Health Council of the Netherlands

Chloramphenicol

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Chloramphenicol*
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Geachte staatssecretaris,

Graag bied ik u hierbij het advies aan over de effecten van *Chlooramfenicol* op de vruchtbaarheid en het nageslacht; het betreft ook effecten op de lactatie en via de moedermelk op de zuigeling.

Dit advies maakt deel uit van een uitgebreide reeks waarin voor de voortplanting giftige stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste commissie van de Gezondheidsraad, de Subcommissie Classificatie Reproductietoxische stoffen. Het is vervolgens getoetst door de Beraadsgroep Gezondheid en omgeving van de raad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

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Evaluation of the effects on reproduction, recommendation for classification

Subcommittee on the Classification of Reproduction Toxic Compounds
A Committee of the Health Council of the Netherlands

to:

the State Secretary of Social Affairs and Employment

No. 2012/18, The Hague, October 30, 2012

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Infrastructure & the Environment, Social Affairs & Employment, Economic Affairs, Agriculture & Innovation, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

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Samenvatting

In het voorliggende advies heeft de Gezondheidsraad chlooramfenicol onder de loep genomen. Chlooramfenicol is een breedspectrum antibioticum, dat in Nederland wordt gebruikt voor de behandeling van ooginfecties in mens en huisdier. Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Subcommissie Classificatie reproductietoxische stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de raad, hierna aangeduid als de commissie, kijkt zowel naar effecten op de vruchtbaarheid van mannen en vrouwen als naar effecten op de ontwikkeling van het nageslacht. Daarnaast worden effecten op de lactatie en via de moedermelk op de zuigeling beoordeeld.

Op basis van Verordening (EG) 1272/2008 van de Europese Unie doet de commissie een voorstel voor classificatie. Voor chlooramfenicol komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie om chlooramfenicol niet te classificeren wegens onvoldoende geschikte gegevens
- voor effecten op de ontwikkeling adviseert de commissie chlooramfenicol in categorie 1B te classificeren (*stoffen waarvan verondersteld wordt dat zij*

toxisch zijn voor de menselijke voortplanting) en met H360D (*kan het ongeboren kind schaden*) te kenmerken

- voor effecten op en via lactatie adviseert de commissie om chlooramfenicol niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report, the Health Council of the Netherlands reviewed chloramphenicol. Chloramphenicol is a broad-spectrum antibiotic that in the Netherlands is used for the treatment of eye infections in humans and dogs and cats. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at the request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. The Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee, evaluates the effects on male and female fertility and on the development of the progeny. Furthermore, the Committee considers the effects of a substance on lactation and on the progeny via lactation.

The Committee recommends classification according to Regulation (EC) 1272/2008 of the European Union. For chloramphenicol, these recommendations are:

- for effects on fertility, the Committee recommends not classifying chloramphenicol
- for effects on development, the Committee recommends classifying chloramphenicol in category 1B (*presumed human reproductive toxicant*) and labelling with H360D (*may damage the unborn child*)
- for effects on or via lactation, the Committee recommends not labelling chloramphenicol due to a lack of appropriate data.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. This classification is performed by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). The classification is performed according to European Union Regulation (EC) 1272/ 2008 on classification, labelling and packaging (CLP) of substances and mixtures. The CLP guideline is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The subcommittee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as reproductive toxicant (category 1A and 1B and 2) or compounds with effects on or via lactation.

1.2 Committee and procedure

This document contains the classification of chloramphenicol by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances, hereafter called the Committee. The members of the Committee are

listed in Annex A. The submission letter (in English) to the State Secretary can be found in Annex B.

In 2012, the President of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft report are listed in Annex C. The Committee has taken these comments into account in deciding on the final version of the report.

The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development as well as lactation of the above mentioned compound.

Classification for reproduction (fertility (F) and development (D)):	
Category 1	Known or presumed human reproductive toxicant (H360(F/D))
Category 1A	Known human reproductive toxicant
Category 1B	Presumed human reproductive toxicant
Category 2	Suspected human reproductive toxicant (H361(f/d))
No classification for effects on fertility or development	
Classification for lactation:	
	Effects on or via lactation (H362)
	No labelling for lactation

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC)1272/2008) presented in Annex D. The classification of compounds is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations (see Annex E).

1.3 Labelling for lactation

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The guideline defines that substances which are absorbed by women and have been shown to interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely

independent of dosage), the labelling for effects on or via lactation is based on risk characterization and therefore, it also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects on or via lactation when it is likely that the substance would be present in breast milk at potentially toxic levels. The Committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration leads to exceeding the exposure limit for the general population, e.g. the acceptable daily intake (ADI).

1.4 Data

Literature searches were conducted in the on-line databases XTOXLINE, MEDLINE and CAPLUS, up to April 2011 without a starting date. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited.

The Committee describes both human and animal studies in the text. The animal data are described in more detail in Annex F as well. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

1.5 Presentation of conclusions

The classification is given with key effects, species and references specified. In case a substance is not classified as toxic to reproduction, one of two reasons is given:

- lack of appropriate data precludes assessment of the compound for reproductive toxicity
- sufficient data show that no classification for toxic to reproduction is indicated.

1.6 Final remark

The classification of compounds is based on hazard evaluation only (Niesink et al., 1995[23]), which is one of a series of elements guiding the risk evaluation process. The Committee emphasizes that for derivation of health-based

occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment, and recommendations of other organizations.

Chapter 2

Chloramphenicol

2.1 Introduction

Chloramphenicol (also known as chloramphenical) is a broad-spectrum antibiotic which inhibits protein synthesis in bacteria by binding reversibly to the 50S ribosomal subunit at the peptidyltransferase site and inhibits the transpeptidation reaction and mitochondrial protein synthesis in mammalian cells.[19,20] It is historically used veterinarily in all major food-producing animals and currently in humans and companion animals. Chloramphenicol has been reviewed several times by the Joint FAO/WHO Expert Committee on Food Additive (JECFA)[19], the International Agency for Research on Cancer (IARC)[18], and the European Committee for Veterinary Medicinal Products (ECVMP).[6] Concerns have been expressed about the genotoxicity of chloramphenicol and its metabolites, its embryo- and foetotoxicity, its carcinogenic potential in humans, and the lack of a dose-response relationship for aplastic anaemia caused by treatment with chloramphenicol in humans. Due to deficiencies in data on carcinogenicity and reproduction toxicity, an acceptable daily intake has never been allocated and consequently, no maximum residue limit has been assigned. In the EU, chloramphenicol is therefore listed among substances prohibited to be administered to food-producing animals.[11] In the Netherlands, chloramphenicol is only registered for the treatment of eye infections in humans and dogs and cats.[5] IARC has classified chloramphenicol as probably carcinogenic to humans

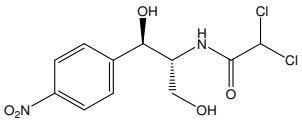
(group 2A)[18], while in the EU chloramphenicol is not classified at all (http://esis.jrc.ec.europa.eu).

