Chloramphenical

Safety Data Sheet

Division of Occupational Health and Safety
National Institutes of Health

WARNING!

This compound is toxic in animals and man.

For skin and eye exposure, wash with water.

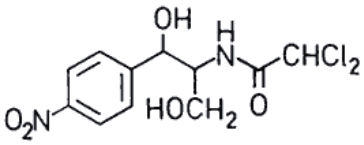
A. Background

Chloramphenicol (chloromycetin) is a white to pale yellow crystalline solid with a bitter taste, moderately toxic to animals and man, particularly to the newborn. Its chief target organ is the blood-forming system. There is no convincing evidence as to its carcinogenicity but it produces teratogenic effects in animals. It is an antibiotic produced by Streptomyces venezuelae, and is used in the treatment of Salmonella and other bacterial infections.

B. Chemical and Physical Data

1. Chemical Abstract No.: 56-75-7
2. Synonyms: Acetamide, 2,2-dichloro-N-[-hydroxy- (hydroxymethyl) p-nitrohenethyl]-D-threo-(-)-;[A] acetamide, 2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-[R-(R*, R*)]-;[B] D-(-)-threo-2-dichloroacetamide-1-p-nitrophenyl-1,3-propanediol; D-threo-N-(1,1'-dihydroxy-1-p-nitrophenyl isopropyl)dichloroacetamide. The most commonly used trade names are Chloramphenicol and Chloromycetin; for a variety of other trade names see the Merck Index (Windholz, 1976).
3. Chemical formula, weight and structure:

$C_{11}H_{12}Cl_2N_2O_5$; 323.1



4. Density: No data.
5. Optical rotation: $[\alpha]^{25}_{D}$= +19.0º in ethanol, -25.5º in ethyl acetate (Rebstock *et al.*, 1949).

[A] Chemical Abstracts name through 8th Index for vols. 66-75.

[B] Chemical Abstracts name in 9th Index for vols. 76-85.

6. Absorption spectroscopy: The ultraviolet spectrum shows a maximum at 278 nm (log ε= 3.99) and a minimum at 240 nm. Infrared, NMR, and mass spectra have been published (Szulczewski and Eng, 1975).
7. Volatility: no data; may be considered to be very low.
8. Solubility: at 25ºC, in mg/ml, the solubility is: water, 2.5, propylene glycol, 150.8; very soluble in methanol, ethanol, ethyl acetate and acetone, fairly soluble in ether, insoluble in benzene and petroleum ether. Water solubility is increased in presence of borax with which chloramphenicol complexes (Szulczewski and Eng, 1975).
9. Description: white to grayish-white or slightly yellow needles or plates with a bitter taste.
10. Boiling Point: no data; melting point: 150.5-151.5ºC
11. Stability: Solid chloramphenicol is stable at room temperature in diffuse light and its aqueous solutions at 5ºC for at least 5 years. At 25ºC, aqueous solutions retain their activity between pH 4.0 and 9.5 for at least 24 hrs (Ehrlich, 1978); there is a loss of 4.8% in 24 days at 20ºC (Moll and Wertz, 1972). The mechanism of hydrolysis depends on pH; below pH 7 the only significant pathway is amide hydrolysis whereas at alkaline pH, dechlorination is the major mechanism. This has been studied in detail (Higuchi and Bias, 1953; Higuchi *et al.*, 1954; Higuchi and Marcus, 1954). Addition of borate buffer to aqueous solutions increases stability (Szulczewski and Eng, 1975). At high temperatures, chloramphenicol decomposes in ultraviolet light, yielding HCl and dichloroacetic acid.
12. Chemical reactivity: The nitro group is readily reduced to the amino group by a number of reducing agents. Rapidly oxidized by alkaline permanganate and other oxidants to p-nitrobenzaldehyde, formic acid, formaldehyde, and ammonia (Moll and Wertz, 1972).
13. Flash point: no data.
14. Autoignition temperature: no data.
15. Explosive limits in air: no data.

*Note: Chlormaphenicol particles from suspensions, and perhaps also from solution, have a tendency to adhere to surfaces of glass containers. This adhesion is increased by neutral and cationic surfactants (*e.g.*, Tweens, benzethonium chloride) and decreased by anionic surfactants (*e.g.*, sodium lauryl sulfate, sodium carboxymethylcellulose) (Uno and Tanaka, 1971).

