramphenicol capsules, and chloramphenicol palmitate oral suspension.
21 CFR 522—PART 522—IMPLANTATION OR INJECTABLE DOSAGE FORM NEW ANIMAL DRUGS. Promulgated: 40 FR 13858 03/27/75. U.S. Codes: 21 U.S.C. 360b.	This part regulates specifications, indications, and conditions of use and limitations of animal drugs. The subpart affects chloramphenicol injection.
21 CFR 524—PART 524—OPHTHALMIC AND TOPICAL DOSAGE FORM NEW ANIMAL DRUGS. Promulgated: 40 FR 13858 03/27/75. U.S. Codes: 21 U.S.C. 360b.	This part identifies specification, indications, and conditions of use and limitations for the animal drug under the heading chloramphenicol ophthalmic and topical dosage forms.
21 CFR 530—PART 530—EXTRALABEL DRUG USE IN ANIMALS. Promulgated: 62 FR 27947, 05/22/97. U.S. Codes: 15 U.S.C. 1453, 1454, 1455; 21 U.S.C. 321, 331, 351, 352, 353, 355, 357, 360b, 371, 379e.	Chloramphenicol is prohibited for extralabel animal and human drug uses in food-producing animals.

The regulations in this table have been updated through the 1999 Code of Federal Regulations 21 CFR, 1 April 1999.

## 3 Human Cancer Studies

### 3.1 Association of chloramphenicol with aplastic anemia or human cancer

#### 3.1.1 IARC evaluations

At the time of the last IARC review (1990), numerous case reports documented the occurrence of aplastic anemia and leukemia following treatment with chloramphenicol, nine case reports documented the occurrence of leukemia following chloramphenicol-induced aplastic anemia (Edwards 1969, Seaman 1969, Goh 1971, Cohen and Huang 1973, Meyer and Boxer 1973, Hellriegel and Gross 1974, Modan *et al.* 1975, Ellims *et al.* 1975, Witschel 1986), and three case reports documented leukemia following chloramphenicol therapy in the absence of intervening aplastic anemia (Humphries 1968, Popa and Iordacheanu 1975, Aboul-Enein *et al.* 1977). However, only one analytic epidemiologic study was available: a case-control study of childhood leukemia in China (Shu *et al.* 1987). The study reported elevated risks of all types of leukemia, increasing significantly with the number of days the medication was taken. The IARC concluded that there was *limited evidence of carcinogenicity in humans* and *inadequate evidence of carcinogenicity in experimental animals*. However, because chloramphenicol was associated with aplastic anemia in case reports, and because aplastic anemia has been related to the development of leukemia (see below), the IARC (1990) considered chloramphenicol *probably carcinogenic to humans* (Group 2A).

#### 3.1.2 Studies published since the IARC (1990) evaluation

Since publication of the IARC (1990) monograph, several case-control studies have evaluated the effects of exposure to chloramphenicol on the risk of aplastic anemia, specific types of leukemia, lymphoma, and soft-tissue sarcoma (Table 3-1). In this review, results are reported as odds ratios (ORs) with 95% confidence intervals.

Two methodologically similar case-control studies were conducted specifically to evaluate association of aplastic anemia with drugs and chemicals. From 1980 to 1995, Laporte *et al.* (1998) conducted a study of aplastic anemia cases in Barcelona, Spain. Cases (n = 145) were identified through the International Agranulocytosis and Aplastic Anemia Study, a multicenter study carried out in Europe and Israel. Controls (n = 1,226) were patients entering the hospital at the same time for reasons other than aplastic anemia. In Thailand, Issaragrisil *et al.* (1997) identified 253 aplastic anemia cases and 1,174 non-anemic controls in hospitals from 1989 to 1994. In both studies, the participants were interviewed in the hospital about their medication use during the period one to six months before their hospital admission. Both studies reported nonsignificant elevated risks associated with the use of chloramphenicol in the six months before disease onset (2.7, 0.7 - 10 [Issaragrisil *et al.* 1997], and 3.8, 0.8 - 16.9 [Laporte *et al.* 1998]). Neither study, however, had more than four exposed cases. In the Spanish study, two of the three exposed individuals also had been exposed to other drugs previously associated with aplastic anemia. The small number of exposed cases in these studies and aggressive attempts to control for confounding resulted in elevated but very imprecise estimates, and neither group of investigators felt confident attributing a significant fraction of the risk for aplastic anemia to chloramphenicol.



Several studies have investigated the possible relationship between chloramphenicol use and cancer. Shu *et al.* (1988) reanalyzed data from their case-control study of childhood leukemia in China, reporting results for different types of leukemia separately. Children under 15 years of age with acute lymphocytic leukemia (n = 171), acute nonlymphocytic (myeloid) leukemia (n = 93), and other types of leukemia (n = 45) were identified and recruited through the Shanghai tumor registry. Control children (n = 618) were randomly recruited through a neighborhood registry and matched to cases by age and sex. An in-person interview revealed that 105 case children and 109 control children had used chloramphenicol, and the associated risk of leukemia increased with increasing duration of drug use. After control for confounding, chloramphenicol use was associated with the following increased risks of acute lymphocytic leukemia: use for one to five days, OR = 1.8 (1.1 - 2.9); use for six to 10 days, OR = 2.1 (1.0 - 4.6); and use for > 10 days, OR = 10.7 (3.9 - 28.7). Chloramphenicol use also was associated with the following increased risks of acute nonlymphocytic leukemia: use for one to five days, OR = 2.8 (1.6 - 4.9); use for six to 10 days, OR = 3.6 (1.5 - 8.7); and use for > 10 days, OR = 12.2 (3.9 - 38.2).

Zahm *et al.* (1989) studied the effects of chloramphenicol, along with other chemical exposures, on the risk of soft-tissue sarcoma in a case-control study of Kansas men (> 21 years of age). Through telephone interviews, 133 men diagnosed with soft-tissue sarcoma, identified through the Kansas tumor registry, and 1,005 controls identified from the population were queried regarding their medication use and occupation. Chloramphenicol use at least five years before the diagnosis or reference date was associated with an increased risk of soft-tissue sarcoma (5.4, 1.2 - 23.9). Despite the magnitude of the reported risk, only four cases and five controls were exposed, which was reflected in the wide confidence limits.

Two case-control studies evaluated the relationship between chloramphenicol and leukemia in adults (> 15 years of age). Zheng *et al.* (1993) interviewed 533 individuals diagnosed with leukemia, identified in the Shanghai tumor registry, along with 502 population controls matched by age and sex. For leukemia patients no longer living and their matched controls (48%), the next-of-kin were interviewed. All interviews collected information regarding medication use more than three years before the diagnosis or reference date. No significant increase was found in risk of acute lymphocytic leukemia (0.8, 0.4 - 1.5), acute nonlymphocytic leukemia (0.7, 0.4 - 1.1), or chronic myeloid leukemia (1.3, 0.7 - 2.3). In this retrospective study, asking next-of-kin about medication use may have increased the potential for exposure misclassification of both cases and controls, which would most likely have biased results toward the null.

Doody *et al.* (1996) abstracted information from medical records of the Kaiser Permanente Medical Care System for cases of non-Hodgkin's lymphoma (n = 94), multiple myeloma (n = 159), multiple types of leukemia (n = 257), and 695 age- and sex-matched controls. These records indicated that few individuals were exposed to chloramphenicol and that exposure before the diagnosis of leukemia was inversely associated with risk of all types of leukemia (exposure more than one year before diagnosis: OR = 0.5, 0.2 - 1.1; exposure more than five years before diagnosis: OR = 0.4, 0.2 - 1.0). Similarly, inverse associations with risk were reported for multiple types of leukemia, non-Hodgkin's lymphoma, and multiple myeloma. The accuracy of

information from medical records is not likely to differ by case status, but if the prescription of chloramphenicol is not routinely noted in the medical record, exposure may be underrepresented in both cases and controls, leading to imprecise results.

### 3.1.3. Summary

Most of these studies are retrospective, and the ability of participants to recall and report complicated drug or chemical names may be imperfect. Although standardized questionnaires were used in these studies, only the study by Doody *et al.* (1996) used objective records to classify exposure. In addition, most of these risk estimates were based on very few exposed cases and controls, making it difficult to draw definitive conclusions about the risk associated with using the drug. Nevertheless, taken together, the many case reports associating chloramphenicol use with aplastic anemia, the positive results in some case-control studies, the significant dose-response trend in the association between chloramphenicol and childhood leukemia reported by Shu *et al.* (1988), and the strong risk of soft-tissue sarcoma (Zahm *et al.* 1993) support the conclusion that increased cancer risk is associated with chloramphenicol exposure. Children may be a susceptible subgroup warranting special consideration (Shu *et al.* 1987, 1988).