Absorption of chloramphenicol results in peak concentrations of 10-20 µg/mL within two to three hours after oral administration of 15 mg/kg bw.[18,20] In infants and neonates, serum (peak) concentrations of 20-24 µg/mL and 14 µg/mL were observed after oral doses of 40 mg/kg bw to neonates and 26 mg/kg bw to infants, respectively.[18] Chloramphenicol is extensively distributed in humans, regardless the route of administration. It penetrates the blood-brain barrier. Concentrations in cerebrospinal fluid can reach approximately 60% of those in plasma. Chloramphenicol may accumulate in the brain and may be present in bile, breast milk and placental fluid. About 50% is bound to plasma proteins. The major route of elimination is hepatic metabolism to the glucuronide.[20] Excretion is primarily via the urine; 15% of the dose as parent compound and the remainder as metabolites, including conjugates.[18] The half-life of chloramphenicol is 1.6-4.6 h in adults, but considerably longer in neonates:
10->48 h in one- to eight-day-old infants and 5-16 h in 11-day- to eight-week-old infants[18], due to limited glucuronyl transferase activity as well as limited renal excretion of unconjugated chloramphenicol.[20]

The immature liver and kidney functions of the newborn may lead to such high plasma levels of chloramphenicol that the so-called ‘gray baby syndrome’, a serious, sometimes fatal, side effect may develop. This may occur at chloramphenicol doses resulting in plasma levels >75 mg/L. In order to prevent such levels, maximum doses no larger than 25 mg/kg bw/day were recommended for children younger than two weeks of age.[20]

The identity and some physicochemical properties of chloramphenicol are presented below.

chemical name	:	chloramphenicol
CAS name	:	acetamide, 2,2-dichloro-N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-
CAS number	:	56-75-7
EC/EINECS number:		200-287-4
synonyms	:	D-threo-2,2-dichloro-N-(β-hydroxy-α-hydroxymethyl-p-nitrophenethyl)acetamide; 2,2-dichloro-N-[(αR,βR)-β-hydroxy-α-hydroxymethyl-4-nitrophenethyl]acetamide; D-threo-2-dichloroacetamido-1-para-nitrophenyl-1,3-propanediol; D-threo-N-dichloroacetyl-1-p-nitrophenyl-2-amino-1,3-propanediol; D-threo-N-(1,1'-dihydroxy-1-p-nitrophenylisopropyl)dichloro-acetamide; D-threo-p-nitrophenyl-1-dichloroacetamido-2-propanediol-(1,3); acetamide, 2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-[R-(R*,R*)]-

colour and physical state	:	white to greyish-white or yellowish-white fine crystalline powder or fine crystals, needles or elongated plates
molecular weight	:	323.14
molecular formula	:	$C_{11}H_{12}C_{l2}N_2O_5$
structure	:	
melting point	:	149-153 °C (sublimes in high vacuum)
optical rotation	:	[α]27D = +18.6° (4.86% in ethanol)
vapour pressure	:	2.31 x 10^{-10} Pa at 25 °C (estimated)
Log $P_{(octanol\text{-}water)}$	:	1.14 (experimental)
solubility	:	2.5 g/L in water at 25 °C; aqueous solutions are neutral; 151 g/L in propylene glycol at 25 °C; very soluble in methanol, ethanol, butanol, ethyl acetate, acetone; fairly soluble in diethyl ether

Data from [4,18,31]

2.2 Human studies

Fertility studies

No studies are available regarding the effects on human fertility.

Developmental toxicity studies

No adverse effects were reported in the children of 22 patients treated with chloramphenicol at various stages of pregnancy.[18]

Czeizel et al. reported a population-based case-control study investigating the teratogenic potential of oral chloramphenicol treatment during pregnancy using the Hungarian Case-Control Surveillance of Congenital Abnormalities from 1980-1996. Of 38,151 pregnant women who had babies without any defects (control group), 51 (0.13%) had been treated with chloramphenicol, while of 22,865 pregnant women who had newborn infants or foetuses with congenital abnormalities, 52 (0.23%) had been treated. Exposure data were derived from maternal self-reported data and from medical documents. For self-reported treatment during the second-third months of gestation (critical period for major congenital abnormalities), only the group of undescended testes showed an increased risk (adjusted OR= 5.9; 95% CI: 1.2-28.7). The risk of cardiovascular congenital abnormalities was increased when comparing treatment during the

entire pregnancy period (adjusted OR=2.1; 95% CI: 1.2-4.0). No risks were found when using only the medically documented treatments.[7]

The Committee notes that there was insufficient information on the extent of exposure information from medical documents and on the over-the-counter availability of chloramphenicol to draw firm conclusions.

Lactation

No studies are available regarding the effects of chloramphenicol on human lactation.

Plomp et al. studied the excretion of chloramphenicol into breast milk in normal puerpera after single and repeated (three times/day, two days) oral administration of 500 mg (approximately 8.5 mg/kg bw/day) of chloramphenicol. After administration of a single dose to four subjects, a peak level of 2.9 µg/mL was reached in breast milk after 1.4 hours. After repeated administration to five subjects, levels in breast milk amounted to 1.7 and 1.6 µg/mL at 24 and 48 hours after the first dose, respectively. From the excretion kinetic data, Plomp et al. calculated a maximum 24-hour excretion into breast milk of approximately 14 mg after single oral administration of 500 mg to the lactating mother.[29]

Havelka et al. reported average minimum and maximum daily milk concentrations of 0.5 and 2.8 µg/mL, respectively, in five subjects after oral administration of doses of 250 mg chloramphenicol (approximately 3-4 mg/kg bw), four times/day, for seven to ten days. Similar administration of doses of 500 mg (approximately 5-8 mg/kg bw) to five subjects resulted in minimum and maximum daily levels of 1.8 and 6.1 µg/mL, respectively.[17]

Vorherr presented breast milk levels of 15-25 µg/mL and stated that the percentage of administered dose in breast milk is 1.3% (no more details given).[32]

2.3 Animal studies

Fertility and developmental toxicity studies in laboratory animals are summarized in Annex F.

