Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

theophylline; 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

EC Number: 200-385-7
CAS Number: 58-55-9

CLH-O-0000006848-58-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

17 September 2010

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: theophylline;

1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

EC Number: 200-385-7

CAS Number: 58-55-9

Index Number: N/A

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON THEOPHYLLINE; 1,3-DIMETHYL-3,7-DIHYDRO-1*H*-PURINE-2,6-DIONE

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Theophylline; 1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione***
EC number:	***200-385-7***
CAS number:	***58-55-9***
Annex VI Index number:	***Not allocated***
Degree of purity:	***97-100% (OECD SIDS, 2001)***
Impurities:	***confidential***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not included in CLP Annex VI
Current proposal for consideration by RAC	Repr. 1B, H360D
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1B, H360D

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Outside scope
2.2.	Flammable gases				Outside scope
2.3.	Flammable aerosols				Outside scope
2.4.	Oxidising gases				Outside scope
2.5.	Gases under pressure				Outside scope
2.6.	Flammable liquids				Outside scope
2.7.	Flammable solids				Outside scope
2.8.	Self-reactive substances and mixtures				Outside scope
2.9.	Pyrophoric liquids				Outside scope
2.10.	Pyrophoric solids				Outside scope
2.11.	Self-heating substances and mixtures				Outside scope
2.12.	Substances and mixtures which in contact with water emit flammable gases				Outside scope
2.13.	Oxidising liquids				Outside scope
2.14.	Oxidising solids				Outside scope
2.15.	Organic peroxides				Outside scope
2.16.	Substance and mixtures corrosive to metals				Outside scope
3.1.	Acute toxicity - oral				Outside scope
	Acute toxicity - dermal				Outside scope
	Acute toxicity - inhalation				Outside scope
3.2.	Skin corrosion / irritation				Outside scope
3.3.	Serious eye damage / eye irritation				Outside scope
3.4.	Respiratory sensitisation				Outside scope
3.4.	Skin sensitisation				Outside scope
3.5.	Germ cell mutagenicity				Outside scope
3.6.	Carcinogenicity				Outside scope
3.7.	Reproductive toxicity	**Repr. 1B; H360D**	**None**	**None**	
3.8.	Specific target organ toxicity –single exposure				Outside scope
3.9.	Specific target organ toxicity – repeated exposure				Outside scope
3.10.	Aspiration hazard				Outside scope

4.1.	Hazardous to the aquatic environment				Outside scope
5.1.	Hazardous to the ozone layer				Outside scope

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger
Hazard statements: H360D (May damage the unborn child)
Precautionary statements: not relevant

Proposed notes assigned to an entry:

: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Theophylline has not previously been assessed for harmonised classification by RAC or the TC C&L.

2.2 Short summary of the scientific justification for the CLH proposal

In accordance with the criteria of the CLP regulation, theophylline should be classified as Repr. 1B (H360D) based on the adverse effects on development (reduced number of pups per litter and increase in resorptions) as observed in studies in mice at dose levels at which no marked maternal toxicity occurred. In addition, a reduction in live pups per litter was observed at maternally toxic dose levels in rats.

2.3 Current harmonised classification and labelling

Not applicable

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not applicable

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

None of the registrants or the notifiers to the C&L inventory classifies for reproductive toxicity.

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Theophylline fulfills the criteria for classification for reproductive toxicity and shall normally be subject to harmonised classification (CLP article 36.1).

RAC general comments

Theophylline is a naturally occurring substance in certain plants, e.g. black tea, coffee and cocoa. It has a wide dispersive use, predominantly as an anti-asthmatic drug in the pharmaceutical sector (99%), while ca. 1% is used in cosmetic applications. Theophylline is a methylxanthine drug with use as a bronchodilator in the therapy for respiratory diseases such as chronic obstructive pulmonary disease and asthma. According to the dossier submitter (DS), therapeutic doses of theophylline are in the range of 2-12 mg/kg bw/d with associated plasma levels between 4-24 µg/mL. Recommended theophylline therapeutic levels are between 5 and 12 µg/mL; plasma levels as low as 1.3 µg/mL have been found to be effective (Health Council of the Netherlands, 2013).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

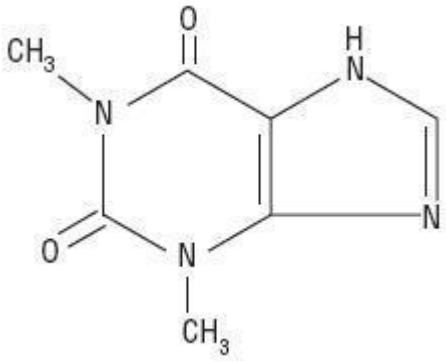
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	200-385-7
EC name:	Theophylline
CAS number (EC inventory):	
CAS number:	58-55-9
CAS name:	1H-Purine-2,6-dione, 3,9-dihydro-1,3-dimethyl-
IUPAC name:	1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione
CLP Annex VI Index number:	Not applicable
Molecular formula:	$C_7H_8N_4O_2$
Molecular weight range:	180.17

Structural formula:



1.2 Composition of the substance

Purity: 97 - 100 % w/w (OECD-SIDS 2001)

No further information available

Current Annex VI entry: not applicable

1.2.1 Composition of test material

Not applicable

1.3 Physico-chemical properties

Table 5: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	white crystalline powder	Health Council of the Netherlands (2013)	
Melting/freezing point	270 – 274 °C	OECD-SIDS 2001	
Boiling point	Not relevant because of chemical decomposition	OECD-SIDS 2001	
Relative density	1.36 mg/cm3	Registration dossier	
Vapour pressure	0.0000007 Pa at 25 °C	OECD-SIDS 2001	Calculated value
Surface tension	Not applicable		
Water solubility	5.5 to 8.3 g/l at 20 °C	OECD-SIDS 2001	
Partition coefficient n-octanol/water	Log K_{ow} =-0.0076 at 23°C	OECD-SIDS 2001	Measured value
Flash point	No data		
Flammability	hardly flammable; ignition temperature > 610°C	OECD-SIDS 2001	
Explosive properties	not explosive	OECD-SIDS 2001	
Self-ignition temperature	No data		
Oxidising properties	No data		
Granulometry	Particle size distribution: D10 = 30.422 µm; D50 = 132.226 µm; D90 = 352.177 µm	ECHA	
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	No data		
Viscosity	No data		

2 MANUFACTURE AND USES

2.1 Manufacture

Theophylline is a naturally occurring substance in certain plants, e.g. black tea (200 – 400 mg/kg dry weight), coffee (about 5 mg/kg in green coffee beans) and cocoa (trace amounts) (The Merck Index 1989).

2.2 Identified uses

Theophylline is a substance with wide dispersive use. It is predominantly used as an anti-asthmatic drug in the pharma sector (99%). 1% is used in cosmetic applications. Theophylline concentrations in cellulite reduction creams are below 1% (OECD-SIDS 2001). Theophylline is a methylxanthine drug with use as a bronchodilator in the therapy for respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma. Therapeutic doses of theophylline are in the range of 2-12 mg/kg/day with associated plasma levels between 4-24 µg/mL; recommended theophylline therapeutic levels are between 5 and 12 µg/mL; plasma levels as low as 1.3 µg/mL have been found to be effective (Health Council of the Netherlands 2013).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Out of scope of this proposal.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The chemical structure of theophylline closely resembles that of caffeine, the only difference being that caffeine has N-CH_3 in the purine 5-ring where theophylline has N-H. In the metabolism of theophylline in mammals interconversion of theophylline and caffeine occurs (IARC 1991).

4.1.1 Non-human information

Only oral data are available. Animal data (dogs, rats, pigs, rabbits) indicate rapid and complete absorption from the GI-tract after oral intake. In blood theophylline is bound to protein but the level of protein-binding is variable across animal species. Theophylline is metabolised in the liver. Excretion takes place in urine, either as unchanged compound (34% of the dose in rats after oral dosing) as 1,3-dimethyluric acid (34%), 1-methyluric acid (18%), 3-methylxanthine (3%) or unidentified polar metabolites (4.8%). In rats, during gestation metabolism was found to be reduced and half time values were longer. Transplacental transfer of theophylline was demonstrated in rats and rabbits (IARC 1991).

4.1.2 Human information

Only oral data are available. Available information is summarised in NTP (1998) and OECD (2001). Theophylline is readily absorbed after oral intake. The absorbed fraction of a dose of about 7.5 mg/kg bw was as high as 99 %. Peak serum levels were reached within 0.5-2 hours. About 50% of theophylline is bound reversibly to plasma proteins in the blood concentration range of 10-20 µg/ml. Theophylline is distributed to erythrocytes, saliva, breast milk, and amniotic fluid, and it can cross the placenta and the blood brain barrier (NTP 1998).

Theophylline is metabolized in humans by ring oxidation and N-demethylation by the liver microsomal mixed-function oxidase system. Metabolites formed are 1,3-dimethyluric acid, 3-methylxanthine, or 1-methylxanthine, which is rapidly converted to 1-methyluric acid by xanthine oxidase. These metabolites are then excreted in urine without further alteration. After administration of a single oral dose of 1 gram to two human volunteers, the following percentages of metabolites were found in the urine: 1,3-dimethyluric acid (35%), 1-methyluric acid (19%), 3-methylxanthine (13%) and unchanged theophylline (10%). Because theophylline is metabolized by liver P450

enzymes, metabolism is subject to variation due to inter-individual genetic differences, to disease state and age. This leads to large variation in plasma concentrations and in elimination half-lives. Half-lives are markedly prolonged in neonates, the latter being reported as five times greater than those in adults. During the first trimester of pregnancy binding to protein is reduced, leading to increased levels of unbound theophylline. In neonates theophylline is excreted as unchanged compound (98%) or as caffeine (2%). In fetuses metabolism was found to be limited to conversion to caffeine. Thus, indications are that metabolic pathways active in children and adults are minimally functional in fetuses and (preterm) neonates (IARC 1991; NTP 1998; OECD 2001).

4.1.3 Summary and discussion on toxicokinetics

Absorption of theophylline after oral intake is fast and complete, as both animal data and human data show. Transport in the blood is as free compound or bound to protein. In the liver ring oxidation and N-demethylation occur under influence of P450-enzymes. Inter-individual variation in these enzymes leads to high inter-individual variation in theophylline plasma concentrations and elimination half-lives. Elimination from the body is in urine as metabolites or as unchanged chemical. During the first trimester of pregnancy binding of theophylline to protein is lower and levels of free theophylline in maternal blood are higher. Theophylline passes the placenta and is also excreted in milk. In fetuses and neonates theophylline metabolism is inactive with only limited conversion occurring to the N-methyl analogue caffeine.

4.2 Acute toxicity

Not relevant for this CLH-report.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not relevant for this CLH-report.

4.4 Irritation

Not relevant for this CLH-report.

4.4.1 Skin irritation

Not relevant for this CLH-report.

4.4.2 Eye irritation

Not relevant for this CLH-report.

4.4.3 Respiratory tract irritation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.5 Corrosivity

Not relevant for this CLH-report.

4.6 Sensitisation

4.6.1 Skin sensititsation

Not relevant for this CLH-report.

4.6.2 Respiratory sensitisation

Not relevant for this CLH-report.

4.7 Repeated dose toxicity

Not relevant for this CLH-report

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not relevant for this CLH-report.

4.9 Germ cell mutagenicity (Mutagenicity)

Not relevant for this CLH-report.

4.10 Carcinogenicity

Not relevant for this CLH-report.