## 3.2 Relationship of aplastic anemia to leukemia and other clonal disorders

### 3.2.1 Studies of clonal disorders following aplastic anemia

Various types of anemia have been shown to be temporal and possibly etiologic predecessors to leukemia. The injury to stem cells that results in aplastic anemia may be either inherited or due to environmental insults. However, it is unclear whether clonal diseases like myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), or acute myeloid leukemia (AML) are a later result of the same injury to the stem cells, whether the presence of aplastic anemia increases susceptibility to a second insult to progenitor cells that leads to carcinogenesis (de Planque *et al.* 1988), or whether specific treatments for aplastic anemia induce carcinogenesis (Ohara *et al.* 1997).

The evolution of aplastic anemia to MDS and leukemia has been described in numerous reports (reviewed in Socié *et al.* 2000). Early reports, which estimated that approximately 5% of aplastic anemia cases evolved to leukemia, were compromised by poor criteria for the diagnosis of aplastic anemia and differentiation of aplastic anemia from leukemia, and by short follow-up times and low survival rates. Patients developing leukemia within a few months following non-severe aplastic anemia might in fact have had a hypocellular MDS or an aplastic phase of leukemia. For example, Hasle *et al.* (1995) reviewed all cases of aplastic anemia reported in children aged up to 16 years in Denmark between 1980 and 1991. Of the 16 cases labeled as aplastic anemia, eight were found instead to be cases of pre-ALL. The presentations of pre-ALL and aplastic anemia are very similar, but symptoms of the former remit within a few weeks of diagnosis. The authors suggested that a multi-step carcinogenic progression of leukemia may include an early phase that is often misdiagnosed as aplastic anemia. (See Section 6 for a discussion of abnormal cytogenetic clones in patients diagnosed with aplastic anemia.)

The introduction of treatments for aplastic anemia, including bone marrow transplantation and immunosuppressive therapies, has improved the prognosis for these

patients, resulting in an increased number of survivors and longer follow-up (Kaito *et al.* 1998). Three single-center studies (Basel, Switzerland; Leiden, the Netherlands; and California) and two multi-center studies conducted by the European Group for Blood Marrow Transplantation Working Party on Severe Aplastic Anemia (EBMT–SAA) reported that the proportion of long-term aplastic anemia survivors with clonal hematological disease was 10% to 57% following immunosuppressive therapy (Tichelli *et al.* 1994, de Planque *et al.* 1988, 1989, Paquette *et al.* 1995, Socié *et al.* 1993). The proportion with malignant disease (MDS or acute leukemia) ranged from 10% to 20% at 10 years of follow-up. The most conclusive evidence of the risk of cancer in long-term survivors of acquired aplastic anemia comes from a multi-center study conducted by the EBMT–SAA (Socié *et al.* 1993). This cohort consisted of 860 patients given immunosuppressive therapy and 748 patients who received bone marrow transplants for severe aplastic anemia. The risk of cancer relative to the general population was analyzed overall and according to treatment. Patients developing acute leukemia arising less than six months after treatment and solid cancer arising less than 12 months after treatment were excluded from the analyses. The overall relative risk of cancer was 5.50 (3.76 - 8.11); the risk was 5.15 (3.26 - 7.94) after immunosuppressive therapy and 6.67 (3.05 - 12.65) after bone marrow transplantation. The relative risk was 85.00 (51.00 - 140.00) for acute leukemia and 2.57 (1.41 - 4.31) for solid tumors.

Evolution of MDS or acute leukemia in aplastic anemia survivors also has been evaluated following other types of treatment, such as treatment with androgens or growth factors. The French Cooperative Group for the study of aplastic and refractory anemia found a lower rate of clonal complications in 137 long-term-surviving patients treated with androgens alone (Najean and Hagenauer 1990). However, most of these patients had moderate aplastic anemia and thus may not have had as extensive stem-cell damage. In Japan, Ohara *et al.* (1997) reviewed the cases of 167 children diagnosed with aplastic anemia between 1988 and 1993. Among these children, the combined incidence of MDS and acute myeloid leukemia (MDS/AML) was 15.9%  $\pm$  6.2%. Incidence rates varied based on the aplastic anemia treatment protocol, with the incidence of MDS/AML as high as 47%  $\pm$  17% among the children who received immunosuppressive therapy combined with recombinant human granulocyte colony-stimulating therapy. Similar increases in MDS/AML (22.2%) were reported in 25 adults receiving granulocyte colony-stimulating factor therapy combined with either cyclosporine A or antithymocyte globulin (Kaito *et al.* 1998), but not in a study of 40 patients (median age 16) treated with a combination of immunosuppressive therapy and granulocyte colony-stimulating factor therapy (Bacigalupo *et al.* 1995).

### 3.2.2 Summary

The higher risk of MDS and leukemia observed after immunosuppressive therapy may be a result of improved survival and a systematic search for MDS in long-term patients (Socié *et al.* 2000). As discussed in Section 6, the increased risk may be related to a persistent defect in hematopoietic stem cells observed in patients recovering from aplastic anemia, which may or may not be enhanced by immunosuppressive therapy.

Although it is possible that certain therapies may contribute to the development of MDS/AML, it also is possible that the disease characteristics that lead clinicians to

choose a specific treatment may represent a different etiologic and prognostic course of disease, independent of therapy regimen.

In general, high rates of leukemia have been reported in aplastic anemic populations. However, it is important to remember that leukemias are etiologically heterogeneous diseases, and the mechanisms for development of each type following a diagnosis of aplastic anemia may differ. These studies also have raised questions about misdiagnosis of early stages of some types of leukemia as aplastic anemia, and the possibility that treatment regimens may influence the progression of anemia to leukemia. Thus, it is unclear where on the multi-step pathway from anemia to leukemia a chemical insult may be most important. Exposure to chloramphenicol has not been assessed in populations followed after diagnosis of aplastic anemia; thus, it is not known from these studies whether or where chloramphenicol may be important in the pathway from anemia to leukemia. However, it is clear that the incidences of diagnosed MDS and leukemia are higher among individuals with a diagnosis of aplastic anemia.

**Table 3-1. Case-control studies of health effects related to chloramphenicol exposure (1986–1998).**

Reference	Health effect/ Study design	Population	Exposure definition and information source	Odds ratio (95% CI) Number of exposed cases	Comments
Laporte <i>et al.</i> 1998 Barcelona, Spain	aplastic anemia case-control (1980–1995)	145 cases identified by hospital surveillance 1,226 hospital controls	interview any use of ocular form 1 to 6 months before hospital admission	3.8 (0.8–16.9) 1 systemic chloramphenicol case 3 ocular chloramphenicol cases	Interviewer not blinded to case status. Only 6-month recall period. Inference made on 3 exposed cases, 2 of which also were exposed to other agents associated with aplastic anemia. Low absolute risk associated with exposure.
Issaragrisil <i>et al.</i> 1997 Thailand	aplastic anemia case-control (1989–1994)	253 cases identified by physician 1,174 hospital controls	interview any use 1 to 6 months before hospital admission	2.7 (0.7–10) 4 cases	Interviewer not blinded to case status. Only 6-month recall period. Inference made on few exposed cases. Aggressive control for confounding.