C. Fire, Explosion, and Reactivity Hazard Data

1. Chloramphenicol does not require special fire-fighting procedures or equipment and does not present unusual fire and explosive hazards.
2. No conditions contributing to instability are known to exist.
3. Hazardous decomposition products under fire conditions, particularly in presence of ultraviolet light, are the irritants dichloroacetic and hydrochloric acids.
4. Chloramphenicol does not require non-spark equipment.

D. Operational Procedures

1. Laboratory operations should be carried out in a fume hood or glove box. Personnel should wear laboratory coats and surgical gloves which should be changed after suspected contamination.
2. Chemical inactivation: no procedures have been specifically validated, but strong oxidants such as alkaline potassium permanganate decompose chloramphenicol rapidly.

3. Decontamination: Wash surfaces and glassware with ethanol or acetone, followed by water. The initial rinses should be collected and treated as liquid toxic waste. (These operations should be carried out in the hood, by operators wearing rubber gloves.) Glassware should then be treated with bichromate cleaning solution followed by the usual washing procedures. Avoid the use of cationic surfactants (Tweens, benzethonium chloride) which promote adhesion of chloramphenicol to glass surfaces. Animal cages should be decontaminated in a similar fashion. Animal carcasses, feces, and litter should be treated as solid toxic waste.
4. Disposal: Aqueous and organic waste solutions may be incinerated. (While there are no specific data for chloramphenicol, temperatures of 1000º or above, with retention time of 2 sec, are satisfactory for most organic compounds.) Waste solids, including animal bedding and carcasses, should be collected in disposal containers and incinerated.
5. Storage: Store in amber bottles, at room temperature. Aqueous solutions may be stored in the refrigerator for long periods of time (no decomposition noted in 5 years). Avoid prolonged exposure to ultraviolet or daylight.

E. Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: no specific methods have been described.
2. Analysis: Microbiological assays (*e.g.*, Louie *et al.*, 1976) have high precision but low specificity (positive response by many other antibiotics) and are not generally used any more unless other interfering substances are known to be absent. Colorimetric methods are based on deproteinization of biological samples, reduction of the nitro group, diazotization, and coupling with Bratton-Marshall reagent [N-(1-naphthyl)ethylene diamine hydrochloride or sulfonate]. The color is read at 555-558 nm. Reducing agents used have been titaneous chloride (Glazko naphthyl)ethylene diamine hydrochloride or sulfonate]. The color is read at 555-558 nm. Reducing agents used have been titaneous chloride (Glazko *et al.*, 1949), stannous chloride (Bessman and Stevens, 1950), or sodium hydrosulfite (Levine and Fischbach, 1951). These methods methods are quite simple the sensitive but are also positive not only with unrelated aromatic nitro compounds (unlikely to be present) but also with biologically inactive derivatives and metabolites of chloramphenicol (succinate, glucuronide) unless these are eliminated by extraction with ethyl acetate, as well as other drugs and bilirubin. The preferred methods are radioenzymatic assay (acetylation with ^{14}C-acetyl coenzyme A in presence of a specific enzyme; linear calibration curve in region 0-100 µg/ml; specific for free and protein-bound unmetabolized chloramphenicol) (Lietman *et al.*, 1976; Robison *et al*., 1978) and gas or liquid chromatography. GC methods using either flame ionization (Least *et al*., 1977) or electron capture sensors (Resnick *et al*., 1966) after trimethylsylylation have sensitivity of 0.5 µg/ml. High pressure liquid chromatography has the advantage of not requiring derivatization and has lower limits of detection (5 ng/sample) (Thies and Fischer, 1978; Koup *et al.*, 1978; Crechiolo and Hill, 1979). Gas chromatography- mass spectrometry has been adapted to simultaneous determination of chloramphenicol and its metabolites (Nakagawa *et al.*, 1975). A comparative study of the radioenzymatic, GC and GC-MS methods has been published (Pickering *et al.*, 1979). Finally, a fluorimetric procedure (reduction of the nitro group and reaction with fluorescamine) has high sensitivity but has the same lack of specificity as the colorimetric procedures (Clarenburg and Rao, 1977).