4.11 Toxicity for reproduction

Table 6: Summary table of relevant reproductive toxicity studies

Method	**Test substance, dose level, duration of exposure**	**Results**	**Reference**
CD-1 mouse 20 animals/sex/dose (controls: 40 pairs) Reproductive Assessment by Continuous Breeding (RACB) Oral (diet)	Theophylline (>99% purity) 0, 0.075, 0.15, 0.3% in the diet Corresponding to 0, 126, 260, 506 mg/kg bw/d Animals are exposed from 1 week prior to cohabitation; during a subsequent 14 wk continuous exposure, animals are housed as breeding pairs and normally 4-5 litters are delivered per adult pair; after delivery of last litter, females are evaluated for vaginal cyclicity for 7 d, and F0 mice in control group and high-dose group killed and necropsied. Additionally, a 1-week crossover mating trial with F0 mice was performed to detect which sex had been affected.	*Continuous breeding phase* Parental toxicity Alopecia in both sexes in all treatment groups (≥126 mg/kg bw/d) Mortality (3 control + 4 low dose female mice) Increased rel. (females) + abs. (males) liver weight (500 mg/kg bw/d) Reduced terminal bw (500 mg/kg bw/d) Reproductive effects Decreased relative seminal vesicle weight (by 19%) in high dose group Reduced epididymal sperm density (by 20%) in high dose Reduced no. of litters/pair (high dose), reduced no. of pups born alive (mid, high dose), reduced no. live pups/litter (low, mid, high dose), reduced pup weight (high dose; adjusted to litter size)	NTP (1985a) Lamb et al. (1997) Morrissey et al. (1988) Klimisch score: 2
Male CD-1 mouse (10/group) Reproduction/ developmental screening assay Oral (gavage)	Theophylline (purity>93%) 0, 20, 60, 200 mg/kg bw/d Vehicle: corn oil 17 days exposure	Mild changes in the testis epithelium at top dose only No general toxicity	Harris et al. (1992) Klimisch score: 2
Female CD-1 mouse (10/group) Reproduction/ developmental screening assay Oral (gavage)	Theophylline (purity>93%) 0, 20, 60, 200 mg/kg bw/d Vehicle: corn oil 19 days exposure	Reduced pregnancy rate at top dose (not significantly) One high dose female killed moribund	Harris et al. (1992) Klimisch score: 2
Mouse, B6C3F1 14 weeks Oral (gavage)	Theophylline (>99% purity) 0, 75, 150, or 300 mg/kg bw/d (vehicle corn oil)	No biologically significant differences in sperm morphology or vaginal cytology parameters between control and exposed animals Decreased absolute testis weight (high dose) Increased mortality (high dose), reduced BW (mid+high)	NTP (1998) Klimisch score: 1
Mouse, B6C3F1 14 weeks	Theophylline (>99% purity) 0, 1000, 2000 or 4000 ppm in the feed, corresponding to 0, 184/229, 401/418, 793/856 (m/f)	No biologically significant differences in sperm morphology or vaginal cytology parameters between control and exposed animals.	NTP (1998) Klimisch

Oral (diet)	mg/kg bw/d		score: 1
Rat F344/N 14 weeks Oral (gavage)	Theophylline (>99% purity) 0, 37.5, 75, 150 mg/kg bw/d (vehicle corn oil)	No biologically significant differences in sperm morphology or vaginal cytology parameters between control and exposed animals Reduced (non-significant) testis weight and uterus weight (rel.) at high dose	NTP (1998) Klimisch score: 1
Rat F344/N 14 weeks Oral (diet)	Theophylline (>99% purity) 0, 1000, 2000 or 4000 ppm in the diet, corresponding to 0, 66/67, 129/135, 258/264 (m/f) mg/kg bw/d	No biologically significant differences in sperm morphology or vaginal cytology parameters between control and exposed animals	NTP (1998) Klimisch score: 1
75 weeks study Osborn Mendel rats, male Oral (diet)	Theophylline (purity >95%) 0 or 0.5% in feed, corresponding to 0 or 250 mg theophylline/kg bw/d (assuming 50 g feed/kg bw) 75 weeks	Non-significant increase in testicular histopathological effects Reduced bw	Weinberger et al, (1978) Klimisch score: 2
19 week study in Holtzman rats, male Oral (diet)	Theophylline (purity >95%) 0 or 0.5% in feed, corresponding to 0 or 250 mg theophylline/kg bw/d (assuming 50 g feed/kg bw) 19 weeks	Significant increase in testicular histopathological effects (testis atrophy, oligospermatogenesis) Marked toxicity (increased mortality, reduced bw gain and food intake)	Weinberger et al, (1978) Klimisch score: 2
Swiss (CD-1) Mouse Prenatal developmental toxicity study Oral (drinking water)	Theophylline (purity >99%) 0, 0.075, 0.15 or 0.20% in drinking water, corresponding to 0, 282, 372 or 396 mg/kg bw/d GD 6-15	<u>Maternal toxicity</u> Maternal growth retardation (mid+high dose) <u>Developmental toxicity:</u> increased no. of resorptions, decreased litter weight (mid+high dose)	NTP (1985b); Lindström et al. (1990) Klimisch score: 1
Sprague-Dawley (CD) Rat Prenatal developmental toxicity study Oral (drinking water)	Theophylline (purity >99%) 0, 0.075, 0.15 or 0.20% in drinking water, corresponding to 0, 124, 218 or 259 mg/kg bw/d GD 6-15	<u>Maternal toxicity</u> Maternal growth retardation at top dose only; <u>Developmental toxicity</u> No. of live fetuses/litter decreased (high dose) fetal weights decreased (mid+high dose)	NTP (1985c); Lindström et al. (1990) Klimisch score: 1
Female CD-1 mouse (13-15/group) Reproduction/ developmental screening	Theophylline (purity>93%) 0, 20, 60, 200 mg/kg bw/d Vehicle: corn oil GD8-14	No adverse effects on development or general toxicity noted	Harris et al. (1992) Klimisch score: 1

assay Oral (gavage)			
Mouse, ICR-JBL strain (20-26 animals/group) Prenatal developmental toxicity study (Intraperitoneal)	Theophylline (purity unknown) 0, 175, 200, 225 mg/kg bw Single exposure GD 12	Maternal toxicity: Slight dyspnea, convulsion (al dose groups, increase in severity with higher dose levels) Mortality (high dose; 40% of animals) Developmental toxicity Reduced foetal BW, induction of subcutaneous hematoma (mid+high dose) Skeletal malformations (low, mid, high dose)	Fujii et al (1969) Klimisch score: 3
Mouse Prenatal developmental toxicity study (Intraperitoneal)	Theophylline (purity unknown) 0, 100, 150, 200 mg/kg bw Single exposure GD 10, or GD11, or GD12, or GD13	Moderate embryo lethality and high incidence of malformations Details lacking on adverse effects in control group (resorptions and malformations)	Tucci et al. (1978) Klimisch score: 3
Rabbit, KbI;JW (20/group) Prenatal developmental toxicity study (intravenous)	Theophylline 0, 15, 30, 60 mg/kg bw/d GD6-18	Maternal toxicity Decreased growth, clinical signs at top dose only Developmental toxicity: fetal toxicity and cleft palate at top dose level only	Shibata et al. (2000) Klimisch score: 2

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Mouse oral continuous breeding study (NTP 1985a; Lamb et al. 1997; Morrissey et al. 1988)
The effect of theophylline on fertility was studied in CD-1 mice using the US National Toxicology Program (NTP) continuous breeding study design (Reproductive Assessment by Continuous Breeding (RACB)). Task 1 of this protocol included a dose-range finding study. Task 2 included the continuous breeding phase. Task 3 of this protocol included a cross-mating between control and high dose animals and was performed after exposure for 19 weeks.

Continuous breeding phase
Groups of 40 (20 males, 20 females) mice were fed diets containing 0.075, 0.15 or 0.3% theophylline during a 1-week premating period and subsequently for a 98 days' cohabitation period (dose levels equalled approximately 126, 260 and 506 mg/kg bw/day respectively, as calculated from feed consumption). The control group consisted of 40 mice per sex. Dose levels were based on a dose-range finding study (task 1). Bodyweights were measured. For all litters produced, the number and sex of newborns was determined. After delivery of the last litter all females were evaluated for vaginal cyclicity for 7 days. Control and high dose mice were then killed and necropsied.

Seven female animals died during cohabitation. Three out of the 40 control females died during week 15 of the study. Four out of the 20 low dose females died during cohabitation (weeks 3, 7, 10 and 12). Their data were excluded. No clear explanation on the cause of death has been provided by NTP (1985a).

A significant number of animals fed theophylline containing diet showed alopecia and the response was dose-dependent (>50% of the animals in the 0.15 and 0.3% dose groups and 20 to 25% of the animals in the 0.075% group). It is however noted that alopecia was also noted in one control animal but the severity was low compared to the treated animals.

There was no difference in daily food consumption between treatment groups. Table 7 presents body weight data during the continuous breeding phase. After 14 weeks of treatment, male mice in the 0 (control), 0.075, 0.15, and 0.3% theophylline groups gained nearly 7, 6, 4, and 3% of their original body weights, respectively. Group mean body weights for the female mice varied with the gestation phase. No further details on maternal body weights in relation to gestational phase were presented for the continuous breeding phase, making it difficult to assess the overall impact of maternal toxicity.

Table 7: Body weight data (group mean (g) ± SE) during continuous breeding phase (NTP 1985a)

		Theophylline dose groups			
		control	**0.075%**	**0.15%**	**0.3%**
Body weight					
Week 1	M	34.7±0.31 (40)[a]	33.8±0.26 (20)	34.3±0.40 (20)	34.3±0.54 (20)
	F	25.9±0.24 (40)	25.6±0.44 (20)	26.1±0.28 (20)	26.0±0.32 (20)
Week 2	M	35.4±0.32 (40)	33.7±0.31 (20)	34.0±0.34 (20)	34.5±0.52 (20)
	F	26.8±0.24 (40)	26.3±0.40 (20)	27.0±0.56 (20)	28.0±0.44 (20)
Week 3	M	34.1±0.29 (40)	33.1±0.28 (20)	33.7±0.43 (20)	32.9±0.65 (20)
	F	29.8±0.30 (40)	28.9±0.38 (20)	30.4±0.32 (20)	30.4±0.50 (20)
Week 6	M	34.4±0.34 (40)	33.6±0.47 (20)	34.0±0.40 (20)	33.7±0.58 (20)
	F	34.4±0.59 (40)	33.8±0.81 (19)[c]	34.1±0.39 (20)	36.1±1.46 (20)
Week 10	M	36.7±0.49 (40)	35.2±0.47 (20)	35.0±0.47 (20)	35.3±0.65 (20)
	F	47.9±1.22 (40)	44.3±1.53 (17)[d]	44.7±1.56 (20)	41.1±1.20 (20)
Week 14	M	37.3±0.53 (40)	35.7±0.53 (20)	35.6±0.49 (20)	35.4±0.56 (20)
	F	37.8±0.61 (40)	40.3±2.01 (16)[e]	40.6±1.46 (20)	43.1±1.39 (20)
Week 18	M	38.1±0.55 (40)	-[f]	-[f]	35.1±0.70 (20)
	F	46.7±1.14 (37)[b]	-[f]	-[f]	41.6±1.52 (20)

a: number of animals providing the data indicated in parenthesis
b: three females died during week 15 of the study
c: one female died during week 3 of the study
d: two females died during weeks 7 and 10 of the study
e: one female died during week 12 of the study
f: animals in the 0.075% and 0.15% dose groups were sacrificed during week 18 of the study

All breeding pairs in the 0 (control), 0.075, and 0.3% dose groups delivered at least one litter (Table 8). In the mid-dose group (0.15% theophylline), 19 out of the 20 breeding pairs delivered at least one litter. Data from pairs in which one or both partners died during cohabitation were excluded for intergroup comparisons and statistical analyses.

There was a significant decrease ($p<0.01$) in the mean number of litters per fertile pair at the 0.3% theophylline level (Table 9). The number of live pups per litter was significantly reduced ($p<0.01$) at all three dose levels relative to the control group. The proportion of pups born alive was significantly decreased ($p<0.05$) at the 0.15 and 0.3% dose levels. The analysis of covariance

indicated that the mean live pup weights when adjusted for the total number of live and dead pups per litter were significantly lower ($p<0.05$) in the high dose group (0.3% theophylline) than the control values (Table 9). One pup delivered by a breeding pair in the 0.3% dose group showed a mid-frontal cephalocele.