Reference	Health effect/ Study design	Population	Exposure definition and information source	Odds ratio (95% CI) Number of exposed cases	Comments
Shu <i>et al.</i> 1987 Shanghai, China	childhood leukemia case-control (1974–1986)	309 childhood leukemia cases identified by registry 618 age- and sex-matched neighborhood controls	interview of parents or guardian for any use for 1–5, 6–10, or > 10 days during period > 2 years before diagnosis/ref. date	1–5 d: 1.7 (1.2–2.5) 57 cases 6–10 d: 2.8 (1.5–5.1) 24 cases 10+ d: 9.7 (3.9–24.1) 24 cases	Significant trends observed for both acute lymphocytic leukemia (56% of cases) and acute nonlymphocytic leukemia. Interview undertaken up to 10 years after diagnosis, which could lead to differential recall between parents of cases and controls. Little information regarding use of other antibiotics given, making it difficult to evaluate the possibility of bias.
Shu <i>et al.</i> 1988 Shanghai, China	childhood leukemia case-control (1974–1985)	171 acute lymphocytic anemia cases 93 acute nonlymphocytic anemia cases 45 other leukemia cases identified by registry 618 age- and sex-matched neighborhood controls boys and girls aged < 15 years	interview of parents or guardian for any use for 1–5, 6–10, or > 10 days during period > 2 years before diagnosis/ref. date	acute lymphocytic leukemia: 1–5 d: 1.8 (1.1–2.9) 32 cases 6–10 d: 2.1 (1.0–4.6) 11 cases 10+ d: 10.7 (3.9–28.7) 15 cases acute nonlymphocytic leukemia: 1–5 d: 2.8 (1.6–4.9) 22 cases 6–10 d: 3.6 (1.5–8.7) 9 cases 10+ d: 12.2 (3.9–38.2) 8 cases	Similar trend for acute lymphocytic and nonlymphocytic leukemia. Aggressive control for confounding. Potential for recall/reporting bias. Reasonable power.

Reference	Health effect/ Study design	Population	Exposure definition and information source	Odds ratio (95% CI) Number of exposed cases	Comments
Zahm <i>et al.</i> 1989 Kansas	soft-tissue sarcoma case-control (1976–1982)	133 soft-tissue sarcoma cases identified through registry 1,005 controls identified through Medicare or random-digit dialing, depending on age men only, aged > 21 years	interview ever use > 5 years before diagnosis or < 1977 for controls	5.4 (1.2–23.9) 4 cases	Recall-reporting biases possible. Inference based on few exposed. Cell type varied among cases, suggesting possible etiologic heterogeneity.
Zheng <i>et al.</i> 1993 China	leukemia case-control (1987–1989)	81 acute lymphocytic leukemia cases 236 acute nonlymphocytic leukemia cases 79 chronic myeloid leukemia cases 28 other leukemia cases identified by registry 502 age- and sex-matched controls identified by resident registry men and women aged > 15 years	interview any use > 3 yrs before diagnosis/ref. date	acute lymphocytic leukemia: 0.8 (0.4–1.5) 12 cases acute nonlymphocytic leukemia: 0.7 (0.4–1.1) 32 cases chronic myeloid leukemia: 1.3 (0.7–2.3) 20 cases	48% of information from next-of-kin, possibly leading to misclassification. Control for demographic and occupational confounding. High exposure prevalence in general population.

Reference	Health effect/ Study design	Population	Exposure definition and information source	Odds ratio (95% CI) Number of exposed cases	Comments
Doody <i>et al.</i> 1996 Northwest U.S.	non-Hodgkin's lymphoma, multiple myeloma, leukemia case-control (1958–1982)	94 non-Hodgkin's lymphoma cases 159 multiple myeloma cases 257 leukemia cases 695 age- and sex-matched controls all races, aged > 15 years. identified through Kaiser Permanente Medical Care System	medical record any use > 1 year or > 5 years before diagnosis/ref. date	total leukemia > 1 yr: 0.5 (0.2–1.1) 12 cases > 5 yr: 0.4 (0.2–1.0) 7 cases acute myeloid leukemia: > 1 yr: 0.8 (0.3–2.3) 7 cases > 5 yr: 0.4 (0.1–1.5) 4 cases non-Hodgkins lymphoma: > 1 yr: 0.9 (0.4–2.1) 9 cases > 5 yr: 1.1 (0.4–2.8) 8 cases multiple myeloma > 1 yr 0.9 (0.4–1.9) 11 cases > 5 yr: 0.7 (0.3–1.7) 9 cases	Similar trend for non-Hodgkin's lymphoma, multiple myeloma, and multiple types of leukemia. Inferences made on few exposed for each cancer type. Aggressive control for confounding. Objective exposure information, not subject to recall bias.





## 4 Studies of Cancer in Experimental Animals

The IARC reviewed studies of the carcinogenic action of chloramphenicol in experimental animals, which included studies of oral and intraperitoneal (i.p.) administration in mice (IARC 1990, see Appendix A). These studies are summarized below.

### 4.1 Oral administration in mice

In a study reported only in an abstract (Sanguineti *et al.* 1983), groups of 50 male and 50 female BALB/c and C57B1/6N mice, six weeks old, were administered chloramphenicol (purity unspecified) at a concentration of 0, 500, or 2,000 mg/L in drinking water for 104 weeks. The incidences of lymphoma in BALB/c mice (sexes combined) were 3% in controls, 6% in the low-dose group, and 12% in the high-dose group ( $P < 0.05$ ). The incidences of other types of tumors were similar in treated and control animals. In the C57B1/6N mice (sexes combined), the incidences of lymphoma were 8% in controls, 22% in the low-dose group ( $P < 0.05$ ), and 23% in the high-dose mice group ( $P < 0.01$ ). The combined (by sex) malignant liver tumor incidences in C57B1/6N mice (sexes combined) were zero in controls (total number of controls not reported), 2/90 (2%) in the low-dose group, and 11/91 (12%,  $P < 0.01$ ) in control, low-dose and the high-dose groups, respectively.

### 4.2 Intraperitoneal injection in mice

A group of 45 six- to eight-week-old BALB/c  $\times$  AF<sub>1</sub> male mice were pretreated 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  $\times$  AF<sub>1</sub> 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. Tumor incidence was not significantly increased in the treated mice (Robin *et al.* 1981).

Two groups of 45 male BALB/c  $\times$  AF<sub>1</sub> mice, six to eight weeks old, were given four i.p. injections of 0.5 mg of busulfan (1,4-butanediol dimethanesulfonate) in 0.25 mL acetone, one injection every two weeks. Two other groups of 45 male BALB/c  $\times$  AF<sub>1</sub> 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  $\times$  AF<sub>1</sub> 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. Five mice each from the chloramphenicol–busulfan and busulfan-only groups died before the end of the experiment and were not evaluated for lymphoma. The incidence of lymphoma was higher in the busulfan–chloramphenicol group (13/37,  $P = 0.02$ ) than in the busulfan-only group (4/35). The incidence of lymphoma in animals treated with chloramphenicol alone was 2/41. No lymphomas were found in the 41 surviving controls (Robin *et al.* 1981). The IARC Working Group noted the short duration of the treatment and observation periods of this study (IARC 1990).

### 4.3 Oral administration of a structural analog in rats

The carcinogenic potential of thiamphenicol, a synthetic antibiotic structurally similar to chloramphenicol, was assessed in male and female F344/DuCrj rats. Thiamphenicol, a methylsulfonyl homologue of chloramphenicol, possesses an  $-SO_2CH_3$  group at the *para* position of the benzene ring in lieu of the  $-NO_2$  group in chloramphenicol. In the study, 150 male and 150 female five-week-old rats were administered thiamphenicol in drinking water at a concentration of 125 or 250 ppm for two years. Control animals received only tap water. The incidence of tumors was no higher in the treated animals than in the controls (Kitamura *et al.* 1997).

### 4.4 Summary

Oral administration of chloramphenicol to mice was reported in an abstract to induce lymphoma and liver tumors in dose-dependent manner. Intraperitoneal injection of chloramphenicol did not induce any significant increase of tumors in mice. When administered i.p. in combination with the known carcinogen busulfan, chloramphenicol increased the incidence of lymphoma over that for busulfan or chloramphenicol alone. A chloramphenicol analog, thiamphenicol, did not induce tumors in rats.

Based on the limitations of these data and the lack of additional data on the carcinogenicity of chloramphenicol in experimental animals there is insufficient evidence to establish a definitive link between chloramphenicol exposure and cancer in experimental animals.

## 5 Genotoxicity

The IARC has reviewed the genetic toxicology of chloramphenicol through 1989. Much of the genotoxicity information presented in IARC (1990) was derived from a review paper (Rosenkranz 1988).

Rosenkranz (1988) noted that the study of genotoxic effects of chloramphenicol is complicated by its broad spectrum of inhibitory effects (e.g., on protein synthesis, cytochrome P-450 isozymes, and mitochondria), general toxicity, and use in human antimicrobial therapy; he prepared a thorough review of this subject. This section contains genotoxicity information from the IARC (1990) and Rosenkranz (1988) reviews and the few applicable studies published after these reviews.