F. Biological Effects (Animal and Human)

1. Absorption: Chloramphenicol is absorbed from the intestinal tract after oral administration, by parenteral injection, and through the lungs. Skin absorption from ointments and from aqueous solution has been demonstrated. It is also transmitted through the placenta.

2. Distribution: After oral or parenteral administration, chloramphenicol is rapidly distributed in body fluids (serum, cerebrospinal fluid, bile) within a few hours. Maximum blood concentration occurs within 2 hours, and disappears in 4-6 hours after intravenous injection and 12-16 hours after ingestion. Tissue distribution is primarily to liver, kidney, and brain, with some binding to tissue protein, mainly in the form of metabolites whose identity is species-dependent. Some distribution also occurs to the bone marrow and this is probably responsible for the toxic effects of chloramphenicol on the hematopoietic system.

3. Metabolism and Excretion: The metabolism of chloramphenicol is complicated. The main pathways are: hydrolysis, reduction, oxidative dehalogenation, and glucuronidation. The relative importance of each of these pathways is species-dependent as outlined below. The metabolic scheme, based on the results of Glazko (1966), Pohl and Krishna (1978), and Martin *et al.* (1980), is depicted below.

   All end products have been identified as metabolites and/or excretion products in one animal species or another, or in in vitro systems. In man, urinary excretion products are mainly the glucoronide and small amounts of free chloramphenicol, and excretion is essentially complete within 24 hours after administration. In the dog and guinea pig large amounts of metabolites are excreted in the bile which in the latter species are reduced to arylamines. Subcutaneous injection in the rat results in excretion into the intestinal tract via bile as inactive nitro compounds which are partially reabsorbed and partially reduced to arylamines which are excreted in the feces.

4. Toxic Effects: The acute LD50 in the adult mouse and rat by oral or subcutaneous administration is in the range of 2,600-5,500 mg/kg, depending on whether the drug is administered in solution or suspension; intravenous LD50 in the mouse for a suspension is 200 mg/kg. It is noteworthy that chloramphenicol is far more toxic in these species to newborn animals (rat and mouse, subcutaneous, 602 and 740 mg/kg respectively; rat oral 693 mg/kg) (Goldenthal, 1971). This parallels the experience that chloramphenicol is more toxic in infants than in adult humans (Weiss *et al.*, 1960). The primary target of toxic activity is the bone marrow, resulting in general cytopenia which leads to decreased hematocrit and platelet count and increase in serum iron. These effects are readily reversed when chloramphenicol administration is discontinued. In epidemiological studies it is found that a small but statistically significant number of patients develop fatal aplastic anemia. The high toxicity of chloramphenicol among infants is ascribed to a much lower rate of conversion to the inactive glucuronide and inadequate renal excretion as compared with adults. The active metabolite, which is bound to bone marrow protein, is believed to be a product of oxidative dehalogenation (see metabolism, above) in the liver.

5. Carcinogenic Effects: There is no evidence for any carcinogenic effects of chloramphenicol in animals or man. In a review of 124 cases of bone marrow depression due to chloramphenicol treatment there were 3 cases of leukemia but only one of these was considered to be possibly causal (Fraumeni, 1967).

6. Mutagenic and Teratogenic Effects: Embryotoxic effects (resorption, inhibition of fetal growth) have been noted in rats, mice and rabbits by some authors but not by others. Teratogenicity has been found in rats.

G. Emergency Treatment

1. In case of skin exposure, remove contaminated clothing and wash with water. (Avoid organic solvents which increase skin penetration.) For eye exposure, irrigate with water.

2. If ingested, drink plenty of milk or water.

3. If inhaled, remove promptly to clean air.

4. Medical surveillance: Pre-employment examination should exclude personnel with liver or kidney impairments (which would lead to decreased inactive glucuronide formation or decreased urinary

clearance, respectively), and dyscrasis of the hematopoietic system. Periodic surveillance during employment should include blood counts, hematocrit, and checks for elevation of serum iron and increased saturation of iron-binding serum globulin. (Yunis and Bloomberg, 1964; Weisberger, 1969).

5. Acute signs and symptoms: in patients undergoing chloramphenicol treatment, nausea, vomiting, unpleasant taste, diarrhea, and perineal irritation have been noted.

H. References

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