Table 8: Fertility of pairs during continuous breeding (NTP 1985a)

Treatment group	No. fertile/No. cohabited	Fertility index (%) [a]
Control	37/37[b]	100
0.075%	16/16[c]	100
0.15%	19/20	95
0.30%	20/20	100

[a] fertility index (%) = no. fertile/no. cohabited × 100
[b] three out of the 40 females died during week 15 of the study, their data were excluded
[c] four out of the 20 females died during the cohabitation (weeks 3, 7, 10 and 12); their data were excluded

Table 9: Reproductive performance of fertile pairs during continuous breeding phase (NTP 1985a)

	Treatment group			
Reproductive parameter[s]	**control**	**0.075%**	**0.15%**	**0.3%**
Litters per pair	4.78±0.096 (37)[c]	4.81±0.136 (16)	4.42±0.221(19)	3.85±0.264(20)[d]
Live pups per litter				
male	6.29±0.289 (37)	4.55±0.341 (16)[e]	3.99±0.364 (19)[e]	3.44±0.310 (20)[e]
female	5.57±0.196 (37)	4.71±0.439 (16)	4.48±0.408 (19)	3.44±0.324 (20)[e]
combined	11.86±0.395 (37)	9.26±0.658 (16)[e]	8.47±0.714 (19)[e]	6.89±0.520 (20)[e]
Proportion of pups born alive	0.98±0.008 (37)	0.92±0.039 (16)	0.88±0.048 (19)[f]	0.90±0.031 (20)[e]
Sex of pups born alive (males/total)	0.52±0.013 (37)	0.50±0.023 (16)	0.47±0.019 (19)[f]	0.51±0.028 (20)
Live pup weight (g)				
male	1.58±0.013 (37)	1.64±0.018 (16)[f]	1.66±0.025 (19)[f]	1.59±0.019 (20)
female	1.54±0.014 (37)	1.57±0.017 (16)	1.58±0.025 (19)	1.58±0.035 (20)
combined	1.56±0.013 (37)	1.61±0.018 (16)[f]	1.61±0.023 (19)[f]	1.59±0.024 (20)
Adjusted live pup weight (g)[b]				
male	1.62±0.014 (37)	1.63±0.019 (16)	1.64±0.017 (19)	1.54±0.019 (20)[e]
female	1.60±0.016 (37)	1.56±0.021 (16)	1.56±0.019 (19)	1.50±0.021 (20)[e]
combined	1.61±0.013 (37)	1.60±0.017 (16)	1.60±0.016 (19)	1.52±0.018 (20)[e]

[a] mean±SE
[b] means adjusted for total number of live and dead pups per litter by analysis of covariance
[c] number of fertile pairs providing the data indicated in parenthesis
[d] significantly different ($p<0.01$) from the control and 0.075% group
[e] significantly different ($p<0.01$) from the control group
[f] significantly different ($p<0.05$) from the control group

Table 10 presents the mean litter data with respect to cumulative days to litter and the number of live pups per litter. The cumulative number of days to litter were consistently longer for the pairs

fed diet with 0.3% theophylline (Table 10A). Further, theophylline feeding resulted in a dose-dependent decrease in the number of live pups per litter (Table 10B).

Table 10: Mean litter data during continuous breeding phase (NTP 1985a)

A. Cumulative days to litter

	Cumulative days to litter				
Litter:	*1st*	*2nd*	*3rd*	*4th*	*5th*
Control	21.4±0.54	43.8±1.49	64.4±1.56	83.5±1.36	103.0±0.81
0.075%	23.7±1.24	44.2±1.44	64.4±1.47	85.5±1.62	104.5±1.23
0.15%	21.3±0.30	44.2±1.23	67.7±2.98	85.5±2.60	102.92±1.08
0.3%	23.5±0.92	50.4±2.61[a]	71.7±3.06[a]	91.2±2.88[a]	108.1±1.42[a]

[a] significantly different ($p<0.05$) compared to control value (Morrisey et al., 1988)

B. No. live pups in each litter

	No. live pups in each litter				
Litter:	*1st*	*2nd*	*3rd*	*4th*	*5th*
Control	11.1±0.43	13.1±0.40	12.5±0.58	12.0±0.65	11.3±0.74
0.075%	9.6±0.89	9.3±0.87[a]	9.5±0.78[a]	9.2±1.33[a]	9.0±0.75
0.15%	8.4±0.80[a]	8.8±1.00[a]	8.7±0.94[a]	7.7±0.99[a]	8.5±1.29[a]
0.3%	8.7±0.74[a]	6.3±0.70[a]	6.9±0.80[a]	6.2±0.89[a]	6.4±1.56

[a] significantly different ($p<0.05$) compared to control value (Morrisey et al., 1988)

Cross-over mating trial

The continuous breeding portion of the protocol, indicated that theophylline treatment significantly affected fertility in CD-l mice. Since this part of the protocol does not discriminate which sex (or sexes) is susceptible to the chemical exposure, it was followed by a 1-week crossover mating trial. This trial was conducted after continuous theophylline treatment for 19 weeks. During this trial, animals from the 0.3% dose group were tested in a crossover mating trial to determine whether the males or females or both sexes had compromised reproductive performance when matched with control animals. The females treated with 0.3% theophylline and cohabited with control males had fewer fertile matings than the control pairs (53% as compared to 72%, respectively, Table 11) but the response was not statistically significant ($p>0.05$). The corresponding value for 0.3% male × control female group was 68% (Table 10). A statistically significant decrease ($p<0.05$) was noted with respect to the proportion of pups born alive and the average live pup weight (absolute as well as adjusted) in the control male × 0.3% female group relative to the control values (Table 12). These parameters were not significantly affected ($p>0.05$) for pups delivered by control females mated with theophylline treated males (Table 12).

Table 11: Mating and fertility of pairs after a cross-over mating trial (NTP 1985a)

Treatment group	**No. with copulatory plugs / no cohabited**	**Mating index (%)[a]**	**No. fertile / no. cohabited**	**Fertility index (%)[b]**
Control male × control female	14/18[c]	78	13/18	72
0.3% male × control female	14/19[d]	74	13/19	68
Control male × 0.3% female	14/19[e,f]	74	10/19	53

[a] mating index (%): no. with copulatory plugs / no. cohabited × 100

[b] fertility index (%)

[c] although not detected by direct means, two females were scored plug-positive based on delivery of litters

[d] although not detected by direct means, nine females were scored plug-positive based on delivery of litters
[e] although not detected by direct means, three females were scored plug-positive based on delivery of litters
[f] one female in the 0.3% dose group died on the second day of cohabitation (week 19); data from this pair were excluded

Table 12: Reproductive performance of fertile pairs after a cross-over mating trial (NTP 1985a)

Reproductive parameter[a]	Control male × control female	0.3% male × control female	Control male × 0.3% female
Live pups per litter			
male	4.31±0.593 (13)[c]	4.23±0.0521 (13)	3.50±0.687 (10)
female	5.08±0.909 (13)	4.46±0.882 (13)	3.30±0.731 (10)
combined	9.38±1.147 (13)	8.69±1.100 (13)	6.80±1.263 (10)
Proportion of pups born alive	1.00±0.000 (13)	0.91±0.077 (13)	0.84±0.102 (10)[e]
Sex of pups born alive (males/total)	0.50±0.054 (13)	0.53±0.054 (12)[d]	0.52±0.058 (9)[d]
Live pup weight (g)			
male	1.75±0.060 (13)	1.69±0.038 (12)	1.53±0.070 (9)[g]
female	1.67±0.068 (13)	1.57±0.040 (12)	1.47±0.058 (9)
combined	1.71±0.062 (13)	1.64±0.034 (12)	1.51±0.070 (9)[e]
Adjusted live pup weight (g)[b]			
male	1.76±0.037 (13)	1.71±0.039 (12)	1.50±0.045 (9)[f]
female	1.68±0.047 (13)	1.58±0.049 (12)	1.43±0.057 (9)[f]
combined	1.72±0.037 (13)	1.66 ± 0.038 (12)	1.47±0.045 (9)[f]

[a] mean±SE
[b] means adjusted for total number of live and dead pups per litter by analysis of covariance
[c] number of fertile pairs providing the data indicated in parenthesis
[d] one litter in this group contained no live pups
[e] significantly different ($p<0.05$) from the control group
[f] significantly different ($p<0.01$) from the control group and 0.3% male × control female group
[g] significantly different ($p<0.05$) from the control group and 0.3% male × control female group

The results of the cross-over mating trail suggested that the female CD-l mice may be more sensitive to the effects of continuous theophylline treatment. Both male and female animals were necropsied. Detailed sperm morphology and vaginal cytology evaluations (SMVCE) were also performed.

The group mean whole body and liver weights in the female mice fed theophylline containing diet were significantly higher than the control values ($p<0.05$) but there was no difference with respect to the average kidneys weight. For male mice, significant differences ($p<0.05$) were noted with respect to the average whole body (decreased), liver (increased), and seminal vesicles (decreased) weights at necropsy. The body and organ weights for individual female mice are presented in Table 13.

SMVCE studies showed that the cauda epididymal sperm counts were reduced in the theophylline exposed mice, and the pair-wise comparisons were statistically significant ($p<0.05$; Table 14). The sperm motility values for the control and treated male mice were essentially the same (83% vs. 86%). The incidence of abnormal sperm was also not affected by theophylline treatment (Table 14).

SMVCE studies with female mice revealed that theophylline treatment does not interfere with the relative frequency of various estrous stages and the average estrous cycle length (Table 15).

Table 13: Organ weight (at necropsy) of CD-1 mice during cross-over mating trial with theophylline (NTP 1985a).

	Treatment group	
Variable[a]	**control**	**0.3%**
females		
Body weight	36.16±0.5829 (37)[b]	38.07±0.5569 (19)[c]
Liver (g)	2.122±0.0549 (37)	2.459±0.0785 (19)[c]
Kidneys (g)	0.581±0.0113 (37)	0.606±0.0147 (19)
males		
Body weight	39.96±0.5991 (40)[b]	37.13±0.6980 (20)[c]
Liver (g)	2.046±0.0403 (40)	2.191±0.0563 (20)[c]
Kidneys (g)	0.763±0.0160 (40)	0.749±0.0231 (20)
R. Epididymis (g)	0.060±0.0010 (40)	0.061±0.0014 (20)
R. Cauda (g)	0.022±0.0012 (40)	0.021±0.0009 (20)
R. Testis (g)	0.136±0.0027 (40)	0.136±0.0039 (20)
Seminal vesicles (g)	0.656±0.0156 (40)	0.497±0.0206 (20)[c]
Prostate gland (g)	0.041±0.0020 (40)	0.038±0.0033 (20)

[a] mean±SE
[b] number of animals providing the data indicated in parenthesis
[c] significantly different ($p<0.05$) from the control group

Table 14: Summary of data of sperm evaluation (NTP 1985a)

	Weight (g)				Sperm motility (%)	Sperm density[a] × 10^6	Abnormal sperm (%)
	bodyweight	**R. Cauda**	**R. Epidid**	**R. testis**			
Control[b]	40.0±0.6[c]	0.022±0.0012	0.060±0.0010	0.136±0.0027	82.8±2.48	927±38.9	3.41±0.34
0.3% [b]	37.1±0.7	0.021±0.0009	0.061±0.0014	0.136±0.0039	85.9±2.80	741±40.8[d]	3.58±0.44

[a]: per g cauda tissue
[b] Number of animals: 40 for controls and 20 for 0.3% theophylline group
[c] Mean ± SE
[d] Significantly different ($p<0.05$) from the control group

Table 15: Summary of vaginal cytology evaluation (NTP 1985a)

	Total no. animals per group	Relative frequency of estrous stages					Average cycle length (days) Group mean±SE
		%P	%E	%M	%D	%NC/NoC	
Control	37	14.7 (37)	25.1 (37)	14.7 (37)	45.6 (37)	0.0 (37)	4.6±0.14 (18)[a.b]
0.3%	19	17.3 (19)	33.1 (19)	21.8 (19)	27.8 (19)	0.0 (19)	4.3±0.16 (15)[c]

[a] number of animals providing the data indicated in parenthesis
[b] in 19 out of the 37 experimental animals, estrous cycle length was >7 days or not clear
[c] in 4 out of the 19 experimental animals, estrous cycle length was not clear
P=proestrous; E=estrous; M=metestrus; D=diestrous, NC/NoC = not clear or no cells

Mouse oral screening study for effect on male fertility (Harris et al. 1992)
Groups of male Swiss CD-1 mice (n=10/group) were exposed by gavage to 0, 20, 60 and 200 mg theophylline/kg bw/day (vehicle corn oil) for 17 days and then necropsied. No adverse clinical signs

were noted and body weights and histology of liver and kidneys were unaffected. No effect on the weights of testes and epididymides, sperm density per cauda and sperm motility were found (table 16). At the high-dose level, theophylline induced mild changes in the testis epithelium, consisting primarily of asynchronous germ cell development and focal loss of germ cells within individual tubules.

Table 16: Male organ weights, sperm parameters and histology scores following exposure with theophylline in a 21-day reproductive/developmental screening study (Harris et al., 1992).

	Theophylline dose (mg/kg bw/d)				
	0	**20**	**60**	**200**	**Trend**
Testis weight (mg)	118±5.5	119±5.0	112±4.9	123±5.3	NS
Epididymis weight (mg)	46.0±1.4	45.9±3.2	44.9±3.3	54.1±3.2	NS
No. sperm/ g cauda ($\times 10^6$)	1080±101	1085±116	887±118	888±134	NS
% motile sperm	78.5±3.2	81.3±4.6	81.8±4.1	80.4±4.5	NS
Histology score	0.2±0.1	0.5±0.2	0.2±0.1	0.7±0.2	

Note: Trend, performed using Jonckheere's test; NS, not significantly different from control; ND, not determined
Mean±SEM (based on n=10 datapoints)

Mouse oral screening study for effect on female and male fertility (Harris et al. 1992)
Groups of female Swiss CD-1 mice (n=10/group) were dosed by gavage with 0, 20, 60 and 200 mg theophylline/kg bw/day (vehicle corn oil) for 19 days. After seven days of dosing these females were cohabited with male mice that had been treated for five days prior to mating (and were continued being treated until day 5 of cohabitation). After 19 days of dosing the females were killed and the numbers of live and dead foetuses and implantation sites were recorded.
No adverse clinical signs were found. One female in the high-dose group was killed moribund. Pregnancy rate was non-significantly decreased in the high-dose group (6/9 vs. 9/10 in all other groups; Table 17). There were no effects on the numbers of live or dead foetuses or the number total implants per female.