Fertility studies

Male and female rats were given 0 or 34 mg/kg bw chloramphenicol intramuscularly during 20 days. Thereafter, rats were mated for 20 days: treated males with treated females, treated males with untreated females, and untreated males with treated females. Females were observed for 30 days and sacrificed. Treated females did not become pregnant and in the group where only males were treated, 6/20 females became pregnant compared to 17/20 in the control group. Morphological investigation of the gonads of 15 treated females and 15 treated males showed cystic degeneration of the Graafian follicles in ovaries in 6/15 females. The oestrus cycle was affected in 14/15 females. No morphological changes were observed in male gonads and uterine mucosa (paper in Polish with summary in English).[25]

Beermann and Hansmann investigated the role of mitochondria in follicular development, oocyte maturation and chromosomal segregation during the first meiotic division. Female NMRI/Han mice were induced for superovulation with pregnant mare serum followed 48 hours later by human chorionic gonadotrophin (HCG) intraperitoneally. Females received an additional intraperitoneal injection of 37.5 mg/kg bw chloramphenicol at 0, 15 or 48 hours after pregnant mare serum injection or 18.8 mg/kg bw chloramphenicol at 0 hour. Controls were injected with saline at the same time points. Females were sacrificed 15-16 hours after HCG injection. Although all females showed follicular maturation and ovulation, chloramphenicol reduced the ovarian weight relative to body weight and the number of ovulated oocytes in females treated with 37.5 mg/kg bw chloramphenicol. The progesterone concentration in the postovulatory ovary was markedly reduced in all treated females. The number of diploid oocytes was increased compared to control at 18.8 mg/kg bw and at 37.5 mg/kg bw at 15 and 48 hours.[2]

Oyeyemi and Adeniji found statistically significant decreases in sperm motility, percentage viability, number of normal spermatozoa and sperm concentration in Wistar rats given daily oral doses of 25 mg/kg bw for 20 and 25 days. Data on general toxicity were not presented.[28]

Oral administration of doses of 28 mg/kg bw four times a day for ten consecutive days to Wistar rats caused statistically significant decreases in sperm motility, percentage viability and sperm count. The percentage of morphologically abnormal sperm was not different from controls. At the end of the experiment,

the treated animals had lost body weight by 7% compared to a body weight gain of 4% in controls. There was no effect on absolute and relative testis weights.[27]

In a dominant lethal assay, male Swiss CD-1 mice (n=7-9/group) were treated with a single intraperitoneal injection of chloramphenicol of 333 or 666 mg/kg bw. A concurrent solvent control group (n=10) was included. Within two hours of injection, each male was paired with three untreated virgin females which were replaced weekly for eight consecutive weeks. Females were sacrificed 13 days from mid-week of their mating, and animals were scored for pregnancy and for numbers of total implants, as comprised by live implants, early foetal deaths and late foetal deaths. As late foetal deaths were extremely rare, total implants and early foetal deaths were the only implant parameters analysed. Chloramphenicol did not produce early foetal deaths and pre-implantation losses exceeding control limits.[9,10]

Developmental toxicity studies

Oral

Mackler et al. reported effects on foetal development in rats when 0, 2 or 3% chloramphenicol (200 or 300 mg/rat) was given in the diet during gestational days 0-20. Food intake was reduced at both dose levels but Mackler et al. did not report on maternal body weight or other effects. The number of resorptions was largely increased (5, 31 and 57%, respectively), foetal and placental weights were reduced as were the numbers of live foetuses at both dose levels. Similar effects were seen when a restricted diet (67% of control diet) was given, except that the number of resorptions was not increased; only the number of live foetuses was decreased. Oedema was found in foetuses of both dose levels and wavy ribs and fused ribs were found in foetuses at the highest dose level.[21]

Additionally, dams were sacrificed on gestational day 20 after treatment with 1.5% chloramphenicol in the diet for the first nine to 12 days of gestation or with 3% chloramphenicol for the first two to eight days of gestation. Increased numbers of dams with no implantations were observed following treatment during gestational days 0-6 and onwards; increased numbers of resorptions following treatment during gestational days 0-5, 0-8, 0-9, 0-10 and 0-11. Treatment during gestational days 0-7 and onwards caused decreased foetal weights.[21]

As part of the above-mentioned study, Mackler et al. tested the implication of electron transport and oxidative energy formation in rat embryos and foetuses

during the period of organogenesis influenced by chloramphenicol. They found that mitochondrial activities of DPNH (reduced diphosphopyridine nucleotide) oxidase, cytochrome c oxidase and ATPase were inhibited, while succinic indophenol dehydrogenase and succinic oxidase were not inhibited suggesting that inhibition of electron transport plays a role in chloramphenicol developmental toxicity.[21]

Fritz and Hess reported a prenatal developmental study in Sprague-Dawley rats, CD-1 mice and rabbits (mixed breed) and compared the embryotoxic and foetal parameters and macroscopic and skeletal abnormalities with the spontaneous rate of abnormalities recorded over a period of four years in untreated controls belonging to the same breed. Rats and mice were given chloramphenicol by gavage at doses of 500-2,000 mg/kg bw and rabbits at doses of 500 and 1,000 mg/kg bw for one or more days during gestation.

The rat dams showed no toxic signs. In the groups treated with daily doses of 500 mg/kg bw on gestational days 5-15, of 1,000 mg/kg bw on gestational days 7-12, or of 2,000 mg/kg bw on gestational days 6-8, 7-9, 9-11, 11-13, 15-17, or on day 8, 9 or 10, the percentages of embryonic or foetal deaths were statistically significantly, sometimes largely, increased ranging from 39 to 100% (controls: 23%). In the groups treated with 1,500 mg/kg bw/day on gestational days 0-6 or with 2,000 mg/kg bw/day on gestational day 15-17, or on day 5, 6 or 7, embryonic or foetal mortality was similar to that in controls. Anomalies observed included omphalocele or umbilical hernia in combination with costal fusion in 8/22 foetuses (1 litter) at 2,000 mg/kg bw/day on gestational day 6-8, 1/26 foetuses (1 litter) on gestational day 7-9, 2/84 foetuses (1 litter) on gestational day 7, 5/46 foetuses (1 litter) on gestational day 8, and 5/64 foetuses (3 litters) on gestational day 9. Two omphaloceles were seen in 6,326 control foetuses. Skeletal development was retarded at 1,000 and 2,000 mg/kg bw: missing ossification of phalangeal nuclei of forelegs and hind legs and of 5th sternebra at 1,000 mg/kg bw/day and 2,000 mg/kg bw/day on gestational day 11-13, a decreased number of ossified cervical vertebrae, and an increased incidence of fusion of sternebra 1+2 and bipartite vertebrae at 1,000 mg/kg bw/day.

Data on toxicity in the mouse dams were insufficiently documented. The number of resorptions (not further specified) was statistically significantly increased at 1,000 mg/kg bw/day administered on gestational days 6-12 (71% vs. 24% in controls). All embryos were resorbed at 2,000 mg/kg bw/day (administered on gestational days 8-10). At 500 mg/kg bw/day, administered on gestational days 5-15, the percentage of embryonic and foetal deaths was 31 (controls: 24%; $p<0.05$); foetal weight was statistically significantly decreased,

no malformations were observed. At 1,000 mg/kg bw/day, one foetus with malformations out of 81 foetuses (0.01%) was noted compared to 4/3,230 (0.001%) in the control group. Skeletal development was retarded as indicated by an increased incidence of missing ossification of phalangeal nuclei of forelegs (37.5 % vs. 9% in controls) and hind legs (45% vs. 18%) and of 5th sternebra (15% vs. 0%), and increased incidence of fusion of sternebrae 1+2 (7.5% vs. 0%).