Table 5-1 summarizes data from IARC (1990) and Rosenkranz (1988). The assays in prokaryotes generally are negative, while the assays in eukaryotes give mixed results. The most consistently positive results seem to be *in vitro* and *in vivo* cytogenetic effects in somatic and germ cells. Most of the data were incidental to studies that used chloramphenicol to investigate the role of protein synthesis in genetic phenomena or as a negative control in genetic assays. Data are lacking on several important genetic end points, such as gene mutation and the induction of unscheduled DNA synthesis in mammalian cells.

**Table 5-1. Genetic and related effects of chloramphenicol exposure reported before 1990**

Test system	End point	Results	References
<b>Prokaryotic systems</b>			
<i>Escherichia coli</i>	reverse mutation	negative (with or without S9 activation)	Hemmerly and Demerec 1955, Mitchell <i>et al.</i> 1980
<i>E. coli</i>	DNA damage ( <i>pol A</i> <sup>+</sup> / <i>pol A</i> <sup>-</sup> )	negative	Slater <i>et al.</i> 1971, Brem <i>et al.</i> 1974, Longnecker <i>et al.</i> 1974, Simmon <i>et al.</i> 1977, 1978, Venturini and Monti-Bragadin 1978, Nestmann <i>et al.</i> 1979, Boyle and Simpson 1980, Süssmuth 1980, Leifer <i>et al.</i> 1981, Levin <i>et al.</i> 1982
<i>E. coli</i>	DNA damage ( <i>pol A</i> <sup>+</sup> <i>lex A</i> <sup>+</sup> / <i>pol A</i> <sup>-</sup> <i>lex A</i> <sup>-</sup> )	negative	Venturini and Monti-Bragadin 1978

Test system	End point	Results	References
<i>E. coli</i>	DNA damage ( <i>B/B<sub>s-1</sub></i> ) ( <i>B/B/r</i> )	negative	Shimizu and Rosenberg 1973
<i>E. coli</i>	DNA damage ( <i>uvrA<sup>+</sup> recA<sup>+</sup>/uvrA<sup>-</sup> recA<sup>-</sup></i> )	negative	Mitchell <i>et al.</i> 1980
<i>E. coli</i>	DNA damage	negative	Mullinix and Rosenkranz 1971, Rosenkranz <i>et al.</i> 1971, Suter <i>et al.</i> 1978, Kubinski <i>et al.</i> 1981
<i>E. coli</i>	DNA damage	negative	Morgan <i>et al.</i> 1967, Dworsky 1974
<i>E. coli</i>	DNA damage ( <i>recA<sup>+</sup>/recA<sup>-</sup></i> ) ( <i>recB<sup>+</sup> recC<sup>+</sup> / recB<sup>-</sup> recC<sup>-</sup></i> ) ( <i>recA<sup>+</sup> recB<sup>+</sup> recC<sup>+</sup> / recA<sup>-</sup> recB<sup>-</sup> recC<sup>-</sup></i> )	positive	Suter and Jaeger 1982
<i>E. coli</i>	DNA damage ( <i>B/r</i> )	positive (breaks)	Jackson <i>et al.</i> 1977
<i>E. coli</i>	induction of SOS functions	negative	Shimada <i>et al.</i> 1975, Ben-Gurion 1978, Mamber <i>et al.</i> 1986
<i>Salmonella typhimurium</i>	reverse mutation (strains TA98, TA100, TA1530, TA1535, TA1537, TA1538)	negative (with or without metabolic activation)	Brem <i>et al.</i> 1974, McCann <i>et al.</i> 1975, Jackson <i>et al.</i> 1977, Mortelmans <i>et al.</i> 1986
<i>S. typhimurium</i>	reverse mutation (strain TA98)	weak positive	Mitchell <i>et al.</i> 1980
<i>S. typhimurium</i>	DNA damage ( <i>uvr<sup>+</sup>/uvrB<sup>-</sup></i> )	negative	Russell <i>et al.</i> 1980, Nader <i>et al.</i> 1981
<i>S. typhimurium</i>	DNA damage (strains TA1976, TA1535, TA100)	positive (breaks)	Jackson <i>et al.</i> 1977
<i>Proteus mirabilis</i>	DNA damage ( <i>rec<sup>+</sup> hcr<sup>+</sup>/rec<sup>-</sup> hcr<sup>-</sup></i> )	negative	Adler <i>et al.</i> 1976
<i>Bacillus subtilis</i>	DNA damage ( <i>rec<sup>+</sup>/rec<sup>-</sup></i> )	negative	Kada <i>et al.</i> 1972, Simmon <i>et al.</i> 1977, 1978, Karube <i>et al.</i> 1981, Sekizawa and Shibamoto 1982, Suter and Jaeger 1982
<i>B. subtilis</i>	DNA damage	negative	Ohtsuki and Ishida 1975

Test system	End point	Results	References
<i>Staphylococcus aureus</i>	induction of SOS functions	negative	Manthey <i>et al.</i> 1975
<b>Lower eukaryotic systems</b>			
<i>Saccharomyces cerevisiae</i>	gene mutation	negative (with or without S9 activation) (diploid strains)	Carnevali <i>et al.</i> 1971, Mitchell <i>et al.</i> 1980
		positive (haploid strains)	Weislogel and Butow 1970, Williamson <i>et al.</i> 1971
<i>Drosophila melanogaster</i>	sex-linked lethal mutation	negative	Clark 1963, Nasrat <i>et al.</i> 1977
<b>Plants</b>			
<i>Arabidopsis</i>	recessive lethal mutation in seeds	negative	Muller 1965
<i>Hordum vulgare</i>	chromosomal aberrations and nondisjunction in root-tip meristematic cells	positive	Yoshida <i>et al.</i> 1972, Yoshida and Yamaguchi 1973
<i>Vicia faba</i>	chromosomal aberrations in seeds	positive	Prasad 1977
<i>Tradescantia paludosa</i>	micronuclei in pollen tetrads	negative	Ma <i>et al.</i> 1984
<i>Spirogyra azygospora</i>	chromosomal aberrations	positive	Vedajanani and Sarma 1978
<b>Mammalian <i>in vitro</i> systems</b>			
Syrian hamster embryo (SHE) cells	unscheduled DNA synthesis, morphological transformation	negative (with or without metabolic activation)	Suzuki 1987
SHE cells	sister chromatid exchange (SCE)	positive	Suzuki 1987
Mouse lymphoma cells	mutation at the <i>tk</i> locus of L5178Y cells	positive (with or without metabolic activation)	Mitchell <i>et al.</i> 1988, Myhr and Caspary 1988
Bovine and porcine lymphocytes	chromosomal aberrations	positive	Quéinnec <i>et al.</i> 1975, Babilé <i>et al.</i> 1978
Human peripheral blood lymphocytes	SCE	negative	Pant <i>et al.</i> 1976a,b
Human peripheral blood lymphocytes	chromosomal aberrations	negative	Jensen 1972

Test system	End point	Results	References
Human peripheral blood lymphocytes	chromosomal aberrations	positive	Mitus and Coleman 1970, Sasaki and Tonomura 1973, Pant <i>et al.</i> 1976a,b, Goh 1979
Human peripheral blood lymphocytes	DNA damage	positive	Yunis <i>et al.</i> 1987
Human lymphoblastoid cells, lymphocytes, bone marrow	DNA damage	negative	Isildar <i>et al.</i> 1988b
Human fibroblasts	chromosomal aberrations	negative	Byarugaba <i>et al.</i> 1975
Human lymphocytes	chromosomal aberrations	positive	Mitus and Coleman 1970
<b>Mammalian <i>in vivo</i> systems</b>			
Rats	chromosomal aberrations in bone marrow cells	negative	Jensen 1972
Mice	dominant lethal mutation	negative (post-meiotic), positive (premeiotic)	Sràm 1972
Mice	dominant lethal mutation	negative	Epstein and Shafner 1968, Ehling 1971, Epstein <i>et al.</i> 1972
Mice	chromosomal aberrations in bone marrow cells	positive	Manna and Bardhan 1972, 1977
Mice	chromosomal aberrations in spermatocytes and spermatogonia	negative	Sràm and Kocisova 1974
Mice	chromosomal aberrations in mitotic and meiotic germ line cells and in F1 progeny	positive	Manna and Roy 1979, Roy and Manna 1981
Mice	aneuploidy in oocytes	positive	Beermann and Hansmann 1986

Source: Rosenkranz 1988, IARC 1990

Genetic toxicology studies that were not included in the IARC (1990) or Rosenkranz (1988) reviews are described in the following sections, and the results are summarized in Table 5-2.