Table 17: Impregnation and uterine implant data from continuously exposed females following chemical exposure in a 21-day reproduction/developmental screening study (Harris et al., 1992)

	Theophylline dose (mg/kg bw/d)				
	0	**20**	**60**	**200**	**Trend**
No. pregnant (no. treated)	9 (10)	9 (10)	9 (10)	6 (9) [1]	NS
No. live implants per female[a]	10.0±0.5	10.0±1.1	10.4±0.4	10.0±1.1	NS
No. dead implants per female[a]	0.4±0.2	0.3±0.2	1.0±0.4	1.2±0.7	NS
Total implants per female[a]	10.4±0.4	10.3±1.1	11.4±0.3	11.2±1.2	NS

Note: Trend, performed using Jonckheere's test; NS, not significantly different from control; [*n*] number of mice dying during theophylline exposure
[a]Mean±SEM

Mouse oral 14-weeks toxicity studies (NTP 1998)

Within the US National Toxicology Program 14 weeks' studies were done in B6C3F1 mice (groups of 10 m, 10f), one with dosing via gavage the other with dosing via the diet. Via gavage, theophylline was applied in dose levels of 0, 75, 150 or 300 mg/kg bw/d (vehicle corn oil). Via feed, theophylline was applied in dose levels of 0, 184/229, 401/418, 793/856 (m/f) mg/kg bw/d. General effects are described under 4.11.3. At the end of the studies, sperm samples were collected from all males for sperm morphology evaluations. The following parameters were evaluated: sperm motility, percent abnormal sperm, and sperm concentration. The right cauda, right epididymis, and right testis were weighed. Vaginal samples were collected for up to 7 consecutive days prior to the end of the studies from all females for vaginal cytology evaluations. The following parameters were evaluated: relative frequency of estrous stages and estrous cycle length.

In the gavage study, mortality was increased at the high dose level (three males and all females), body weights were reduced at 150 and 300 mg/kg bw/day and absolute testes weights were decreased at 300 mg/kg bw/day. There were no biologically significant differences in sperm morphology or vaginal cytology parameters between control and dosed mice.

In the feeding study body weights were decreased at all dose levels (200, 400, 800 mg/kg bw/day) but no mortality occurred. There were no biologically significant differences in sperm morphology or vaginal cytology parameters between control and exposed mice.

Tables 18 and 19 provide an overview of the effects of theophylline (gavage and feed, respectively) on terminal body weight and reproductive organ weights, sperm characteristics, and estrous cycle.

Table 18: Effects of theophylline (gavage) on terminal body weight and reproductive organ weights, sperm characteristics, and estrous cycle of B6C3F1 mice (NTP 1998)*

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
n	10	9	10	7
Weights (g)				
Necropsy body wt	37.9 ± 1.4	35.6 ± 1.2	33.6 ± 0.6**	33.1 ± 0.8**
R. Cauda epididymis	0.019 ± 0.001	0.018 ± 0.001	0.020 ± 0.001	0.016 ± 0.001
R. Epididymis	0.044 ± 0.001	0.042 ± 0.001	0.045 ± 0.001	0.042 ± 0.001
R. Testis	0.119 ± 0.001	0.113 ± 0.002*	0.117 ± 0.002	0.110 ± 0.002**
Epididymal spermatozoal measurements				
Sperm motility (%)	81.82 ± 0.57	81.02 ± 1.27	81.03 ± 0.59	81.30 ± 0.48
Abnormal sperm (%)	0.88 ± 0.14	1.29 ± 0.20	1.02 ± 0.12	1.40 ± 0.20
Concentration (10^6/g cauda epididymal tissue)	888 ± 33	934 ± 60	849 ± 35	1038 ± 74
Female				
n	9	10	10	1
Weights (g)				
Necropsy body wt (g)	30.4 ± 0.6	27.9 ± 0.4*	29.0 ± 0.8	—[b]
R. Ovary	0.013 ± 0.001	0.012 ± 0.001[c]	0.012 ± 0.001	—
Uterus	0.161 ± 0.015	0.148 ± 0.009	0.151 ± 0.012	—
Estrous cycle length (days)	4.44 ± 0.18	4.80 ± 0.20	4.70 ± 0.21	—[d]
Estrous stages[e] (% of cycle)				
Diestrus	20.6	20.0	20.0	0.0
Proestrus	23.8	22.9	20.0	0.0
Estrus	34.9	37.1	38.6	57.1
Metestrus	20.6	20.0	21.4	42.9

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' (male necropsy body weights) or Dunnett's (right testis weight) test
[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the vehicle control group were not significant by Dunnett's test (right cauda weight, right epididymis weight, female organ weights) or Dunn's test (epididymal spermatozoal measurements and estrous cycle lengths).
[b] One female survived to the end of dosing but died prior to necropsy. Necropsy body weights and organ weights are not available for this animal.
[c] n=9
[d] Estrous cycle was longer than 7 days or unclear in the surviving mouse in this dose group.
[e] Evidence shows that the surviving female exposed to 300 mg/kg differed significantly (Wilk's Criterion, $P \leq 0.05$) from vehicle control females in the relative length of time spent in the estrous stages. This female spent more time in estrus and metestrus and less time in diestrus and proestrus than vehicle control females.

* a discrepancy is noted related to the presentation of the results of the repeated dose studies by NTP (1998) and by Morrissey et al. (1988). It is noted that different statistical analyses were applied to the data. In this CLH-dossier the results as presented and evaluated by NTP (1998) have been primarily used.

Table 19: Effects of theophylline (feed) on terminal body weight and reproductive organ weights, sperm characteristics, and estrous cycle of B6C3F1 mice (NTP 1998)*

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
n	10	10	10	10
Weights (g)				
Necropsy body wt	34.4 ± 1.0	31.4 ± 0.4**	29.7 ± 0.5**	29.5 ± 0.3**
R. Cauda epididymis	0.019 ± 0.001	0.022 ± 0.001	0.022 ± 0.001*	0.020 ± 0.001
R. Epididymis	0.045 ± 0.001	0.048 ± 0.001	0.050 ± 0.001*	0.049 ± 0.002
R. Testis	0.116 ± 0.001	0.121 ± 0.001	0.116 ± 0.003	0.115 ± 0.001
Epididymal spermatozoal measurements				
Sperm motility (%)	76.34 ± 0.71	75.81 ± 0.57	76.63 ± 0.75	76.37 ± 0.75
Abnormal sperm (%)	1.18 ± 0.18	1.28 ± 0.20	1.08 ± 0.14	1.46 ± 0.29
Concentration (10^6/g cauda epididymal tissue)	876 ± 39	798 ± 23	776 ± 42	817 ± 44
Female				
n	10	10	10	10
Weights (g)				
Necropsy body wt	30.0 ± 0.6	27.8 ± 0.6*	28.0 ± 0.4*	27.9 ± 0.3*
R. Ovary	0.013 ± 0.001	0.012 ± 0.001[b]	0.013 ± 0.001	0.013 ± 0.001
Uterus	0.161 ± 0.010	0.157 ± 0.011	0.143 ± 0.010	0.153 ± 0.013
Estrous cycle length (days)	4.38 ± 0.18[c]	4.78 ± 0.22[d]	4.11 ± 0.26[d]	4.44 ± 0.18[d]
Estrous stages (% of cycle)				
Diestrus	30.0	24.3	28.6	24.3
Proestrus	18.6	22.9	20.0	21.4
Estrus	28.6	32.9	30.0	32.9
Metestrus	22.9	20.0	21.4	21.4

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** (P≤0.01)
[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the control group were not significant by Dunnett's test (right testis weights and female organ weights) or Dunn's test (epididymal spermatozoal measurements and estrous cycle lengths). By multivariate analysis of variance, exposed females did not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] n=9
[c] n=8; estrous cycle was longer than 7 days or unclear in 2 of 10 animals.
[d] n=9; estrous cycle was longer than 7 days or unclear in 1 of 10 animals.

* a discrepancy is noted related to the presentation of the results by NTP (1998) and by Morrissey et al. (1988). It is noted that different statistical analyses were applied to the data. In this CLH-dossier the results as presented and evaluated by NTP (1998) have been primarily used.

Rat oral 14-weeks toxicity studies (NTP 1998)

Within the US National Toxicology Program 14 weeks' studies were done in F344 rats (using groups of 10m, 10f), one with dosing via gavage the other with dosing via the diet. Via gavage, theophylline was applied in dose levels of 0, 37.5, 75 or 150 mg/kg bw/d (vehicle corn oil). Via feed, theophylline was applied in dose levels of 0, 66/67, 129/135, 258/264 (m/f) mg/kg bw/d. General effects are described under 4.11.3. At the end of the studies, sperm samples were collected from all males for sperm morphology evaluations. The following parameters were evaluated: sperm motility, percent abnormal sperm, and sperm concentration. The right cauda, right epididymis, and right testis were weighed. Vaginal samples were collected for up to 7 consecutive days prior to the

end of the studies from all females for vaginal cytology evaluations. The following parameters were evaluated: relative frequency of estrous stages and estrous cycle length.

In the gavage study testes weights and relative uterus weights were slightly (non-significantly) decreased at the highest dose level of 150 mg/kg bw/day only. There were no significant differences in sperm morphology or vaginal cytology parameters between control and dosed rats. Survival, body weight, feed consumption were not affected. Mean cell volume and mean cell haemoglobin levels were increased in males at 150 mg/kg bw/day. There was a dose related increase in periarteritis in both sexes that was significant in females at the high dose.

In the feeding-study absolute epididymis weights were increased at 130 mg/kg bw/day (but not at 260 mg/kg, the highest dose level); the cauda epididymis weights were (non-significantly) decreased at 260 mg/kg only. There were no significant differences between control and exposed rats in sperm morphology or vaginal cytology parameters. The percentage of abnormal sperm was increased at the high dose, though this was not significant. Oestrus cycle length was not affected in any group. Mean cell volume and mean cell haemoglobin levels were increased in males at 130 and 260 mg/kg bw/day. Kidney weight was increased in males and lung weight in females at 260 mg/kg bw/day. There was a dose related increase in kidney nephropathy in males and an increase in periarteritis in both sexes that was significant in females at the high dose. Survival, body weight, feed consumption were not affected..

Tables 20 and 21 provide an overview of the effects of theophylline (gavage and feed, respectively) on terminal body weight and reproductive organ weights, sperm characteristics, and estrous cycle.

Table 20: Summary of reproductive tissue evaluations and estrous cycle characterization for F344 rats in the 14-week *gavage* study of theophylline (NTP 1998)*

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg
Male				
n	10	10	10	9
Weights (g)				
Necropsy body wt	336 ± 5	334 ± 5	329 ± 5	321 ± 5
R. Cauda epididymis	0.216 ± 0.012	0.225 ± 0.005	0.216 ± 0.004	0.199 ± 0.004
R. Epididymis	0.442 ± 0.013	0.451 ± 0.009	0.447 ± 0.006	0.421 ± 0.005
R. Testis	1.471 ± 0.020	1.553 ± 0.039	1.496 ± 0.011	1.429 ± 0.027
Epididymal spermatozoal measurements				
Sperm motility (%)	78.67 ± 0.97	80.26 ± 1.08	81.53 ± 1.96	79.58 ± 0.94
Abnormal sperm (%)	0.72 ± 0.12	0.96 ± 0.18	0.82 ± 0.11	0.80 ± 0.12
Concentration (10^6/g cauda epididymal tissue)	394 ± 20	382 ± 13	359 ± 16	413 ± 21
Female				
n	10	10	10	10
Weights (g)				
Necropsy body wt	203 ± 2	198 ± 2	209 ± 3	216 ± 3**[b]
R. Ovary	0.046 ± 0.004	0.062 ± 0.006	0.057 ± 0.004	0.049 ± 0.006[b]
Uterus	0.595 ± 0.072	0.525 ± 0.044	0.587 ± 0.067	0.416 ± 0.034[b]
Estrous cycle length (days)	4.40 ± 0.16	4.89 ± 0.20[c]	4.56 ± 0.29[c]	4.86 ± 0.14[d]
Estrous stages[e] (% of cycle)				
Diestrus	30.0	21.4	35.7	35.7
Proestrus	14.3	25.7	14.3	11.4
Estrus	34.3	35.7	25.7	24.3
Metestrus	21.4	15.7	24.3	27.1
Uncertain diagnoses	0.0	1.4	0.0	1.4

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test

[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the vehicle control group were not significant by Dunnett's test (male necropsy body weights and male and female organ weights) or Dunn's test (epididymal spermatozoal measurements and estrous cycle lengths).