In rabbits, no toxic signs were noted in the dams. The number of embryonal resorptions was statistically significantly increased at 1,000 mg/kg bw/day given on gestational day 6-9 or gestational day 8-11 (25 and 58%, respectively; controls: 10%). Administration of 500 mg/kg bw given on gestational days 6-15 did not affect the percentage of prenatal deaths (12% vs. 10% in controls:) or average foetal body weight. No increased incidence of malformations in the live foetuses was noted. Skeletal development was delayed: at 500 mg/kg bw/day as indicated by an increased incidence of missing ossification of phalangeal nuclei of forelegs (50% vs. 33% in controls) and at 1,000 mg/kg bw/day given on gestational day 6-9 by an increased incidence of phalangeal nuclei of fore- and hind legs (51.5 and 12%, respectively; controls: 33 and 5%, respectively) and given on gestational day 8-11 by an increased incidence of the 5th sternebra (33% vs. 26%).[13]

Al-Hachim & Al-Baker performed a prenatal developmental study in mice administering five to seven oral doses of 0, 25, 50, 100 or 200 mg/kg bw during the third stage of pregnancy and investigated behavioural parameters of the pups (conditioned avoidance response in 30-36-day-old, electroshock seizure threshold in 38-day-old, and open-field in 42-48-day-old pups). Conditioned avoidance response was statistically significantly decreased at all dose levels with a dose-response relation. Electroshock seizure threshold was not statistically significantly increased at 50-200 mg/kg bw/day with a dose-response relation. Open-field performance was statistically significantly decreased at all dose levels without a dose-response relation. The authors report that no gross congenital abnormalities were noted.[1]

Subcutaneous injection

Bertolini and Poggioli investigated the conditioned avoidance response in 60-day-old rats. Four groups of pregnant Wistar rats were treated as follows: in one group, 50 mg/kg bw chloramphenicol (hemisuccinate) was given subcutaneously on gestational days 7-21; in two other groups, 50 or 100 mg/kg

bw was injected subcutaneously into pups for the first three days after birth; the fourth group, receiving saline, served as controls. No effects on pregnancy, litter size, pup weight, postnatal weight gain, or incidence of gross malformations were seen; no mortality was observed. When 60-days old, animals were selected, trained, then examined for avoidance learning at days 5, 10, 15 and 20 from the start of the conditioning procedure. No difference in pain threshold was noted between the groups. Conditioned avoidance response was statistically significantly decreased in all treated groups, generally more marked in males than in females and in intrauterine treated than in postnatally treated rats.[3]

Neumann reported a study in rats given 800-2,500 mg/kg bw chloramphenicol sodium succinate subcutaneously on gestational days 6-10 or 11-14 compared to a control group. In the group treated on gestational days 6-10, all live foetuses showed retarded development, one foetus was malformed (clinodactyly), and mean foetal weight was markedly reduced. In the group treated on gestational days 11-14, an increased number of resorptions, reduced number of normal live foetuses, and increased number of retarded live foetuses (predominantly haemorrhages and oedemas) were observed compared to controls. Six foetuses were malformed (all at 1,500 mg/kg bw; five from 1 litter); five had cleft palate and one had cleft palate and anomalies of the limbs. The control group contained no retarded or malformed foetuses. Foetal weight was again markedly reduced compared to controls. The number of resorptions and reduced number of normal live foetuses were dose related.[22]

The Committee notes that the malformations were almost all found in one litter at a high subcutaneous dose.

Other studies

Chloramphenicol affected mitochondrial function or morphology following intravenous injection into pregnant rats[26], intraperitoneal injection into pregnant mice[24] or into newborn rats (0-two-hours and up to eight-days old)[16,15], or incubation in *in vitro* systems such as rat yolk sacs[12] and perfused, isolated hearts from one-four-day-old piglets[33].

Lactation

No studies were found regarding the effects of chloramphenicol on lactation in animals.

Following a single intravenous dose of chloramphenicol of 100 mg/kg bw to four goats, a maximum level of chloramphenicol of 15 mg/L was detected in milk one hour after administration (the first measurement point).[29]

Intramuscular administration of a single dose of 10 mg/kg bw to five cows resulted in maximum levels in milk between 0.5 and 1.3 mg/L, six and nine hours after injection. In a separate experiment, chloramphenicol was determined in both whole and skimmed milk after a single intramuscular dose of 10 mg/kg bw (n=5 cows). Average concentrations in skimmed milk were almost similar to those in whole milk amounting to 2.0 and 1.9 mg/L, three and six hours after injection, respectively (whole milk: 1.9 and 1.8 mg/L, respectively). After oral administration of 10 mg/kg bw, no chloramphenicol was detected in milk measured up to 24 hours after administration.[8,30]

2.4 Conclusion

No human studies on fertility effects of chloramphenicol were available.

No guideline studies were available regarding the effects of exposure to chloramphenicol on (functional) fertility in laboratory animals.

Oral administration of chloramphenicol to rats affected certain sperm characteristics.[27,28] In one of these studies, treatment caused decreased body weight.[27]

The Committee could not assess the relevance of effects on several stages on fertility observed in rats and mice[2,14,25] for workers occupationally exposed to chloramphenicol. In these studies, no information was reported on general toxicity which could have caused or contributed to these effects. Furthermore, administration was through routes less relevant to occupational exposure (i.e. intramuscular and intraperitoneal injections).

Overall, the Committee proposes not to classify chloramphenicol for effects on fertility due to a lack of appropriate human and animal data.

Both human and animal data were available to evaluate the developmental toxicity of chloramphenicol.

A population-based case-control study[7] investigating the teratogenic potential of oral chloramphenicol treatment during pregnancy suggested increased risks on undescendent testes and cardiovascular congenital abnormalities.

In laboratory animal experiments in which high, not maternally toxic, oral doses of chloramphenicol (500-2,000 mg/kg bw) were administered during gestational days 0-20 to rats [21], or during selected gestational day(s) to rats, mice

or rabbits[13], the main effect was a, sometimes large, increase in embryonic and/or foetal mortality while delayed development or malformations (in rats only) were seen in the survivors. Lower doses (25-200 mg/kg bw) given orally to mice during the third stage of gestation[1] or subcutaneously to rats during gestational days 7-21[3] affected neurobehavioural parameters in their offspring.

Following intravenous injection into pregnant rats[26], intraperitoneal injection into pregnant mice[24] or into newborn rats (0-two-hours and up to eight-days old)[16,15], or incubation in *in vitro* systems such as rat yolk sacs[12] and perfused isolated hearts from one-four-day-old piglets[33], affected mitochondrial function or morphology was observed.

Overall, the Committee concludes that the human data are not sufficient for classification. Based on the prenatal and postnatal effects found in laboratory animals, the Committee proposes to classify chloramphenicol for developmental effects in category 1B (*presumed human reproductive toxicant*).