### 5.1 Non-mammalian systems

No new information on the genotoxicity of chloramphenicol in prokaryotic systems, plants, or lower eukaryotic systems was found in the literature published after the IARC (1990) review.

**Table 5-2. Genetic and related effects of chloramphenicol exposure reported in studies not reviewed in IARC (1990)**

Test System	End point	Results	References
<b>Mammalian <i>in vitro</i> systems</b>			
Chinese hamster V79 cells	gene mutation (6-thioguanine resistance)	positive (without metabolic activation)	Martelli <i>et al.</i> 1991
Mouse primary bone marrow cells	chromosomal aberrations	weak positive	Sbrana <i>et al.</i> 1991
Human peripheral blood lymphocytes	chromosomal aberrations	positive	Sbrana <i>et al.</i> 1991
Bovine lymphocytes	SCE	positive	Catalan <i>et al.</i> 1993
Mouse primary bone marrow cells	SCE	weak positive	Sbrana <i>et al.</i> 1991
Chinese hamster V79 cells	SCE	weak positive	Sbrana <i>et al.</i> 1991
Human peripheral blood lymphocytes	SCE	weak positive	Sbrana <i>et al.</i> 1991
Chinese hamster V79 cells	DNA damage	positive	Martelli <i>et al.</i> 1991
Rat hepatocytes	DNA damage	weak positive	Martelli <i>et al.</i> 1991
Rat hepatocytes	DNA damage	negative	Martelli 1997
Human hepatocytes	DNA damage	positive	Martelli <i>et al.</i> 1991, Martelli 1997
Human peripheral blood lymphocytes	DNA damage	positive (only for certain metabolites) <sup>a</sup>	Isildar <i>et al.</i> 1988a, Lafarge-Frayssinet <i>et al.</i> 1994, Robbana-Barnat <i>et al.</i> 1997
Human Raji cells	DNA damage	positive (only for certain metabolites) <sup>a</sup>	Isildar <i>et al.</i> 1988a, Lafarge-Frayssinet <i>et al.</i> 1994
Human bone marrow cells	DNA damage	positive (only for certain metabolites) <sup>a</sup>	Isildar <i>et al.</i> 1988a, Robbana-Barnat <i>et al.</i> 1997
Mouse A-31-1-13 BALB/c-3T3 cells	morphological transformation	negative	Matthews <i>et al.</i> 1993



Test System	End point	Results	References
<b>Mammalian <i>in vivo</i> systems</b>			
Male Sprague-Dawley rats	micronuclei (bone marrow and hepatocytes)	negative	Martelli <i>et al.</i> 1991

<sup>a</sup> See section 5.2.1.4

## 5.2 Mammalian systems

### 5.2.1 In vitro assays

#### 5.2.1.1 Gene mutations at specific loci (aprt, hprt, ouabain) in rodent cells

A small but significant dose-related increase in the frequency of 6-thioguanine-resistant (6-TG<sup>r</sup>) clones was observed in Chinese hamster V79 cells after exposure to chloramphenicol at a concentration of 1 mM or 2 mM for one hour in the absence of an exogenous metabolic activation system. Exposure of co-cultures of V79 cells and rat hepatocytes to chloramphenicol for 20 hours did not increase the observed frequency of 6-TG<sup>r</sup> variants in the V79 cells (Martelli *et al.* 1991).

#### 5.2.1.2 Chromosomal aberrations in human peripheral blood lymphocytes

An increased frequency of chromosomal aberrations was observed in human peripheral blood lymphocytes exposed to chloramphenicol for 24 hours at concentrations ranging from 2.4 mg/mL to 4.8 mg/mL (Sbrana *et al.* 1991). In this study, lymphocytes exposed for two hours during either the G<sub>1</sub> or the G<sub>2</sub> phase did not develop chromosomal aberrations. Intrachromosomal vacuoles occurred in mouse bone marrow cells, but only a few structural aberrations were observed. The authors concluded that short exposures to high concentrations of chloramphenicol did not result in chromosome breaks, and that chromatid-type chromosomal aberrations were induced only after prolonged exposures. Cells that were exposed for extended periods prior to G<sub>2</sub> but were not exposed during G<sub>2</sub> recovered rapidly. Based on a marked decrease in aberrations in lymphocytes after a few hours' recovery, the authors suggested that processes occurring during G<sub>2</sub>, rather than DNA replication, were the targets for chloramphenicol action, and that in order to induce chromosomal aberrations, exposure must continue until the cells enter mitosis (Sbrana *et al.* 1991).

#### 5.2.1.3 Sister chromatid exchange in bovine, rodent, and human cells

Bovine lymphocytes exposed to chloramphenicol at concentrations of 5 to 40 µg/mL showed a small but statistically significant increase in SCEs. However, the highest SCE frequency was observed at the lowest dose. This peculiar response could not be explained by heterogeneity of lymphocyte populations, but may have been related to the inhibitory effects of chloramphenicol on cellular kinetics (Catalan *et al.* 1993).

Cultured human peripheral blood lymphocytes, mouse primary bone marrow cells, and V79 Chinese hamster cells were exposed to chloramphenicol at concentrations ranging from 2.4 to 4.8 mg/mL for 24 hours (one cell cycle) or 2.8 to 8 mg/mL for two hours (G<sub>1</sub> and G<sub>2</sub> phases). The frequencies of SCEs were only slightly increased in the human

lymphocytes and in V79 cells, in each case after exposure during the entire cell cycle (Sbrana *et al.* 1991). Based on these results, the authors suggested that inhibition of cell proliferation was not caused by direct or indirect interference of chloramphenicol with DNA replication; otherwise, a high frequency of SCEs would be expected.

#### 5.2.1.4 DNA damage and repair in rodent and human cells

Isildar *et al.* (1988a) examined DNA damage induced by chloramphenicol and four of its bacterial metabolites, aminochloramphenicol, *p*-nitrobenzaldehyde, *p*-nitrophenyl-2-amino-3-hydroxy-propanone·HCl, and 2-dichloroacetamido-3-hydroxypropio-*p*-nitrophenone (DHCAP). Although this study was included in the IARC review, the results for the metabolites were not reported. Human Raji cells (a lymphoblastoid cell line), peripheral blood lymphocytes, and bone marrow cells were exposed to the test substances at concentrations of  $2 \times 10^{-5}$  to  $8 \times 10^{-4}$  M for three hours. Viability of exposed cells was comparable to that of concurrent negative controls. Only one metabolite, DHCAP at  $10^{-4}$  M, induced DNA single-strand breaks, and it did so in all three cell types. Chloramphenicol and the other three metabolites did not induce DNA damage.

Martelli *et al.* (1991) reported a small but statistically significant increase in single-strand breaks in Chinese hamster V79 cells exposed to chloramphenicol at 4 mM for one hour and in rat hepatocytes exposed to chloramphenicol at 2 mM for 20 hours. The authors also reported a statistically significant slight increase in unscheduled DNA synthesis (repair) in rat hepatocytes exposed to chloramphenicol at 2 mM for 20 hours. An even greater increase in DNA repair was observed in human hepatocytes originating from two donors and exposed to chloramphenicol at 1 mM. Although statistically significant, the observed increase in DNA repair in rat hepatocytes did not meet the minimal biological criteria defining a frankly positive response. This also was the case for DNA repair observed in hepatocytes from a third human donor (Martelli *et al.* 1991). In a more recent study (Martelli 1997), unscheduled DNA synthesis was induced in human hepatocytes but not in rat hepatocytes after exposure to chloramphenicol for 20 hours.

Lafarge-Frayssinet *et al.* (1994) investigated the ability of chloramphenicol and its metabolites to induce DNA damage in human peripheral blood lymphocytes and Raji cells. Cells were exposed to the test substances at concentrations of  $10^{-5}$  to  $4 \times 10^{-3}$  M for three hours. Chloramphenicol and three of its metabolites (the glucuronide conjugate, an alcohol derivative, and the chloramphenicol base) did not induce DNA single-strand breaks in either cell type. Three other chloramphenicol metabolites (dehydrochloramphenicol, dehydrochloramphenicol base, and nitrosochloramphenicol) induced DNA single-strand breaks in both cell types at concentrations of  $10^{-4}$  M and above. Of these three, the nitroso metabolite was the most potent.