[b] n=9

[c] n=9; estrous cycle was longer than 7 days or unclear in 1 of 10 animals.

[d] n=7; estrous cycle was longer than 7 days or unclear in 3 of 10 animals.

[e] Evidence shows that females exposed to 37.5 or 150 mg/kg differ significantly (Wilk's Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages. Females in the 37.5 mg/kg group spent more time in proestrus and less time in diestrus and metestrus than vehicle control females. Females in the 150 mg/kg group spent more time in diestrus and metestrus and less time in estrus than the vehicle control females.

* a discrepancy is noted related to the presentation of the results by NTP (1998) and by Morrissey et al. (1988). It is noted that different statistical analyses were applied to the data. In this CLH-dossier the results as presented and evaluated by NTP (1998) have been primarily used.

Table 21: Summary of reproductive tissue evaluations and estrous cycle characterization for F344 rats in the 14-week *feed* study of theophylline (NTP 1998)*

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
n	10	10	10	10
Weights (g)				
Necropsy body wt	351 ± 8	368 ± 6	364 ± 4	344 ± 6
R. Cauda epididymis	0.202 ± 0.006	0.205 ± 0.007	0.213 ± 0.004	0.185 ± 0.005
R. Epididymis	0.412 ± 0.011	0.428 ± 0.007	0.441 ± 0.005*	0.417 ± 0.007
R. Testis	1.440 ± 0.046	1.484 ± 0.025	1.491 ± 0.018	1.441 ± 0.029
Epididymal spermatozoal measurements				
Sperm motility (%)	77.61 ± 0.55	78.16 ± 0.41	77.70 ± 0.82	77.48 ± 0.77
Abnormal sperm (%)	0.84 ± 0.09	0.96 ± 0.11	1.16 ± 0.21	1.32 ± 0.16
Concentration (10^6/g cauda epididymal tissue)	438 ± 23	395 ± 20	400 ± 21	450 ± 18
Female				
n	10	10	10	10
Weights (g)				
Necropsy body wt	207 ± 3	222 ± 3	206 ± 5	202 ± 8
R. Ovary	0.055 ± 0.006	0.053 ± 0.002	0.064 ± 0.009	0.046 ± 0.003
Uterus	0.509 ± 0.037	0.589 ± 0.067	0.584 ± 0.045	0.449 ± 0.061
Estrous cycle length (days)	4.67 ± 0.29[b]	4.67 ± 0.29[b]	5.13 ± 0.30[c]	5.22 ± 0.28[b]
Estrous stages (% of cycle)				
Diestrus	35.7	34.3	34.3	37.1
Proestrus	18.6	15.7	12.9	15.7
Estrus	25.7	27.1	30.0	31.4
Metestrus	20.0	15.7	22.9	15.7
Uncertain diagnoses	0.0	7.1	0.0	0.0

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test

[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the control group were not significant by Dunnett's test (necropsy body weights, right cauda weights, right testis weights, and female organ weights) or Dunn's test (epididymal spermatozoal measurements and estrous cycle lengths). By multivariate analysis of variance, exposed females did not differ significantly from the control females in the relative length of time spent in the estrous stages.

[b] n=9; estrous cycle was longer than 7 days or unclear in 1 of 10 animals.

[c] n=8; estrous cycle was longer than 7 days or unclear in 2 of 10 animals.

* a discrepancy is noted related to the presentation of the results by NTP (1998) and by Morrissey et al. (1988). It is noted that different statistical analyses were applied to the data. In this CLH-dossier the results as presented and evaluated by NTP (1998) have been primarily used.

Rat oral 75-weeks toxicity study (Weinberger et al, 1978)

Groups of 20 male Osborne-Mendel rats received diet containing 0 or 0.5% theophylline. Conversion from 0.5%, assuming 50 g feed / kg bw results in 250 mg theophyline /kg bw/day. After 14 weeks 6 rats were sacrified for hematology, limited clinical chemistry, limited organ weights and histopathology. The remaining rats were sacrificed after 75 weeks. The same parameters were determined with the addition of blood cholesterol and cytogenetic analyses of sperm cells.

Survival and body weight were not affected at week 14. There was no effect on testis weight or histopathology.

Survival, hematology, clinical chemistry, cholesterol and sperm cell cytogenetic analysis was not affected at week 75 but body weight was reduced. There was an increase in relative kidney, adrenal

and pituitary weights. There was no effect on relative testis weight but the incidence of testis histopathological effects was non-significantly increased.

Rat oral 19-weeks toxicity study (Weinberger et al, 1978)
Male Holtzman rats received diet containing 0% (n=35) or 0.5% (n=24) theophylline for 19 weeks. Conversion from 0.5%, assuming 50 g feed / kg bw results in 250 mg theophyline /kg bw/day. Examinations were limited to testes, accessory sexual organs, and some clinical chemistry parameters.
Mortality was increased whereas weight gain, food intake and food efficiency were decreased. Mortality occurred mainly during the first 5 weeks and appeared to be due to pulmonary disease. Relative testicular weight was not affected but histopathology revealed an increase in testes atrophy and oligospermatogenesis. Blood triglyceride levels were increased.

Table 22: Incidence of testicular atrophy and impaired spermatogenesis in rats fed theophylline (250 mg/kg bw/d) (Weinberger et al., 1978).

		Testes			
	No. of rats	**normal**	**atrophy**	**Oligosperma-togeneisis**	**Aspermato-genesis**
14 weeks[a]					
control	6	6 (100)	-	-	-
theophylline	6	6 (100)	-	-	-
19 weeks[a]					
control	15	15 (100)	-	-	-
theophylline	7	1 (14)[b]	6 (86)[b]	5 (71)[b]	1 (14)
75 weeks[a]					
control	6	6 (100)	-	-	-
theophylline	6	3 (50)	3 (50)	2 (33)	1 (17)

Percentages are given in parentheses
[a] Osborne-Mendel rats used for the 14- and 75 week exposures, Holtzman rats used for the 19 week exposure
[b] Significantly different from control value, $p<0.05$

4.11.1.2 Human information

No data available

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

MOUSE STUDIES

Mouse oral continuous breeding study (NTP 1985a; Lamb et al 1997; Morrissey et al. 1988)
Theophylline was tested for its effect on reproduction in Swiss CD-1 mice according to the Reproductive Assessment by Continuous Breeding (RACB) design used by the National Toxicology Program. See section 4.11.1.1 for a detailed description of the study characteristics and results Adverse effects on development were noted in the continuous breeding phase. There was a significant decrease ($p<0.01$) in the mean number of litters per fertile pair at the 0.3% theophylline

level (19% reduction; see the table under 4.11.1.1). The number of live pups per litter was significantly reduced ($p<0.01$) at all three dose levels relative to the control group. The proportion of pups born alive was significantly decreased ($p<0.05$) at the 0.15 and 0.3% dose levels. The analysis of covariance indicated that the mean live pup weights when adjusted for the total number of live and dead pups per litter were significantly lower ($p<0.05$; 6% decrease) in the high dose group (0.3% theophylline) than the control values (see the table under 4.11.1.1). Also in the crossover mating trial, some adverse effects on development were noted. In the group cohabiting control males and high-dose females, the proportion of pups born alive was reduced by 16% and the adjusted pup weight was reduced by 15%.

Mouse oral developmental toxicity study (NTP 1985b; Lindström et al. 1990)
Theophylline was administered to groups of 23-33 pregnant Swiss (CD-1) mice in the drinking-water at concentrations of 0, 0.075, 0.15 or 0.2% from day 6 through 15 of gestation (dose levels equal to 0, 282, 372 or 396 mg theophylline/kg bw/day based on water consumption). Dose levels were based on a dose-range finding study. Clinical signs were recorded. Maternal bodyweights were measured daily. At day 17 the mice were killed. Gravid uterine weights were measured. The numbers of implantation sites, resorptions, dead fetuses, live fetuses and fetal weights were recorded. All live foetuses were examined for visceral abnormalities. Half of the fetuses were decapitated prior to dissection and the heads were fixed in Bouin's solution, sectioned and examined. All foetuses were examined for skeletal malformations.
Approximately equal numbers of CD-l females were assigned to each treatment group within each replicate of the teratology study. Due to a low pregnancy rate in the mice, a three replicate design was necessary in order to provide a minimum of 20 pregnant animals per dose group. During treatment (i.e., GD 6 through 15), females were observed once daily for clinical signs.

There were no maternal deaths. The primary clinical signs associated with theophylline treatment were piloerection, rough coat and weight loss. Other signs of toxicity observed during and after treatment were lethargy, hunched back, and dehydration. Clinical signs associated with the use of mesh-covered feed jars included alopecia at various sites on the body, sores on legs and at the corner of the eye, ruffled fur, and discolored fur. A total of 4 animals were removed from the study due to delivery of pups prior to scheduled sacrifice (1), death due to drowning because of a leaking water bottle (1), death due to starvation and/or dehydration as a result of refusal to eat or drink (2) . Upon verification of nonpregnant status at scheduled sacrifice, 29 animals were removed from further evaluation. At sacrifice, pregnancy was confirmed in 74.3% (26/35), 78.8% (26/33), 91.7% (33/36) and 69.7% (23/33) of the females from the vehicle, 0.075, 0.15, and 0.20% theophylline groups, respectively.

Table 23 presents an overview of the general maternal effects. Maternal feed consumption was not affected by treatment, whereas drinking water consumption was reduced during gestation and treatment in the 0.15% and 0.20% group. Measures of maternal weight gain (i.e. gestation period, treatment period, or maternal body weight on GD17 corrected for gravid uterine weight) showed a treatment-related decreasing trend. Maternal body weight gain during gestation and corrected for gravid uterine weight were decreased at 0.15 and 0.2% (statistically significant), while weight gain during treatment was significantly decreased only in the 0.2% group. Gravid uterine weight was decreased at 0.2% (statistically significant). Absolute maternal liver weight was significantly decreased in the 0.15% and 0.20% groups, while relative maternal liver weight was unaffected by treatment.

Table 24 presents an overview of the pregnancy parameters and the developmental toxicity data.

The numbers of corpora lutea per dam (being 12.6±0.75, 11.88±0.71, 11.97±0.83 and 10.05±1.28 at control, low, mid and high dose group, respectively (not presented in this table)) and the numbers of implantation sites were not affected. The percentage of resorptions per litter was increased at 0.15 and 0.2% (statistically significant) (percentages 10, 14, 27 and 34 at 0, 0.075, 0.15 and 0.2% respectively). There were no differences in the percentages pre-implantation loss.

The average male and female fetal weight per litter was decreased at 0.15 and 0.2% (statistically significant). The number of externally malformed foetuses (mainly cleft palate) was slightly increased (not statistically significant) at 0.15 and 0.2% (incidences 1/296, 2/278, 5/300, 4/197 at 0, 0.075, 0.15 and 0.2% respectively). Incidences of visceral or skeletal malformations and variations were not increased. Lindström et al. (1990) concluded that the NOAEL for maternal and developmental toxicity was 282 mg/kg bw/day.

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Table 23: Maternal Toxicity of theophylline in mice (NTP 1985b; Lindstrom, 1990)

MATERNAL TOXICITY IN SWISS (CD-1) MICE CONSUMING THEOPHYLLINE IN DRINKING WATER ON DAYS 6 THROUGH 15 OF GESTATION

	Theophylline (% in drinking water)			
	0	0.075	0.15	0.20
Subjects (Dams)				
Total treated	35	34	37	35
No. removed	0	1[a]	1[b]	2[c]
Nonpregnant	9	7	3	10
No. (%) pregnant at termination	26 (74)	26 (79)	33 (92)	23 (70)
Maternal weight gain (g)[d]				
Gestation (0–17)[f]	22.7 ± 1.1	20.4 ± 1.2	17.9 ± 1.2*	15.9 ± 1.8*
Treatment (6–15)[f]	14.7 ± 0.9	14.5 ± 1.0	12.7 ± 0.9	8.0 ± 1.6*
Corrected[e,f]	6.1 ± 0.5	4.9 ± 0.4	4.6 ± 0.4*	4.2 ± 0.5*
Gravid uterine weight (g)[f]	16.7 ± 0.9	15.5 ± 1.0	13.3 ± 0.9	11.7 ± 1.5*
Maternal liver weight				
Absolute (g)[f]	2.75 ± 0.08	2.63 ± 0.06	2.51 ± 0.06*	2.37 ± 0.09*
Relative (% body weight)	5.36 ± 0.11	5.41 ± 0.07	5.34 ± 0.09	5.33 ± 0.09
Maternal feed consumption (g/kg/day)				
Gestation[f]	197 ± 6	199 ± 5	208 ± 9	216 ± 9
Treatment[f]	177 ± 4	181 ± 6	192 ± 8	196 ± 8
Maternal water consumption (g/kg/day)				
Gestation[f]	360 ± 11	374 ± 10	309 ± 11*	295 ± 12*
Treatment[f]	352 ± 9	376 ± 13	248 ± 12*	198 ± 10*
Calculated dose (mg/kg/day)	0	282	372	396

[a] One dam delivered early.
[b] One dam died due to accidental causes.
[c] Two dams died (GD 12, GD 14) due to refusal to eat or drink.
[d] Includes all dams pregnant at termination mean ± SEM.
[e] Corrected body weight gain = weight at termination minus initial weight and gravid uterine weight.
[f] Test for linear trend ($p < 0.05$).
* Statistically significant ($p < 0.05$) relative to the vehicle control group.