There were no human or animal data on effects on or via lactation. There were no data on background concentrations of chloramphenicol in breast milk or on concentrations in breast milk in occupationally exposed women. Chloramphenicol was found in milk following oral administration to women[17,29,32] and intravenous and intramuscular administration to animals[8,30].

In the absence of data on the toxicity of chloramphenicol in breast milk, the Committee is not able to calculate a safe level for chloramphenicol in human breast milk. Therefore, the Committee proposes not labelling chloramphenicol for effects on or via lactation due to a lack of appropriate data

Proposed classification for fertility

Lack of appropriate data precludes the assessment of chloramphenicol for effects on fertility.

Proposed classification for developmental toxicity

Category 1B; H360D

Proposed labelling for effects on or via lactation

Lack of appropriate data precludes the assessment of chloramphenicol for labelling for effects on or via lactation.

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A	The Committee
B	The submission letter (in English)
C	Comments on the public draft
D	Regulation (EC) 1272/2008 of the European Community
E	Additional considerations to Regulation (EC) 1272/2008
F	Fertility and developmental toxicity studies

Annexes

Annex **A**

The Committee

- A.H. Piersma, *chairman*
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  Professor of Medical Genetics, Paediatrician (not practising), Clinical Geneticist, University Medical Centre, Utrecht
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- J.T.J. Stouten, *scientific secretary*
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The first draft of this report was prepared by Dr. H.M. Barentsen, from the Regulatory Affairs Department of NOTOX BV, Den Bosch, by contract with the Ministry of Social Affairs and Employment.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

The submission letter (in English)

Subject : Submission of the advisory report *Chloramphenicol*
Your reference : DGV/MBO/U-932342
Our reference : U 7401 /HS/fs/543-V12
Enclosed : 1
Date : October 30, 2012

Dear State Secretary,

I hereby submit the advisory report on the effects of *Chloramphenicol* on fertility and on the development of the progeny; it also concerns effects on lactation and on the progeny via lactation. This advisory report is part of an extensive series in which reproduction toxic substances are classified in accordance with European guidelines. This involves substances to which people may be exposed occupationally.

The advisory report was prepared by a permanent committee of the Health Council of the Netherlands, the Subcommittee on the Classification of Reproduction Toxic Compounds. The advisory report was consequently reviewed by the Health Council's Standing Committee on Health and the Environment.

Today I sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their information.

Yours sincerely,

(signed)
Prof. dr. W.A. van Gool,
President

Annex **C**

Comments on the public draft

- T.J. Lenz, Q. Ma. National Institute for Occupational Safety and Health, Cincinnati OH, USA.

Annex **D**

Regulation (EC) 1272/2008 of the European Community

3.7 Reproductive toxicity

3.7.1 Definitions and general considerations

3.7.1.1 Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. The definitions presented below are adapted from those agreed as working definitions in IPCS/EHC Document No 225, Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. For classification purposes, the known induction of genetically based heritable effects in the offspring is addressed in Germ Cell Mutagenicity (section 3.5), since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity.

In this classification system, reproductive toxicity is subdivided under two main headings:

(a) adverse effects on sexual function and fertility;

(b) adverse effects on development of the offspring.

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. Nonetheless, substances with these effects, or mixtures containing them, shall be classified as reproductive toxicants.

3.7.1.2 For the purpose of classification the hazard class Reproductive Toxicity is differentiated into:

- adverse effects
  - on sexual function and fertility, or
  - on development;
- effects on or via lactation.

3.7.1.3 Adverse effects on sexual function and fertility

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

3.7.1.4 Adverse effects on development of the offspring

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

3.7.1.5 Adverse effects on or via lactation are also included in reproductive toxicity, but for classification purposes, such effects are treated separately (see Table 3.7.1 (b)). This is because it is desirable to be able to classify substances specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

3.7.2 Classification criteria for substances

3.7.2.1 Hazard categories

3.7.2.1.1 For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Table 3.7.1(a) Hazard categories for reproductive toxicants.

Categories		Criteria
CATEGORY 1		Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
	Category 1A	Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.
	Category 1B	Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
CATEGORY 2		Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Table 3.7.1(b) Hazard category for lactation effects.

EFFECTS ON OR VIA LACTATION
Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

3.7.2.2 Basis of classification

3.7.2.2.1 Classification is made on the basis of the appropriate criteria, outlined above, and an assessment of the total weight of evidence (see 1.1.1). Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects.

The classification of a substance is derived from the hazard categories in the following order of precedence: Category 1A, Category 1B, Category 2 and the additional Category for effects on or via lactation. If a substance meets the criteria for classification into both of the main categories (for example Category 1B for effects on sexual function and fertility and also Category 2 for development) then both hazard differentiations shall be communicated by the respective hazard statements. Classification in the additional category for effects on or via lactation will be considered irrespective of a classification into Category 1A, Category 1B or Category 2.

3.7.2.2.2 In the evaluation of toxic effects on the developing offspring, it is important to consider the possible influence of maternal toxicity (see section 3.7.2.4).

3.7.2.2.3 For human evidence to provide the primary basis for a Category 1A classification there must be reliable evidence of an adverse effect on reproduction in humans. Evidence used for classification shall ideally be from well conducted epidemiological studies which include the use of appropriate controls, balanced assessment, and due consideration of bias or confounding factors. Less rigorous data from studies in humans shall be supplemented with adequate data from studies in experimental animals and classification in Category 1B shall be considered.

3.7.2.3 Weight of evidence

3.7.2.3.1 Classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence, see section 1.1.1. This means that all available information that bears on the determination of reproductive toxicity is considered together, such as epidemiological studies and case reports in humans and specific reproduction studies along with sub-chronic, chronic and special study results in animals that provide relevant information regarding toxicity to reproductive and related endocrine organs. Evaluation of substances chemically related to the substance under study may also be included, particularly when information on the substance is scarce. The weight given to the available evidence will be influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects, the presence of maternal toxicity in experimental animal studies, level of statistical significance for inter-group differences, number of endpoints affected, relevance of route of administration to humans and freedom from bias. Both positive and negative results are assembled together into a weight of evidence determination. A single, positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification (see also 3.7.2.2.3).

3.7.2.3.2 Toxicokinetic studies in animals and humans, site of action and mechanism or mode of action study results may provide relevant information which reduces or increases concerns about the hazard to human health. If it is conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals should not be classified.

3.7.2.3.3 If, in some reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments.

3.7.2.3.4 Data from animal studies ideally shall provide clear evidence of specific reproductive toxicity in the absence of other systemic toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects shall be assessed to the extent possible. The preferred approach is to consider adverse effects in the embryo/foetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternally toxic doses shall not be automatically discounted. Discounting devel-

opmental effects that are observed at maternally toxic doses can only be done on a case-by-case basis when a causal relationship is established or refuted.