Similar results were reported by Robbana-Barnat *et al.* (1997) for human bone marrow cells and peripheral blood lymphocytes. Dose-related DNA single-strand breaks were induced in human bone marrow cells by the nitroso metabolite at concentrations of  $10^{-4}$  M or greater and by the dehydro metabolites at concentrations of  $2 \times 10^{-4}$  M or greater. The nitroso metabolite was the most cytotoxic. Chloramphenicol, the glucuronide

conjugate, an alcohol derivative, and the chloramphenicol base did not induce DNA single-strand breaks in bone marrow cells at concentrations as high as  $4 \times 10^{-3}$  M. Similar but more intense responses were observed in human peripheral blood lymphocytes exposed to chloramphenicol and its metabolites (Robbana-Barnat *et al.* 1997).

#### 5.2.1.5 Morphological transformation of mouse BALB/c-3T3 cells

Exposure of A-31-1-13 BALB/c-3T3 cells *in vitro* to chloramphenicol did not induce morphological transformation (Matthews *et al.* 1993).

#### 5.2.2 In vivo assays

No increases in the micronucleus frequencies in either hepatocytes or bone marrow cells were observed in male Sprague-Dawley rats given a single oral dose of chloramphenicol at 1,250 mg/kg b.w. (Martelli *et al.* 1991).

### 5.3 Summary

The genotoxicity data show predominantly negative results in bacterial systems and mixed results in mammalian systems. The most consistently positive results were observed for cytogenetic effects in mammalian cells, including DNA single-strand breaks and increases in the frequencies of SCEs and chromosomal aberrations. The nitroso metabolite of chloramphenicol was the most potent test substance *in vitro*. Overall, chloramphenicol appears to be a genotoxin.

## 6 Other Relevant Data

Chloramphenicol induces bone marrow injury in humans. The most common type is a dose-dependent reversible bone marrow suppression that most often affects the erythroid cells. This response has been linked to inhibition of mitochondrial protein synthesis and suppression of heme synthetase (Yunis *et al.* 1980). However, chloramphenicol treatment also has been associated with some rare but serious side effects. These include an irreversible idiosyncratic aplastic anemia and leukemia (IARC 1990, Turton *et al.* 1999). Although production of DNA-damaging metabolites, inhibition of mitochondrial protein synthesis, genetic traits, and biochemical and immune system defects have been suggested as factors in bone marrow toxicity, the exact mechanisms leading to aplastic anemia and leukemia are complex and not well understood (Malkin *et al.* 1990, Holt *et al.* 1997). The following sections discuss the pharmacokinetics of chloramphenicol and mechanisms related to bone marrow toxicity, including possible links between aplastic anemia and leukemia.

### 6.1 Absorption, distribution, metabolism, and excretion

IARC (1990) reviewed the pertinent literature regarding the absorption, distribution, metabolism, and excretion of chloramphenicol. In addition, several studies published after the IARC Monograph were reviewed and are included in this section.

Chloramphenicol is rapidly absorbed from the gastrointestinal tract in humans and animals, with peak values in plasma being reached within two to three hours of administration (Kauffman *et al.* 1981, Bartlett 1982, Mulhall *et al.* 1983, Cid *et al.* 1983). It is extensively distributed throughout the human body, regardless of its administration route, and has been found in the heart, lung, kidney, liver, spleen, pleural fluid, seminal fluid, ascitic fluid, and saliva (Gray 1955, Ambrose 1984). About 50% of chloramphenicol in the blood is bound to albumin (Gilman *et al.* 1980, cited in HSDB 1995). Chloramphenicol penetrates the brain-blood barrier, and its concentration in cerebrospinal fluid can reach about 60% of that in plasma (Friedman *et al.* 1979). The concentration attained in brain tissue equals or exceeds that in plasma. Chloramphenicol easily crosses the placenta and also is secreted in breast milk (Kramer *et al.* 1969, Havelka *et al.* 1968).

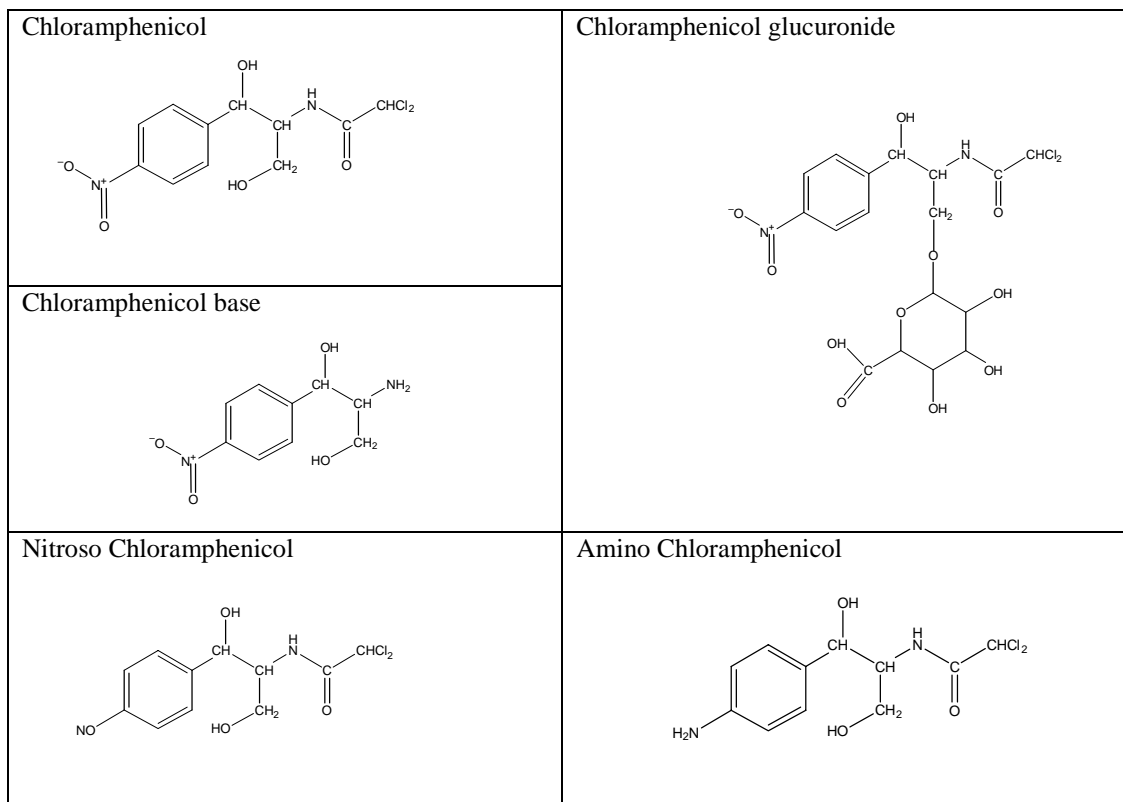
Chloramphenicol has a half-life ranging from 1.6 to 4.6 hours (longer in neonates), with an apparent volume of distribution ranging from 0.2 to 3.1 L/kg (Ambrose 1984, Rajchgot *et al.* 1983). The half-life was longer following oral than following intravenous administration (Butler *et al.* 1994). Patients with chloramphenicol-induced bone marrow depression experienced reduced clearance rates (Suhrland and Weisberger 1969).

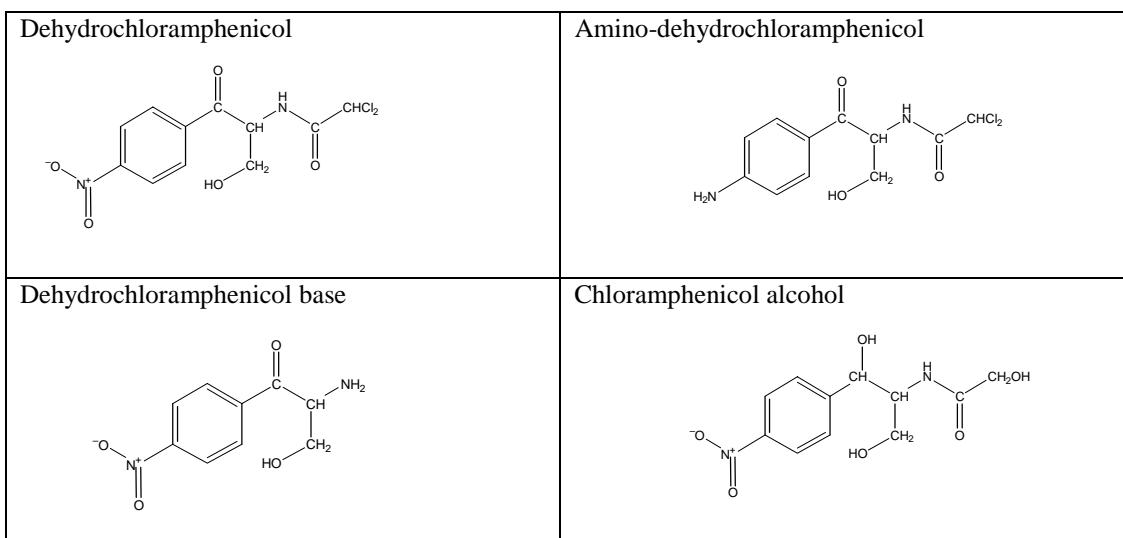
The primary metabolite of chloramphenicol is the glucuronide conjugate. Chloramphenicol arylamide is formed by intestinal bacterial reduction of the nitro group of chloramphenicol to an amine, which is acetylated and excreted in the urine (Meissner and Smith 1979). Human liver microsomes can reduce the nitro group of chloramphenicol (Salem *et al.* 1981). Oxamic acid, oxamylethanolamine, and aldehyde derivatives also have been identified as metabolites of chloramphenicol (Corpet and Bories 1987, Cravedi *et al.* 1995, Holt 1995, Holt *et al.* 1997). Chloramphenicol, its