Table 24: Developmental Toxicity of theophylline in mice (NTP 1985b; Lindstrom, 1990)

DEVELOPMENTAL TOXICITY IN SWISS (CD-1) MICE FOLLOWING MATERNAL EXPOSURE TO THEOPHYLLINE IN THE DRINKING WATER ON DAYS 6 THROUGH 15 OF GESTATION

	Theophylline (% in drinking water)			
	0	0.075	0.15	0.20
All litters (No.)[a]	26	26	33	23
Implantation sites/litter[b]	12.4 ± 0.6	12.6 ± 0.5	11.9 ± 0.6	11.2 ± 0.8
% Preimplantation loss[b,c]	7 ± 2	6 ± 2	7 ± 2	3 ± 2
% Resorptions/litter[c]	10 ± 4	14 ± 4	27 ± 5*	34 ± 9*
No. (%) litters with resorptions[f]	14 (54)	18 (69)	29[g] (88)	18 (78)
No. (%) litters totally resorbed	1 (4)	1 (4)	4 (12)	6 (26)
No. litters with live fetuses[d]	25	25	29	17
Live fetuses/litter[b]	11.8 ± 0.5	11.1 ± 0.6	10.3 ± 0.4	11.6 ± 0.5
Average male fetal body weight per litter[b,c]	1.05 ± 0.03	1.02 ± 0.02	0.92 ± 0.03*	0.88 ± 0.02*
Average female fetal body weight per litter[b,c]	1.03 ± 0.03	0.98 ± 0.02	0.90 ± 0.03*	0.86 ± 0.03*
% Fetuses malformed/litter[b,c]	0.3 ± 0.3	0.7 ± 0.5	3.0 ± 1.5	2.3 ± 1.4
No. (%) litters with malformed fetuses[e]	1 (4)	2 (8)	5 (17)	3 (18)

[a] Includes all dams with implantation sites at termination.
[b] Reported as mean ± SEM.
[c] Defined as [(No. corpora lutea − No. implantation sites) ÷ No. corpora lutea] × 100.
[d] Includes only dams with live fetuses; litter size = number live fetuses per dam.
[e] Test for linear trend ($p < 0.05$).
[f] χ^2 test ($p < 0.05$).
[g] Fisher's exact test ($p < 0.05$).
* Statistically significant ($p < 0.05$) relative to the vehicle control group.

Table 25: Developmental Toxicity of theophylline in mice (NTP 1985b; Lindstrom, 1990)

MORPHOLOGIC DEFECTS IN SWISS (CD-1) MOUSE FETUSES FOLLOWING MATERNAL EXPOSURE TO THEOPHYLLINE ON GESTATIONAL DAYS 6 THROUGH 15[a]

	Theophylline (% in drinking water)			
	0	0.075	0.15	0.20
Total fetuses examined[b]	296	278	300	197
Total litters examined[c]	25	25	29	17
External malformations				
No. fetuses with defects[d]	1	2	5	4
No. litters with defects[e,h]	1	2	3	2
Cleft palate	1		2	4
Exencephaly		1	2	
Adactyly (missing all digits)		1		
Ectrodactyly (missing one or more digits, but not all)			1	
Micromelia (disproportionately short limb)		1		
Visceral malformations				
No. fetuses with defects[d]	0	0	1	1
No. litters with defects[e]	0	0	1	1
Hydroureter (bilateral)				1
Pulmonary artery half normal diameter			1	
Skeletal malformations				
No. fetuses with defects[d]	0	0	1	0
No. litters with defects[e]	0	0	1	0
Ribs fused to each other			1	
Variations				
No. fetuses with defects[f]	9	13	20	8
No. litters with defects[g]	7	12	13	7
Misaligned sternebrae	7	6	12	5
Hematoma (head)		2	2	
Hematoma (upper limb)		2	1	
Black spots on liver lobes	1			
Clubbed limb (without bone change)		1	1	
Hematoma (face)			1	1
Hematoma (lower limb)		1	1	
Bipartite centra				1
Displaced testes (bilateral)	1			
Distended ureter(s)		1		
Extra ossification site(s) (sternebrae)	1			
Hematoma (neck)			1	
Incomplete ossification (interparietal)				1
Open eye bilateral			1	

[a] A single fetus may be represented more than once in listing individual defects. Defects are listed in order of frequency (high to low) for all dose groups combined.
[b] Only live fetuses were examined for malformations.
[c] Includes only litters with live fetuses.
[d] Fetuses with one or more malformations.
[e] Litters with one or more malformed fetuses.
[f] Fetuses with one or more variations.
[g] Litters with one or more fetuses with variations.
[h] χ^2 test ($p < 0.05$).

Mouse oral screening study for developmental effects (Harris et al. 1992)
Groups of 10 female Swiss CD-1 mice were dosed by gavage with 0, 20, 60 and 200 mg theophylline/kg bw/day for 19 days. After seven days of dosing, the females were cohabited with male mice that had been treated for five days prior to mating and were treated further until day 5 of cohabitation. At day 19 the females were killed and the numbers of live and dead fetuses and implantation sites were recorded.
There were no adverse clinical signs. One female in the high-dose group was killed moribund. Pregnancy rate was decreased in the high-dose group (6/9 versus 9/10 in all other groups, not statistically significant). There were no effects on the numbers of live and dead fetuses or the numbers of total implants per female (see section 4.11.1.1, table 17).

Mouse oral screening study for developmental effects (Harris et al. 1992)
Groups of 13-15 mated female Swiss CD-1 mice were dosed by gavage with 0, 20, 60 and 200 mg theophylline/kg bw/day from gestation day 8 through 14. The dams were allowed to deliver and litters were evaluated on postnatal day 0, 1 and 4 (no. of litters, no. of implantations per female, number of live pups, total litter weight).
No effects were observed.

Table 26: Neonatal and uterine implant data for females exposed during gestation following theophylline exposure during a 21-day reproduction/developmental screening study (Harris et al., 1992)

	Theophylline dose (mg/kg bw/d)				
	0	**20**	**60**	**200**	**Trend**
No. females littering (No. Rx)	14 (14)	13 (13)	15 (15)	14 (14)	
No. live neonates					
PND0	11.4±0.4	9.4±0.8	11.0±0.7	10.9±0.5	NS
PND1	11.4±0.4	9.3±0.8	11.0±0.7	10.8±0.5	NS
PND4	11.4±0.4	9.3±0.8	11.0±0.7	10.8±0.5	NS
Total litter wt.					
PND1	20.4±0.8	17.8±0.5	19.9±1.3	18.7±0.9	NS
PND4	34.8±1.2	31.4±0.8	32.9±1.5	32.5±1.2	NS
No. implantation sites per female	12.2±0.5	11.1±0.3	12.0±0.6	11.3±0.5	NS

Mouse ip developmental study (Fujii et al, 1969)
Groups of 20-26 ICR-JCL pregnant mice were treated ip with 0, 175, 200 or 225 mg/kg bw theophylline on day 12 of gestation. Fetal external, internal and skeletal examination was performed on day 18. Mortality in dams occurred at the highest dose (40%). Dams at the low and mid dose levels showed slight dyspnea and convulsions, this increased to severe dyspnea and convulsions at the high dose levels. A dose related increase in malformation was observed at all dose levels including mainly cleft palate but also digital defects and micrognathia. A decrease in fetal body weight and an increase in subcutaneous hematoma was observed at the two highest dose levels. As this study concerns a route of exposure that is not relevant for human exposure except for therapeutic treatment, this study and the observed effects are considered less relevant for classification.

Mouse ip developmental study (Tucci et al, 1978)

Groups of 10 pregnant mice were injected ip with 100, 150 or 200 mg/kg bw theophylline on either day 10, 11, 12 or 13 after observation of a mating plug. Fetuses were counted and examined for external malformations including cleft palate on day 17. Although a control group was included, no information was provided on the percentage of resorptions and malformations in this group. There was a dose-dependent increase in resorptions on almost all days with the highest percentage after injection on day 13 (31%). There was also a dose-dependent increase in malformations with the highest percentage after injection on day 11. The main effect on all injection days and exposure levels was cleft palate. In addition, also increases were observed in polydactyly, ectrodactyly, syndactyly and micromelia. No information is provided on maternal toxicity. As this study concerns a route of exposure that is not relevant for human exposure except for therapeutic treatment, this study and the observed effects are considered less relevant for classification.

RAT STUDIES

Rat oral developmental toxicity study (NTP 1985c; Lindström et al. 1990)
Theophylline was administered to groups of 20-21 pregnant Sprague-Dawley (CD) rats via the diet at 0, 0.15, 0.3 or 0.4% from day 6 through 15 of gestation. The dose levels equalled 0, 124, 218 and 259 mg theophylline/kg bw/day respectively based on diet consumption. Clinical signs were recorded. Maternal bodyweights were measured daily. At day 20 the rats were killed. Gravid uterine weights were measured. The numbers of implantation sites, resorptions, dead foetuses and live fetuses and fetal weights were recorded. All live foetuses were examined for visceral abnormalities. Half of the fetuses were decapitated prior to dissection and the heads were fixed in Bouin's solution, sectioned and examined. All foetuses were examined for skeletal malformations.
Approximately equal numbers of CD females were assigned to each treatment group within each replicate of the teratology study. During treatment (i.e., GD 6 through 15), females were observed once daily for clinical signs.
There were no maternal deaths. The primary clinical signs associated with theophylline treatment were piloerection, weight loss, and rough coat. Incidences of piloerection were increased at 0.3 and 0.4%. Other clinical signs, including alopecia or sores on various parts of the body, may be attributed to the use of mesh-topped food jars. A total of 26 animals were removed from further evaluation upon verification of non-pregnant status at sacrifice. At sacrifice, pregnancy was confirmed in 77.8% (21/27), 76.9% (20/26), 77.8% (21/27), and 72.4% (21/29) of the animals in the vehicle through high dose groups.
Table 27 presents an overview of the general maternal effects. Maternal body weight gain (during gestation and during treatment) and maternal body weight on gestational day 20 corrected for gravid uterine weight were decreased at 0.4% (no effect at other dose levels). Maternal feed consumption was decreased at 0.4%. Water consumption was increased in all theophylline-treated groups.

Tables 28+29 present an overview of the pregnancy parameters and the developmental toxicity data. There were no differences among the groups in numbers of implantation sites per litter, percentages pre-implantation loss, litters with resorptions or percentage resorptions per litter. The number of live foetuses per litter was decreased at 0.4% (statistically significant). Average male and female foetal weights per litter were decreased at 0.3 and 0.4% (statistically significant). The percentage of malformed foetuses per litter was not affected. External, visceral or skeletal malformations and variations were not affected by theophylline. Lindström et al. (1990) concluded that the NOAEL for maternal toxicity was 218 mg/kg bw/day and for developmental toxicity 124 mg/kg bw/day.
It is noted that OECD (2001) considers 124 mg/kg bw/d to be a NOAEL for maternal toxicity. They state that "….maternal toxicity (reduced corrected body weight gain of 10% at 218 mg/kg bw/d, and clinical signs like piloerection and rough coat) which was more pronounced at 259 mg/kg bw/d than

at 218 mg/kg bw". It is however noted that the reduction in body weight gain reach only statistical significance at the high dose (256 mg/kg bw) only, and clinical signs such as piloerection and rough coat have been observed in all groups (NTP 1985c; Lindstrom et al., 1990).