3.7.2.3.5 If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is especially the case when the effects in the offspring are significant, e.g. irreversible effects such as structural malformations. In some situations it can be assumed that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, if the substance is so toxic that dams fail to thrive and there is severe inanition, they are incapable of nursing pups; or they are prostrate or dying.

3.7.2.4 Maternal toxicity

3.7.2.4.1 Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms. In the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification.

3.7.2.4.2 Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.

3.7.2.4.3 Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity.

3.7.2.4.4 Some of the end points used to assess maternal effects are provided below. Data on these end points, if available, need to be evaluated in light of their statistical or biological significance and dose response relationship.

Maternal mortality:

an increased incidence of mortality among the treated dams over the controls shall be considered evidence of maternal toxicity if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.

Mating index

(no. animals with seminal plugs or sperm/no. mated × 100) (*)

Fertility index

(no. animals with implants/no. of matings × 100)

Gestation length

(if allowed to deliver)

Body weight and body weight change:

Consideration of the maternal body weight change and/or adjusted (corrected) maternal body weight shall be included in the evaluation of maternal toxicity whenever such data are available. The calcula-

* () It is recognised that the Mating index and the Fertility index can also be affected by the male.

tion of an adjusted (corrected) mean maternal body weight change, which is the difference between the initial and terminal body weight minus the gravid uterine weight (or alternatively, the sum of the weights of the foetuses), may indicate whether the effect is maternal or intrauterine. In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.

Food and water consumption (if relevant):

The observation of a significant decrease in the average food or water consumption in treated dams compared to the control group is useful in evaluating maternal toxicity, particularly when the test material is administered in the diet or drinking water. Changes in food or water consumption need to be evaluated in conjunction with maternal body weights when determining if the effects noted are reflective of maternal toxicity or more simply, unpalatability of the test material in feed or water.

Clinical evaluations (including clinical signs, markers, haematology and clinical chemistry studies):

The observation of increased incidence of significant clinical signs of toxicity in treated dams relative to the control group is useful in evaluating maternal toxicity. If this is to be used as the basis for the assessment of maternal toxicity, the types, incidence, degree and duration of clinical signs shall be reported in the study. Clinical signs of maternal intoxication include: coma, prostration, hyperactivity, loss of righting reflex, ataxia, or laboured breathing.

Post-mortem data:

Increased incidence and/or severity of post-mortem findings may be indicative of maternal toxicity. This can include gross or microscopic pathological findings or organ weight data, including absolute organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio. When supported by findings of adverse histopathological effects in the affected organ(s), the observation of a significant change in the average weight of suspected target organ(s) of treated dams, compared to those in the control group, may be considered evidence of maternal toxicity.

3.7.2.5 Animal and experimental data

3.7.2.5.1 A number of internationally accepted test methods are available; these include methods for developmental toxicity testing (e.g. OECD Test Guideline 414), and methods for one or two-generation toxicity testing (e.g. OECD Test Guidelines 415, 416).

3.7.2.5.2 Results obtained from Screening Tests (e.g. OECD Guidelines 421 — Reproduction/ Developmental Toxicity Screening Test, and 422 — Combined Repeated Dose Toxicity Study with

Reproduction/Development Toxicity Screening Test) can also be used to justify classification, although it is recognised that the quality of this evidence is less reliable than that obtained through full studies.

3.7.2.5.3 Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads.

3.7.2.5.4 Evidence from in vitro assays, or non-mammalian tests, and from analogous substances using structure-activity relationship (SAR), can contribute to the procedure for classification. In all cases of this nature, expert judgement must be used to assess the adequacy of the data. Inadequate data shall not be used as a primary support for classification.

3.7.2.5.5 It is preferable that animal studies are conducted using appropriate routes of administration which relate to the potential route of human exposure. However, in practice, reproductive toxicity studies are commonly conducted using the oral route, and such studies will normally be suitable for evaluating the hazardous properties of the substance with respect to reproductive toxicity. However, if it can be conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals shall not be classified.

3.7.2.5.6 Studies involving routes of administration such as intravenous or intraperitoneal injection, which result in exposure of the reproductive organs to unrealistically high levels of the test substance, or elicit local damage to the reproductive organs, including irritation, must be interpreted with extreme caution and on their own are not normally the basis for classification.

3.7.2.5.7 There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also, due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model.

3.7.2.5.8 In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would

not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Please also refer to the section on maternal toxicity (3.7.2.4) for further guidance in this area.

3.7.2.5.9 However, specification of the actual 'limit dose' will depend upon the test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by the oral route, an upper dose of 1 000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level.

3.7.3 Classification criteria for mixtures

3.7.3.1 Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.7.3.1.1 The mixture shall be classified as a reproductive toxicant when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 reproductive toxicant and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for Category 1A, Category 1B and Category 2 respectively.

3.7.3.1.2 The mixture shall be classified for effects on or via lactation when at least one ingredient has been classified for effects on or via lactation and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for the additional category for effects on or via lactation.

Table 3.7.2 Generic concentration limits of ingredients of a mixture classified as reproduction toxicants or foreffects on or via lactation that trigger classification of the mixture.

Ingredient classified as:	Generic concentration limits triggering classification of a mixture as:			
	Category 1A reproductive toxicant	Category 1B reproductive toxicant	Category 2 reproductive toxicant	Additional category for effects on or via l actation
Category 1A reproductive toxicant	≥ 0,3 % [Note 1]			
Category 1B reproductive toxicant		≥ 0,3 % [Note 1]		
Category 2 reproductive toxicant			≥ 3,0 % [Note 1]	
Additional category for effects on or via lactation				≥ 0,3 % [Note 1]

Note The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).
Note 1 If a Category 1 or Category 2 reproductive toxicant or a substance classified for effects on or via lactation is present in the mixture as an ingredient at a concentration above 0,1 %, a SDS shall be available for the mixture upon request.

3.7.3.2 Classification of mixtures when data are available for the complete mixture

3.7.3.2.1 Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients of the mixture. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual components. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of reproduction test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.7.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles

3.7.3.3.1 Subject to paragraph 3.7.3.2.1, where the mixture itself has not been tested to determine its reproductive toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.7.4 Hazard Communication

3.7.4.1 Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.7.3

Table 3.7.3 Label elements for reproductive toxicity.

Classification	Category 1A or Category 1B	Category 2	Additional category for effects on or via lactation
GHS Pictograms			No pictogram
Signal Word	Danger	Warning	No signal word
Hazard Statement	H360: May damage fertility or the unborn child (state specific effect if known)(state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H361: Suspected of damaging fertility or the unborn child (state specific effect if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H362: May cause harm to breast-fed children.
Precautionary Statement Prevention	P201 P202 P281	P201 P202 P281	P201 P260 P263 P264 P270
Precautionary Statement Response	P308 + P313	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405	
Precautionary Statement Disposal	P501	P501	

Annex **E**

Additional considerations to Regulation (EC) 1272/2008

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC)1272/2008) presented in Annex B. The classification of compounds is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations:

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the offspring, the compound will be classified in category 1A, irrespective of the general toxic effects (see Annex B, 3.7.2.2.1.).
- Adverse effects in a reproductive study, reported without information on the parental or maternal toxicity, may lead to a classification other than category 1B, when the effects occur at dose levels which cause severe toxicity in *general* toxicity studies.
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.