glucuronide conjugate, chloramphenicol base, and the oxamic acid, alcohol, acetylarlyamine, and arylamine metabolites were found in the urine of rats administered  $^3\text{H}$ -chloramphenicol intramuscularly. The major metabolites were assumed to be chloramphenicol base (~26%) and the acetylarlyamine derivative (~20%) on the basis of recovered radioactivity (Bories *et al.* 1983). Similarly, chloramphenicol, its glucuronide conjugate, and the oxamic acid, acetylarlyamine, arylamine, and base derivatives were found in the urine of goats administered chloramphenicol intramuscularly (Bories *et al.* 1983).

Some of the chloramphenicol metabolites are more toxic than the parent compound and may be toxic to the bone marrow (Robbana-Barnat *et al.* 1997). For example, reactive nitroreduction intermediates have been associated with DNA damage (Isildar *et al.* 1988a, Robbana-Barnat *et al.* 1997). Dehydrochloramphenicol, a metabolite produced by intestinal bacteria, can undergo nitroreduction in the bone marrow. Individuals producing more of the toxic metabolites, or having a greater capacity for nitroreduction, could be predisposed to stem-cell damage ultimately resulting in aplastic anemia and/or leukemia (Isildar *et al.* 1988a,b). This possibility is discussed further in Section 6.2.3. The chemical structures of chloramphenicol and selected metabolites are provided in Figure 6-1.

**Figure 6-1. Chemical structures of chloramphenicol and some of the metabolites**





Source: Robbana-Barnat *et al.* 1997 and Isildar *et al.* 1988a

The primary route of chloramphenicol excretion is through the kidneys, with about 70% and 90% eliminated in the urine of experimental animals and humans, respectively (Glazko *et al.* 1949, Javed *et al.* 1984, Yunis 1988, Burke *et al.* 1980, Ambrose 1984). In humans, about 15% is eliminated as the parent compound and the remaining 75% in the form of metabolites (Yunis 1988, Burke *et al.* 1980, Ambrose 1984).

## 6.2 Toxicity

Chloramphenicol is a broad-spectrum antibiotic that binds to the 50S subunit of the 70S ribosome in bacteria and blocks peptidyl transfer (Smyth and Pallett 1988). Mammalian cells contain 80S ribosomes, which are not affected by chloramphenicol; however, mammalian mitochondria do contain 70S ribosomes. Therefore, chloramphenicol preferentially inhibits mitochondrial protein synthesis and cell replication in mammalian systems (Rosenkranz 1988, Mehta *et al.* 1989). Other factors potentially affecting the toxicity of chloramphenicol include the production of reactive oxygen species, the production of reactive metabolites, and genetic susceptibility.

### 6.2.1 Mitochondrial effects

In addition to their role in cellular respiration and energy production, mitochondria are involved in cell growth regulation, cell differentiation, and apoptosis. Therefore, impairment of mitochondrial function can stop or decrease cell proliferation and differentiation (Kaneko *et al.* 1988, Rochard *et al.* 2000). Leiter *et al.* (1999) exposed K562 human erythroleukemia cells to chloramphenicol at a concentration of 10  $\mu\text{g}/\text{mL}$  for four days and reported marked decreases in cell surface transferrin receptor expression, *de novo* ferritin synthesis, cytochrome *c* oxidase activity, ATP levels, respiratory activity, and cell growth due to mitochondrial dysfunction.

Transcription and translation of mitochondrial DNA into functional proteins is a key component of normal hematopoiesis. Chloramphenicol blocks mitochondrial protein synthesis in eukaryotes, resulting in hematological abnormalities similar to those associated with preleukemia or secondary myelodysplastic syndrome (sMDS). Secondary

MDS typically precedes the onset of acute myeloid leukemia by months or years. These hematological abnormalities include ineffective erythropoiesis, reticulocytopenia, and erythroblasts with abnormally structured mitochondria. Patients with the greatest risk of developing leukemia have a high proportion of bone marrow cells in the G<sub>0</sub> and G<sub>1</sub> phases of the cell cycle and a low labeling index (Hatfill *et al.* 1993). Chloramphenicol treatment is associated with a gradual increase in the percentage of cells in G<sub>1</sub> phase and a gradual decrease in the percentage of cells in S phase (Leiter *et al.* 1999). Hatfill *et al.* (1993) suggested that mitochondrial abnormalities could account for many of the clinical features associated with sMDS and that the pathogenesis of MDS is associated with adult acute leukemias. For example, mitochondrial DNA has been shown to be a target for benzene, a chemical that induces myeloid dysplasia in occupationally exposed workers and is a known leukemogen. Inhibition of mitochondrial protein synthesis leads to hypoplastic bone marrow injury, which may progress to aplastic anemia, sMDS, error-prone DNA repair, and ultimately, acute leukemia (Hatfill *et al.* 1993).

### 6.2.2 Reactive oxygen species and apoptosis

An aldehyde metabolite of chloramphenicol has been identified in human urine (Holt 1995, Holt *et al.* 1997). Oxidation of aldehydes by xanthine and aldehyde oxidases is known to generate free radicals. Holt *et al.* (1997) proposed a possible link between free radical production, apoptosis, bone marrow suppression, and aplastic anemia. Monkey kidney cells were exposed to chloramphenicol at concentrations ranging from 0.5 mM to 2.0 mM for 24 hours or 48 hours. In a separate experiment, human hematopoietic progenitor cells from neonatal cord blood were exposed to chloramphenicol at concentrations ranging from 0.005 mM to 1.0 mM for 3 to 14 days (Holt *et al.* 1997). Chloramphenicol induced apoptosis in both cell lines within 24 hours, and a free radical was thought to be the proximal toxicant. Concurrent treatment of the cell cultures with antioxidants decreased toxicity; however, it was not clear whether these effects would be observed *in vivo*.

Turton *et al.* (1999) observed increased apoptosis in bone marrow cells of female CD-1 mice administered chloramphenicol at 1,400 mg/kg b.w. per day for 10 days and in mice administered a single 2,200-mg/kg dose. However, apoptosis was not increased in bone marrow cells of male Wistar rats given a single 4,000-mg/kg dose. Bone marrow cells from patients with aplastic anemia had a higher proportion of apoptotic cells within the CD34<sup>+</sup> progenitor population than did cells from controls (Philpott *et al.* 1995). Although the mechanisms underlying increased apoptosis in aplastic anemia are unknown, these researchers speculated that a deficiency in local production of survival factors (e.g., granulocyte colony-stimulating factor), increased levels of interferon- $\gamma$  and tumor necrosis factor- $\alpha$ , or changes in the bone marrow microenvironment may be responsible. They concluded that apoptosis was an important factor contributing to the stem-cell deficiency of aplastic anemia. MDS also is considered to be hyperapoptotic disorder (Kliche and Hoffken, 1999).