Table 27: Maternal toxicity in Sprague-Dawley rats (NTP 1985c; Lindstrom et al, 1990)

MATERNAL TOXICITY IN SPRAGUE–DAWLEY (CD) RATS CONSUMING DIETARY THEOPHYLLINE ON DAYS 6 THROUGH 15 OF GESTATION

	Theophylline (% in feed)			
	0	0.15	0.30	0.40
Subjects (dams)				
Total treated	27	26	27	29
Nonpregnant	6	6	6	8
No. (%) pregnant at termination	21 (78)	20 (77)	21 (78)	21 (72)
Maternal weight gain (g)[a]				
Gestation (GD 0–20)[c]	133 ± 7	134 ± 4	122 ± 6	107 ± 4*
Treatment (GD 6–15)[c]	47 ± 2	56 ± 2*	41 ± 4	22 ± 3*
Corrected[b,c]	58 ± 4	62 ± 2	52 ± 3	45 ± 3*
Gravid uterine weight (g)[c]	74 ± 4	73 ± 3	70 ± 4	62 ± 2
Maternal liver weight				
Absolute (g)[c]	15.9 ± 0.5	16.3 ± 0.3	15.4 ± 0.4	14.9 ± 0.3
Relative (% body wt)	4.3 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1
Maternal feed consumption (g/kg/day)				
Gestation[c]	82 ± 1	85 ± 2	81 ± 1	76 ± 1*
Treatment[c]	78 ± 1	83 ± 2*	73 ± 2	65 ± 3*
Calculated dose (mg/kg/day)	0	124	218	259
Maternal water consumption (g/kg/day)				
Gestation[c]	151 ± 6	171 ± 5*	178 ± 7*	174 ± 5*
Treatment[c]	138 ± 5	174 ± 6*	187 ± 9*	177 ± 6*

[a] Includes all dams pregnant at termination; mean ± SEM.
[b] Corrected body weight gain = weight at termination minus initial weight and gravid uterine weight.
[c] Test for linear trend ($p < 0.05$).
* Statistically significant ($p < 0.05$) relative to the vehicle control group.

Table 28: Developmental toxicity in Sprague-Dawley rats (NTP 1985c; Lindstrom et al, 1990)

DEVELOPMENTAL TOXICITY IN SPRAGUE–DAWLEY (CD) RATS FOLLOWING MATERNAL EXPOSURE TO DIETARY THEOPHYLLINE ON DAYS 6 THROUGH 15 OF GESTATION

	Theophylline (% in feed)			
	0	0.15	0.30	0.40
All litters (No.)[a]	21	20	21	21
Implantation sites/litter[b]	13.7 ± 0.7	13.4 ± 0.6	14.6 ± 0.7	13.8 ± 0.4
% Preimplantation loss[b,c]	3 ± 1	10 ± 4	4 ± 2	7 ± 2
% Resorptions/litter[b]	8 ± 5	4 ± 1	11 ± 5	11 ± 2
No. (%) litters with resorptions	8 (38)	8 (40)	12 (57)	15 (71)
No. (%) litters totally resorbed	1 (5)	0 (0)	1 (5)	0 (0)
No. litters with live fetuses[d]	20	20	20	21
Live fetuses/litter[b]	13.8 ± 0.3	12.9 ± 0.6	14.2 ± 0.4	12.0 ± 0.4*
Average male fetal body weight per litter[b,e]	3.6 ± 0.1	3.6 ± 0.1	3.3 ± 0.1*	3.2 ± 0.1*
Average female fetal body weight per litter[b,e]	3.5 ± 0.0	3.5 ± 0.1	3.1 ± 0.1*	3.0 ± 0.0*
% Fetuses malformed/litter[b]	1.4 ± 0.6	0.9 ± 0.7	0.3 ± 0.3	1.6 ± 0.7
No. (%) litters with malformed fetuses	4 (20)	2 (10)	1 (5)	4 (19)

[a] Includes all dams with implantation sites at termination.
[b] Reported as mean ± SEM.
[c] Defined as [(No. corpora lutea − No. implantation sites) ÷ No. corpora lutea] × 100.
[d] Includes only dams with live fetuses; litter size = No. live fetuses per dam.
[e] Test for linear trend ($p < 0.05$).
* Statistically significant ($p < 0.05$) relative to the vehicle control group.

Table 29: Developmental Toxicity of theophylline in rats (NTP 1985b; Lindstrom, 1990)

	Theophylline (% in feed)			
	0	0.15	0.30	0.40
Total fetuses examined[b]	276	258	283	253
Total litters examined[c]	20	20	20	21
External malformations				
No. fetuses with defects[d]	0	1	1	3
No. litters with defects[e]	0	1	1	3
Partial fetus[f]			1	
Cleft palate		1		
Curly tail (with a cartilage change)				1
Edema (severe)				1
Short tail				1
Visceral malformations				
No. fetuses with defects[d]	2	2	1	2
No. litters with defects[e]	2	2	1	2
Partial fetus[f]			1	
Hydronephrosis (right)	2			
Hydroureter (left)		1		
No tricuspid papillary muscles				1
Abnormal tricuspid valve				1
Aorta and pulmonary artery 4 × normal size				1
Aorta behind trachea and/or esophagus		1		
Common truncus				
Skeletal malformations				
No. fetuses with defects[d]	2	0	1	0
No. litters with defects[e]	2	0	1	0
Partial fetus[f]				
Branched rib	1		1	
Short rib	1			
Variations				
No. fetuses with defects[g]	29	46	61	60
No. litters with defects[h]	13	18	18	17
Bipartite ossified centra, cartilage normal	16	37	43	41
Hematoma (back)	6	4	11	7
Distended ureter(s)	4	2	3	7
Centra and cartilage split		4	2	8
Hematoma (head)	1	1	1	2
Misaligned sternebrae			3	1
Abnormal organ[i]	1	1		1
Hematoma (face)			2	1
Sternebrae misaligned and cartilage irregular		1		1
Globular heart		1	1	
Hematoma (neck)	1	1		1
Hematoma (upper limb)	1		1	
Hemicentrum				1
			1	1

Table continued

	Theophylline (% in feed)			
	0	0.15	0.30	0.40
Variations (*continued*)				
Unossified centra, cartilage				
normal				1
Very soft heart muscle tissue				1

[a] A single fetus may be represented more than once in listing individual defects. Defects are listed in order of frequency (high to low) for all dose groups combined.
[b] Only live fetuses were examined for malformations.
[c] Includes only litters with live fetuses.
[d] Fetuses with one or more malformations.
[e] Litters with one or more malformed fetuses.
[f] The partial fetus was alive at time of dissection and shared another fetus' placenta. Only the hind limbs, genital papilla, and bottom portion of the thorax were present. No viscera were present. The ilium was misshapen and toes 2 and 3 were fused on the right foot.
[g] Fetuses with one or more variations.
[h] Litters with one or more fetuses with variations.
[i] One fetus in the control group had a cream-colored appendix-type growth on the liver; one fetus in the 0.15 group had a black spot on the left median liver lobe; one fetus in the 0.40 group had a polyp on the right median liver lobe.

RABBIT STUDIES

Rabbit intravenous developmental toxicity study (Shibata et al. 2000)
Groups of 20 mated KbI:JW rabbits were dosed intravenously into the auricular vein using an automatic infusion pump at 0, 15, 30 or 60 mg theophylline/kg bw/day from gestational day 6 through 18. Body weights were measured daily. On day 29 of gestation the rabbits were killed and submitted to macroscopy. The numbers of implantations and of dead and live fetuses were determined. Organs and tissues of live foetuses were examined externally for malformations. All fetuses were examined for skeletal variations and malformations. Blood samples were taken on day 6 directly after dosing and 2, 6 12 and 24 hours later; the same was done after the last dose application at day 18 of gestation. C_{max} and the AUC were determined from these data.
The C_{max} of theophylline was similar on gestational day 6 and 18, namely 30, 56 and 106 µg/mL in the low-, mid- and high-dose group, respectively. Decreases in body weight (figure only) and in feed intake and reversible toxicity (accelerated respiration, sluggish startle reactions, dilation of the auricular vessels, polyurea) were noted in dams at 60 mg/kg bw. One animal died and four animals aborted at 60 mg/kg bw.
Fetal toxicity as evident from increased incidences of abortions and late fetal deaths observed at 60 mg/kg bw. There were no differences in numbers of implantations, numbers of live foetuses or sex ratio. Cleft palate was observed in eight fetuses (two litters) at 60 mg/kg but not in control animals. Increased incidences of skeletal variations (13^{th} rib) were noted at 60 mg/kg. There were no differences in the incidence of visceral or skeletal anomalies or of ossifications.

4.11.2.2 Human information

Schatz et al. (1997) carried out a prospective monitoring study among pregnant women concerning the relation between the use of asthma medication and perinatal outcome. The cohort consisted of 824 pregnant women suffering from asthma and 678 women without asthma. Medicine use was documented at the first trimester of pregnancy and followed thereafter. No associations were identified between major congenital malformations and first trimester exposure (prevalence: 4.5% in 292 exposed vs. 5.3% in 1208 non-exposed) or any time exposure (prevalence: 4.7% in 429 exposed vs. 5.3% in 1061 non-exposed) to theophylline. An association was found, however, between theophylline use and preterm birth (6% in exposed vs. 3.6% in non-exposed; p=0.034). According to the study-authors this finding may have been confounded by the presence and the severity of the asthma (Schatz et al. 1997).

Schatz et al. (2004) evaluated the associations between the use of asthma medication and perinatal outcomes including gestational hypertension, preterm birth, low birth weight, small for gestational age and major congenital malformations. The asthmatic participants recruited had completed an asthma observational cohort study or a randomized controlled trial of beclomethasone versus theophylline for moderate asthma during pregnancy. The final cohort included 2,123 asthmatic participants. No differences in perinatal outcomes were found comparing theophylline-using participants (n=273) and participants on other types of medication (n=1,850).

Heinonen et al. (1982) studied possible developmental effects of drugs used in a cohort of 50,282 mother-child pairs recruited in 12 centres in the US during the years 1959-1965. For theophylline 117 mother-child pairs were identified. “In this group, ten children had any malformation in relation to exposure to theophylline during the first four months of pregnancy (hospital standardized relative risk: 1.38; survival and race standardized relative risk: 1.29). The authors concluded that the data provided no evidence for a teratogenic effect” (Abstract only)

In a prospective cohort study 51,830 singleton pregnancies at 12 medical centres in USA between 1959 and 1966 were evaluated for a possible association between theophylline use and stillbirth. Theophylline use during pregnancy was not associated with increased risk of stillbirth. This applied both to theophylline-using women suffering from asthma (n=392) and to those not so labelled (n=814; it was not clear why subjects without a diagnosis of asthma received medication). Details on the amount of theophylline received were not available. Due to the low incidence of stillbirth, the power of the study was approximately 50 % (Neff and Leviton 1990).

In a Finnish case-control study covering the years 1982-1990, 212 pregnant asthmatics with theophylline treatment were compared with findings in 292 pregnant asthmatics without theophylline treatment and 237 non-asthmatic pregnant control subjects. No differences between groups as to gestational age, birth weight, Apgar score or perinatal deaths were found. Theophylline treatment was not associated with premature contractions or premature rupture of membranes, haemorrhage, placenta previa, abruption of the placenta, abnormal foetus position, augmentation of labour, prolonged third phase of delivery or increased haemorrhage post-partum. Three infants with malformations were born in 121 patients (2.5%) treated with theophylline during the first trimester and four in the 91 patients (4%) treated with theophylline during the second and third trimester only. Corresponding figures in the asthmatic and healthy control group were three (1%) and two (0.8%), respectively. The average frequency of malformations in Finland was 2% at that time (Stenius-Aarniala et al. 1995).

Effects of asthma or various asthma therapies were prospectively examined in 872 pregnant women with diagnosis asthma (778 of whom experienced asthma symptoms or took medication during pregnancy) and 1333 women without a diagnosis of asthma (of whom 884 had neither symptoms

nor used medication, whereas 449 had symptoms or used medication during pregnancy). Asthma severity during pregnancy was determined for each subject, regardless of a diagnosis of asthma, by cross-classifying them on their symptoms and medication steps, to arrive at four severity categories (intermittent, mild persistent, moderate persistent, severe persistent) and a category with neither symptoms nor treatment. When specific medication was considered, theophylline use was associated with an increased risk of preterm delivery (OR= 5.0; 95%CI: 1.6-16.0) but not with intra-uterine growth restriction. More detailed analyses showed that theophylline use increased the risk of premature delivery by 5% (95%CI: 1-9%) for every increase in dose per month and decreased the gestational age by 1.1 weeks (p=0.002) for once-daily use across pregnancy, adjusted for asthma severity and other confounding factors (Bracken et al. 2003).