- The Committee does not only use guideline studies (studies performed according to OECD* standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

* Organisation for Economic Cooperation and Development

Annex **F**

Fertility and developmental toxicity studies

Table 1 Fertility studies in laboratory animals with chloramphenicol: oral administration.

authors	species	experimental period/design	dose	general toxicity	effects on reproductive organs/effects on reproduction
Oyeyemi/ Adeniji (2010)	male Wistar rats (n=5/group)	20, 25 d	0, 25 mg/kg bw/d	not reported	decreases ($p<0.05$) in sperm motility, % viability, number of normal sperm, sperm concentration
Oyagbemi et al. (2010)	male Wistar rats (n=6/group)	4 times/d, 10 consecutive d; sacrifice 24 h after last treatment	0, 28 mg/kg bw	bw loss of 7% vs. bw gain of 4% in controls	no effect on % of morphologically abnormal sperm; decreases ($p<0.05$) in sperm motility, number of normal sperm, sperm concentration

bw=body weight; d=day(s); h=hour(s); ; n=number

Table 2 Fertility studies in laboratory animals with chloramphenicol: parental administration.

authors	species	experimental period/design	dose/ route	general toxicity	effects on reproductive organs/effects on reproduction
Nowkunski (1963)	white rats (n=10 males; 20 females	20 d; then mating: treated males with treated females, or treated males with untreated females, or untreated males with treated females; morphology of gonads of 15 males and 15 females	0, 34 mg/kg bw; im	not reported	male gonads, uterine mucosa not affected; in ovaries, cystic degeneration of Graafian follicles; oestrus cycle affected in 6/15 treated females; no pregnant females when treating both sexes or only females; when treating only males, 6/20 female pregnant vs. 17/20 in controls

Beermann/ Hansmann (1986)	female NMRI/Han mice (n=8-18)	mice induced for superovulation with pregnant mare serum followed 48 h later by ip HGC; groups received saline or chloramphenicol at 0, 15 or 48 h after pregnant mare serum; sacrificed 15-16 h after HCG	0, 18.8 (t=0 only), 37.5 mg/kg bw; ip	not reported	relative ovarian weight and the number of ovulated oocytes reduced at 37.5 (all time points); progesterone concentration markedly reduced in all treated females; number of diploid oocytes increased compared to control at 18.8 mg/kg bw and at 37.5 mg/kg bw at 15 and 48 h.
Epstein/ Shafner (1968); Epstein et al. (1972)	male Swiss CD-1 mice (n=7-9/ group)	single injection; males mated with 3 untreated females which were replaced weekly for 8 consecutive wk; females sacrificed 13 d from mid-week of their mating	0, 333, 666 mg/kg bw; ip	not reported	no effect on number of early foetal deaths and on pre-implantation losses

bw=body weight; d=day(s); h=hour(s); HGC=human chorionic gonadotrophin; im=intramuscular; ip=intraperitoneal; wk=week(s); n=number

Table 3 Developmental toxicity studies in laboratory animals with chlorampenicol: oral administration.

authors	species	experimental period/ design	dose	general toxicity	developmental toxicity
Fritz/Hess (1971)	Sprague-Dawley rats (n=5-15; controls: n=553 over a period of 4 y)	sacrifice gd 21; skeletal development only examined in foetuses at 1,000 mg/kg bw/d and 2,000 mg/kg bw/d on gd 11-13	gd 5-15: 0, 500 mg/kg bw/d gd 7-12: 0, 1,000 mg/kg bw/d gd 0-6: 1,500 mg/kg bw/d gd 5, 6, 7, 8, 9, 10, or gd 6-8, 7-9, 9-11, 11-13, 13-15, 15-17: 0, 2,000 mg/kg bw/d in 2% CMC; gavage	no toxic signs	500 mg/kg bw: reduced number of live foetuses ($p<0.05$); increased % of embryonic or foetal deaths (63% vs. 22.6%; $p<0.05$) 1,000 mg/kg bw: decreased average foetal weight ($p<0.05$); % embryonic or foetal deaths: 38.5% (vs. 22.6%; $p<0.05$); 2 litters totally aborted 1,500 mg/kg bw: no effects 2,000 mg/kg bw/d: gd 6-8, 7-9, 11-13: increased % of embryonic or foetal deaths (67-75%; $p<0.05$); decreased number of live foetuses/dam ($p<0.05$); decreased average foetal weights ($p<0.05$) gd 9-11: % embryonic or foetal deaths: 100%; 3 litters totally aborted gd 13-15: % embryonic or foetal deaths: 95.9%; decreased average foetal weights ($p<0.05$) gd 15-17: decreased average foetal weights ($p<0.05$) gd 5: no effects gd 6, 7: decreased average foetal weight ($p<0.05$) gd 8: % of embryonic or foetal deaths: 45.9%; $p<0.05$); decreased number of live foetuses/dam ($p<0.05$); decreased average foetal weights ($p<0.05$); 1 litter totally aborted gd 9: % of embryonic or foetal deaths: 43,9%; $p<0.05$); decreased average foetal weights; 2 litters totally aborted gd 10: % of embryonic or foetal deaths: 46.4%; $p<0.05$); decreased number of live fetuses/dam ($p<0.05$); decreased average foetal weights ($p<0.05$)