### 6.2.3 Reactive metabolites and DNA damage

Jimenez *et al.* (1990) demonstrated that two chloramphenicol metabolites produced by intestinal bacteria, dehydrochloramphenicol and nitrophenylaminopropane, are

considerably more cytotoxic than the parent compound. These metabolites are stable enough to reach the bone marrow, where they may serve as substrates for nitroreduction. Although the nitroso metabolite produced by the liver is cytotoxic and damages DNA *in vitro*, it is not stable *in vivo* and degrades before reaching the bone marrow (Isildar *et al.* 1988a,b). Generation of nitroso intermediates within the bone marrow may interfere with the production of hematopoietic growth- and colony-stimulating factors and mediate chloramphenicol-induced bone marrow injury (Jimenez *et al.* 1990). In other studies, nitrosochloramphenicol, dehydrochloramphenicol, and the dehydrochloramphenicol bases have induced DNA single-strand breaks in human lymphocytes, bone marrow cells, and Raji cells. However, chloramphenicol, the glucuronide conjugate, the alcohol derivative, and the chloramphenicol base have not (Robbana-Barnat *et al.* 1997, Lafarge-Frayssinet *et al.* 1994, Isildar *et al.* 1988a,b). Nitroreduction of dehydrochloramphenicol by human and rabbit bone marrow cells and other tissues was reported by Isildar *et al.* (1988b). Holt and Bajoria (1999) demonstrated that aerobic and anaerobic nitroreduction of chloramphenicol by human fetal and neonatal liver produces the amine derivative. Therefore, nitroreduction of dehydrochloramphenicol or other toxic metabolites within the bone marrow could cause stem-cell damage leading to aplastic anemia and leukemia (Isildar *et al.* 1988a).

Further evidence that reactive nitroreduction metabolites of chloramphenicol are responsible for its carcinogenicity is provided by Kitamura *et al.* (1997), who assessed the carcinogenicity of thiamphenicol, a synthetic drug structurally similar to chloramphenicol. Thiamphenicol has a methylsulfonyl group instead of the *p*-nitro group present in chloramphenicol, and, although it induces reversible bone marrow suppression, it does not induce DNA damage or carcinogenicity.

#### 6.2.4 Genetic susceptibility and clonal disorders

Although aplastic anemia is not considered a genetic disease, there is evidence that genetic susceptibility may play a role in idiosyncratic disease. A few reports suggest a familial relationship, but not as many as would be expected if genetic factors were very important. Nevertheless, many researchers believe that individual susceptibility to idiosyncratic drug reactions has some genetic basis (Malkin *et al.* 1990).

Recent data indicate that some patients with aplastic anemia have a defect in the glycosyl-phosphatidylinositol molecule, due to alterations within the *PIG-A* gene, leading to an abnormal clonal hematopoiesis (Socié 1996, Socié *et al.* 2000). Although aplastic anemia has generally been viewed as a nonclonal disorder, clonal cytogenetic abnormalities have been reported in some patients. Furthermore, the *PIG-A* mutations recently described in aplastic anemia are identical to those in paroxysmal nocturnal hemoglobinuria (PNH), which is considered a clonal hemopathy (Hotta 1997).

Tooze *et al.* (1999) and Socié *et al.* (2000) reported an association between aplastic anemia and several clonal disorders, including PNH, myelodysplastic syndrome, and acute myelogenous (myeloid) leukemia. Clonality in aplastic anemia has been investigated. Cytogenetic analysis has found abnormal karyotypes in some patients with aplastic anemia, including monosomy 7, trisomy 6, and trisomy 8. Abnormal cytogenetic clones in patients with aplastic anemia may disappear following immunosuppressive



therapy. A monoclonal pattern has been detected in some female patients with aplastic anemia through the use of X-inactivation analysis; however, the majority of patients demonstrated polyclonal hemopoiesis (Tooze *et al.* 1999).

### 6.3 Aplastic anemia and leukemia

An *in vivo* model of chloramphenicol-induced aplastic anemia has not been developed (Holt *et al.* 1998). Drug-induced aplastic anemia is independent of dose, affects all three hematopoietic cell lines, may take months to develop, and persists even after use of the drug has stopped. Survivors of aplastic anemia generally suffer permanent bone marrow damage (Malkin *et al.* 1990). Da (1988) described eight pathogenic categories in 30 patients with aplastic anemia. Chloramphenicol was identified as a likely etiologic agent in nine of the 30 patients. Of these nine, all had low growth of granulocyte-macrophage colony-forming units, and six had no growth of fibroblast colony-forming units in marrow cells. These results suggest that the hemopoietic stem cells and the hemopoietic microenvironment were affected by chloramphenicol exposure.

The nitroso chloramphenicol metabolite may be the mediator of chloramphenicol-induced aplastic anemia. Yunis *et al.* (1980) compared the effects of chloramphenicol and its nitroso derivative on DNA synthesis, CFU-culture (CFU-c) growth, and cell viability in human bone marrow. Both compounds inhibited DNA synthesis and CFU-c growth. Higher concentrations were required for chloramphenicol, and the effects were mainly reversible, whereas the effects induced by lower concentrations of the nitroso derivative were irreversible, implying that the nitroso compound may be important in aplastic anemia. However, the nitroso derivative is an intermediate of chloramphenicol nitroreduction and is unstable; therefore, it is not clear whether these effects would occur *in vivo*. The end product of nitroreduction, chloramphenicol amine, has been detected in children treated with chloramphenicol (Holt and Bajoria 1999). These researchers have proposed that bone-marrow damage may be related to the nitroreduction of chloramphenicol and the subsequent production of nitric oxide. Individuals with a greater capacity for nitroreduction may be predisposed to chloramphenicol-induced aplastic anemia, which would explain its rare occurrence (Holt and Bajoria 1999).

A possible link between aplastic anemia and leukemia has been suggested from case reports and other clinical evidence (Schmitt-Gräff 1981, de Planque *et al.* 1988, Orlandi *et al.* 1988, IARC 1990, Sbrana *et al.* 1991, Socié 1996); however, the relationship of chemically induced bone marrow suppression, aplastic anemia, and leukemia is not clear (Adamson and Seiber 1981). Some chemicals that are potent bone marrow depressants (e.g., methotrexate and *Vinca* alkaloids) have not been associated with leukemia (Adamson and Seiber 1981). Conversely, there are case reports of leukemia occurring after chloramphenicol therapy without aplastic anemia (IARC 1990). There have been no case reports of patients developing both dose-dependent bone marrow suppression and aplastic anemia (Malkin *et al.* 1990).

Recent clinical data suggest that with improved treatment and increased survival of patients with aplastic anemia, about 5% to 15% of long-term survivors will develop myelodysplastic syndrome and acute myeloid leukemia (see Section 3). Several reports suggest that aplastic anemia could be considered as a preleukemic syndrome (Auerbach

and Allen 1990, Socié 1996, Tooze *et al.* 1999). Aplastic anemia patients with clonal cytogenetic abnormalities of bone marrow cells have an increased risk of developing clonal disorders, including leukemia (Auerbach and Allen 1990). The increased risk may be related to a persistent defect in hemopoietic stem cells observed in patients recovering from aplastic anemia. Tooze *et al.* (1999) suggest that the later evolution of clonal disorders could be a manifestation of the natural history of aplastic anemia, which may or may not be enhanced by immunosuppressive therapy. Data reviewed by Socié *et al.* (2000) and human studies described in Section 3 support this conclusion. One study reported that about 10% of aplastic anemia patients who were not treated with immunosuppressive therapy or bone marrow transplants later developed paroxysmal nocturnal hemoglobinuria (Socié *et al.* 2000).

#### **6.4 Summary**

Regardless of its route of administration to humans, chloramphenicol is readily absorbed and extensively distributed. The major metabolite is the chloramphenicol glucuronide conjugate; however, several studies indicate that the dehydrochloramphenicol metabolite produced by intestinal bacteria may be responsible for DNA damage and carcinogenicity. This metabolite can undergo nitroreduction in the bone marrow, where it may cause DNA single-strand breaks and stem-cell damage leading to aplastic anemia and leukemia. Chloramphenicol inhibits mitochondrial protein synthesis, a process essential for normal hematopoiesis. Mitochondrial abnormalities induced by chloramphenicol are similar to those observed in preleukemia, suggesting that mitochondrial DNA is involved in the pathogenesis of secondary leukemia.

An association between aplastic anemia and subsequent leukemia has been investigated. Persistent stem-cell defects occur in long-term survivors of aplastic anemia, which may predispose these patients to later clonal disorders. Clinical evidence suggests that 5% to 15% of long-term survivors of aplastic anemia will develop clonal disorders, including leukemia. Clonal cytogenetic abnormalities and accumulation of mutational events may lead to progression of the mutated clone and subsequent development of MDS and leukemia.



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