					malformations: controls: 1 brachygnathia, 1 oxygcephaly+agnathia, 1 brachymelia, 2 omphaloceles in 6,326 foetuses 500 mg/kg bw: no malformations 1,000 mg/kg bw: hypognatia in 1/83 foetuses 1,500 mg/kg bw: harelip in 1/65 2,000 mg/kg bw: gd 6-8: omphalocele (umbilical hernia) + unilateral/ bilateral costal fusion in 8/22 foetuses (1 litter) gd 7-9: idem in 1/26 gd 7: idem in 2/84 (1 litter) gd 8: idem in 5/46 (1 litter) gd 9: idem in 5/64 (3 litters) gd 10: no malformations *skeletal effects*: 1,000 mg/kg bw: increased missing ossification of phalangeal nuclei of forelegs and hind legs and of 5th sternebra; decreased number of ossified cervical vertebrae; increased fusion sternebra 1+2 and bipartite vertebrae 2,000 mg/kg bw, gd 11-13: increased missing ossification of phalangeal nuclei of forelegs and hind legs and of 5th sternebra
Fritz/Hess (1971)	CD-1 mice (n=7-19; controls: n=307 over a period of 4 y)	sacrifice gd 18; skeletal development only examined in foetuses at 1,000 mg/kg bw/d	gd 5-15: 0, 500 mg/kg bw/d gd 6-12: 0, 1,000 mg/kg bw/d gd 8-10: 0, 2,000 mg/kg bw/d in 2% CMC; gavage	bw effects insuffi-ciently documented	500 mg/kg bw: decreased average foetal weight ($p<0.05$) 1,000 mg/kg bw: % embryonic or foetal deaths: 71.1% (vs. 24.4% in controls; $p<0.05$); decreased number of live fetuses/dam ($p<0.05$); decreased average foetal weight ($p<0.05$) 2,000 mg/kg bw: gd 8-10: % embryonic or foetal deaths: 100% *malformations*: controls: 2 cranioschisis+exophthalmos, 1 exencephaly, 1 median cleft palate in 3,230 foetuses 1,000 mg/kg bw: cranioschisis+exophthalmos in 1/ 81 foetuses *skeletal effects*: 1,000 mg/kg bw: increased missing ossification of phalangeal nuclei of forelegs and hind legs and 5th sternebra; iincreased fusion sternebra 1+2
Fritz/Hess (1971)	mixed breed rabbit (n=5-8; controls: n=192 over a period of 4 y)	sacrifice gd 28; skeletal development examined in all foetuses	gd 6-15: 0, 500 mg/kg bw/d gd 6-9, 8-11: 0, 1,000 mg/kg bw/ d in 2% CMC; gavage	no toxic signs	500 mg/kg bw: no effects 1,000 mg/kg bw: gd 6-9: % embryonic or foetal deaths: 24.6% (vs. 10% in controls; $p<0.05$); decreased average foetal weight ($p<0.05$) gd 8-11: % embryonic or foetal deaths: 58.1% (vs. 10% in controls; $p<0.05$); decreased number of live fetuses/ dam ($p<0.05$); decreased average foetal weight ($p<0.05$) *malformations*: no malformations observed

					skeletal effects: 500 mg/kd bw: increased missing ossification of phalangeal nuclei of forelegs 1000 mg/kg bw: gd 6-9: increased missing ossification of phalangeal nuclei of forelegs and hind legs; gd 8-11: increased missing ossification of 5th sternebra
Al-Hachim/ Al-Baker (1974)	albino mice (n=8/ group)	5-7 doses during 3rd stage of pregnancy; offspring: 10/ group tested for conditioned avoidance response (pnd 30-36), electroshock seizure threshold (pnd 38), open-field (pnd 42-48)	0, 25, 50, 100, 200 mg/kg bw/d; gavage	not reported	conditioned avoidance response: statistically significant decrease in response at all dose levels with a dose-response relationship electroshock seizure threshold: non-significant increase at 50-200 mg/kg bw/d with a dose-response relationship open-field performance: statistically significant decrease at all dose levels without a dose-response relationship; no gross congenital abnormalities
Mackler (1975)	Sprague-Dawley rats (n not specified)	gd 0-20; sacrifice gd 20	0, 2, 3%; diet (i.e. 200, 300 mg/rat); additional group with restricted diet (67% of control)	decreased food intake at both dose levels	200 mg/rat: % resorptions: 31.4% (controls: 4.7%); n of live foetues: 117 (controls: 201); decreased foetal weight ($p<0.001$) 300 mg/rat: % resorptions: 57.0%; n live fetuses: 31; decreased foetal weight ($p<0.001$); decreased placental weight ($p<0.001$) [restricted diet group: % resorptions: 1.5%; n live foetuses: 64] *developmental effects*: 200 mg/rat: oedema (12%) 300 mg/rat: oedema (71%); wavy ribs (7%); fused ribs (7%) control diet: no effects [restricted diet: no effects] mitochondrial activities in homogenates of gd 14 foetuses: 200 mg/rat: decreased activities of DPNH oxidase ($p<0.001$), cytochrome c oxidase ($p<0.001$), ATPase ($p<0.01$); no effect on succinic oxidase and succinic indophenol dehydrogenase 300 mg/rat: decreased activities of DPNH oxidase ($p<0.001$); no effect on succinic oxidase (cytochrome c oxidase, ATPase, and succinic indophenol dehydrogenase activities not measured)

Mackler (1975)	Sprague-Dawley rats (n=3-11)	sacrifice gd 20	1.5 % on gd 0-9, 0-10, 0-11, 0-12, 3% on gd 0-2, 0-3, 0-4, 0-5, 0-6, 0-7, 0-8; diet (i.e.150, 300 mg/rat)	not reported	increased number of females without implantations when treated during gd 0-6 and onwards (18-67%); increased number of resorptions when treated during gd 0-5 (8%) and gd 0-8, 0-9, 0-10, 0-11 (16-40%); decreased foetal weights when treated during gd 0-7 and onwards

CMC=carboxymethyl cellulose; gd: gestational day(s); n=number; pnd=postnatal day(s); y=year(s)

Table 4 Developmental toxicity studies in laboratory animals with chlorampenicol: subcutaneous administration.

authors	species	experimental period/ design	dose/route	general toxicity	developmental toxicity
Neumann (1976)	Wistar rats	gd 6-10 or 11-14; sacrifice gd 21	0, 800-2500 mg/kg bw; as sodium succinate	not reported	controls (n=15): % resorptions: 7.6%; % dead foetuses: 0.6%; no retarded or malformed foetuses gd 6-10 group (n=13): % resorptions: 9.6%; % dead foetuses: 1.9 %; % retarded fetuses: 87.9%; % malformed fetuses: 0.6% (clinodactily); reduced average foetal weight (2.4 g vs. 4.4 g) gd 11-14 group (n=43): % resorptions: 59.3%; % dead foetuses: 0.6 %; % retarded foetuses: 19.4% (predominantly haemorrhages and oedemas); % malformed foetuses: 1.3% (n=6; all at 1500 mg/kg bw; 5 cleft palate, 1 cleft palate+limb anomalies; 5 in 1 litter); reduced average foetal weight (3.0 g vs. 4.4 g) (increased number of resorptions, reduced number of normal live foetuses are dose related)
Bertolini/ Poggioli (1981)	Wistar rats (n=15/ group)	dams: gd 7-21 pups: pnd 1-3; intrauterine exposed pups and pups exposed on pnd 1-3 (n=10/group/ sex) trained for conditioned avoidance response at pnd 60; response tests at pnd 65, 70, 75, 80; pain threshold also tested	dams: 0, 50 mg/kg bw/d; pups: 0, 50 100 mg/kg bw/d; as hemisuccinate	course of pregnancy not affected	dams: no effect on litter size, pup weight, postnatal weight gain; no gross malformations in offspring; no mortality in all groups; pups: statistically significantly decreased conditioned avoidance response in all treated groups (more marked in males than in females and in intrauterine treated than in postnatally treated pups); no difference in pain threshold; no mortality

bw=body weight; gd=gestational day(s); n=number; pnd=postnatal day(s)