4.11.3 Other relevant information

Oral repeated dose toxicity

Limited information on the oral repeated dose toxicity is provided to assess the toxicological effects of theophylline in males and the possible toxicological effects in females. For females, an extrapolation from the non-pregnant females to dams is required.
The table below presents a schematic overview of the repeated dose data of the NTP (1998; Klimisch score 1).

Table 30: Effect levels in oral short term toxicity studies with theophylline (NTP 1998)

Study	Species	Dose levels (mg/kg bw/day)	Effects	LOAEL (mg/kg bw/day)
16 Days feeding	Mouse	0, 250, 475, 950, 1,800 and 2,000 in males 0, 300, 450, 1,225, 2,000 and 4,375 in females	No effect seen	>4375
16 Days gavage	Mouse	0, 25, 50, 100, 200 and 400	No effect seen	> 400
16 Days feeding	Rat	0, 50, 100, 250, 450 and 1,000 in males 0, 75, 150, 250, 450 and 1,100 in females	Reduced body weight gain Increased testes weight Increased uterine hypoplasia	1000 250 75
16 Days gavage	Rat	0, 25, 50, 100, 200 and 400	Reduced body weight gain Decreased uterus weight	50 100
14 Weeks feeding	Mouse	0, 175, 400 and 800 in males 0, 225, 425 and 850 in females	Reduced body weight gain Increased leucocytes, neutrophils and lymphocytes	175 425
14 Weeks gavage	Mouse	0, 75, 150 and 300	Reduced body weight gain	150
14 Weeks feeding	Rat	0, 75, 125 and 250 in males 0, 75, 125 and 275 in females	Increased segmented neutrophils Increased kidney weight Increased incidence of nephropathy Increased incidence of mesenteric and/or pancreatic periarteritis	75 250 75 75
14 Weeks gavage	Rat	0, 37.5, 75 and 150 mg	Slightly increased incidence of mesenteric periarteritis	37.5

16-day feed study in rats (NTP 1998)

Groups of five male and five female F344/N rats were given 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm theophylline in feed for 16 days, which resulted in approximate daily doses of 50, 100, 250, 450, or 1,000 mg theophylline/kg body weight to males and 75, 150, 250, 450, or 1,100 mg/kg to females. All rats survived until the end of the study. The final mean body weights and body weight gains of 8,000 ppm males and females were significantly less than those of the controls. Increases in RBC, hematocrit and Hb were observed at 2000 ppm and above and considered secondary to the diuretic effect of theophylline. The absolute and relative testis weights of 4,000 ppm males were significantly greater than those of the controls. Increased incidences of uterine hypoplasia were observed microscopically in exposed groups of females.

16-day gavage study in rats (NTP 1998)

Groups of five male and five female F344/N rats were given 0, 12.5 (twice daily), 25 (once daily), 50 (once daily), 50 (twice daily), 100 (once daily), 200 (once daily), 200 (twice daily), or 400 (once daily) mg theophylline/kg body weight in corn oil by gavage. All rats receiving 400 mg/kg once daily and all but one female receiving 200 mg/kg twice daily died during the study. In groups dosed once daily, final mean body weights and body weight gains of males receiving 100 or 200 mg/kg and mean body weight gains of females receiving 50, 100, or 200 mg/kg were less than those of controls. The final mean body weights and body weight gains of groups receiving theophylline twice daily were generally similar to those of groups receiving the same daily dosages once daily. Clinical findings included rapid or labored respiration, hunched posture, and squinting. In groups dosed once daily, absolute and relative uterus weights of females receiving 100 or 200 mg/kg once daily were significantly less than those of the controls, and the absolute and relative uterus weights of females receiving 100 mg/kg once daily were significantly less than those of females receiving 50 mg/kg twice daily. Uterine atrophy was observed in three females receiving 200 mg/kg twice daily. Periarteritis of the mesenteric arteries was observed in two males and two females receiving 400 mg/kg once daily.

16-day feed study in mice (NTP 1998)

Groups of five male and five female B6C3F1 mice were given 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm theophylline in feed for 16 days, resulting in approximate daily doses of 250, 475, 950, 1,800, or 2,000 mg theophylline/kg body weight to males and 300, 450, 1,225, 2,000, or 4,375 mg/kg to females. All mice survived until the end of the study. Final mean body weights of 4,000 and 8,000 ppm females and mean body weight gains of 2,000, 4,000, and 8,000 ppm females were significantly greater than those of the controls. Feed consumption by exposed groups was similar to that by the controls, except that by the 8,000 ppm males, which was approximately 40% the amount of feed consumed by the control group. Histopathologic examinations were not performed due to the absence of mortality and significant exposure-related lesions.

16-day gavage study in mice (NTP 1998)

Groups of five male and five female B6C3F1 mice were given 0, 12.5 (twice daily), 25 (once daily), 50 (once daily), 50 (twice daily), 100 (once daily), 200 (once daily), 200 (twice daily), or 400 (once

daily) mg theophylline/kg body weight in corn oil by gavage. Three males and all females receiving 400 mg/kg once daily died on day 1. There were no significant differences in final mean body weights or body weight gains. There were no histopathologic findings attributed directly to theophylline.

14-week feed study in rats (NTP 1998)

Groups of 10 male and 10 female F344/N rats were given 0, 1,000, 2,000, or 4,000 ppm theophylline in feed for 14 weeks, which resulted in approximate daily doses of 75, 125, or 250 mg theophylline/kg body weight to males and 75, 125, or 275 mg/kg to females. The final mean body weight of 1,000 ppm females was significantly greater than that of the control group. Feed consumption by exposed groups was similar to that by the controls. Mean cell volume and mean cell hemoglobin were significantly greater in males exposed to 2,000 or 4,000 ppm than those in the control group. Segmented neutrophil counts of all groups of exposed females were significantly greater than that of the control group. The absolute and relative kidney weights of 4,000 ppm males were significantly greater than those of the controls, and there was an exposure-related increase in the severity of nephropathy in males. Exposure-related increases in the incidences of mesenteric and/or pancreatic periarteritis were observed in males and females.

14-week gavage study in rats (NTP 1998)

Groups of 10 male and 10 female F344/N rats were given 0, 37.5, 75, or 150 mg theophylline/kg body weight in corn oil by gavage for 14 weeks. One male and one female receiving 150 mg/kg died before the end of the study. The mean body weight gain of 150 mg/kg females was significantly greater than that of the controls. Mean cell volume of 150 mg/kg males and mean cell hemoglobin of all groups of dosed males were significantly greater than those of the control group. There were slight dose-dependent increases in the incidences of mesenteric periarteritis in dosed males and females.

14-week feed study in mice (NTP 1998)

Groups of 10 male and 10 female B6C3F mice were given 0, 1,000, 2,000, or 4,000 ppm theophylline in feed for 14 weeks, resulting in approximate daily doses of 175, 400, or 800 mg theophylline/kg body weight to males and 225, 425, or 850 mg/kg to females. All mice survived until the end of the study. The final mean body weights and body weight gains of all exposed groups of males and females were significantly less than those of the controls. Feed consumption by exposed groups was similar to that by the controls. Leukocyte, segmented neutrophil, and lymphocyte counts of 4,000 ppm males were significantly greater than those of the controls. Leukocyte and segmented neutrophil counts of 2,000 or 4,000 ppm females were significantly greater than those of the controls. There were no histopathologic findings attributed directly to theophylline exposure.

14-week gavage study in mice (NTP 1998)

Groups of 10 male and 10 female B6C3F mice were given 0, 75, 150, or 300 mg theophylline/kg body weight in corn oil by gavage for 14 weeks. Three males and all females receiving 300 mg/kg,

one 75 mg/kg male, and one control female died before the end of the study. Final mean body weights and body weight gains of 150 and 300 mg/kg males were significantly less than those of the controls. Mean cell volume and mean cell hemoglobin of 300 mg/kg males were significantly greater than those of the controls. There were no histopathologic findings attributed directly to theophylline treatment.

In the subsequent 2-year gavage studies in rats and mice the only effect observed was chronic inflammation of the mesenteric artery, seen in rats at the highest dose level of 75 mg/kg bw/day (NOAEL 25 mg/kg bw/day; NOAEL in mice >150 mg/kg bw/day) (NTP 1998).

Pharmacodynamics

Theophylline (dimethylxanthine) has been used to treat airway diseases for more than 80 years. It was originally used as a bronchodilator, but the relatively high doses required are associated with frequent side effects, so its use declined as inhaled b2-agonists became more widely used. More recently it has been shown to have anti-inflammatory effects in asthma and chronic obstructive pulmonary disease (COPD) at lower concentrations. The molecular mechanism of bronchodilatation is inhibition of phosphodiesterase (PDE) 3, but the anti-inflammatory effect may be due to inhibition of PDE4 and histone deacetylase-2 activation, resulting in switching off of activated inflammatory genes. Through this mechanism, theophylline also reverses corticosteroid resistance, and this may be of particular value in severe asthma and COPD, wherein histone deacetylase-2 activity is reduced. Theophylline antogonizes adenosine A1 and A2 receptors. Theophylline is given systemically (orally as slow-release preparations for chronic treatment and intravenously for acute exacerbations of asthma). Efficacy is related to blood concentrations, which are determined mainly by hepatic metabolism, which may be increased or decreased in several diseases and by concomitant drug therapy. Theophylline is now usually used as an add-on therapy in patients with asthma not well controlled on inhaled corticosteroids with or without long-acting b2-agonists and in patients with COPD with severe disease not controlled by bronchodilator therapy. Side effects are related to plasma concentrations and include nausea, vomiting, and headaches due to PDE inhibition and at higher concentrations to cardiac arrhythmias and seizures due to adenosine A1-receptor antagonism. In the future, low-dose theophylline may be useful in reversing corticosteroid resistance in COPD and severe asthma (Barnes, 2013).

Effect on lactation

Non-human information

Theophylline was administered to groups of 5 or 6 pregnant female Wistar rats via the drinking water at 0 or 1 mg/kg bw/day throughout pregnancy up to lactation day 14. The dose of 1 mg/kg bw/day was chosen to mimic the theophylline intake that occurs when drinking tea. Dam bodyweights were measured three times per week until day 14 after birth. Milk samples were collected from the dams on days 7 and 14 of lactation; milk volume was measured on days 12-13. The dams were killed at day 14. Litter weights were determined and dam carcass fat was measured. Theophylline had no effect on maternal weight and carcass fat during pregnancy/lactation, the volume or composition of the milk, or on litter weight (Hart and Grimble 1990a and 1990b).

Human information

Yurchak and Jusko studied the transfer of theophylline to breast milk following single oral doses of theophylline of 4.25 mg/kg bw in three asthmatic patients. They did this also in two further patients after four daily doses of 200 mg aminophylline (i.e. theophylline with ethylenediamine in 2:1 ratio). Blood, saliva and breast milk samples were collected at frequent intervals over a period of 6 to 14 hours. Peak concentrations were observed in serum at or within 30 minutes and in breast milk two to three hours after administration and amounted in one patient to 6.8 mg/L (blood) and 4.0 mg/L (milk) (not reported for other subjects). The average milk to serum concentration was about 0.7; milk concentration paralleled the time-course of serum concentrations. Irritability and fretful sleeping were observed in one infant only on days when the mother was taking theophylline while no such effects were seen in the other infant (Yurchak and Jusko 1976).

Stec et al. investigated the kinetics of transfer to breast milk in three nursing patients following single intravenous doses of 3-5 mg/kg bw of theophylline. Serum and milk theophylline concentrations were measured up to 6 hours after dosing. Serum and milk concentrations paralleled. The breast milk : serum concentration ratio was about 0.7 (Stec et al. 1980).

Reinhardt et al. (1983) investigated the kinetics of the transfer of theophylline from breast-feeding mothers to their infants. Following administration of two oral doses (300 mg followed by 200 mg after four hours) of theophylline to 12 lactating mothers. Concentrations in blood and milk were determined in samples obtained at 1-2 hours intervals up to 10 hours after the first dose. Breast milk : plasma ratios between 0.6-0.9 were calculated. The mean levels obtained within one to ten hours after the first dose were in the range of 6-10 mg/L in plasma and in the range of 3-7 mg/L in milk (Reinhardt et al. 1983).

Gardner et al. (1987) studied the kinetics of theophylline in 11 asthmatics throughout pregnancy and post-partum. On four occasions they determined theophylline concentrations in breast milk samples collected prior to treatment and at three time points after treatment and in infant plasma samples obtained prior and after feeding. Concentrations in milk roughly paralleled those in plasma. The breast milk:plasma concentration ratios varied between 0.54 and 1.08. According to the authors, characterization of the theophylline acquisition by the nursing neonates was hampered by an inadequate number of neonatal plasma samples. In all cases, however, detectable levels of theophylline were present in the neonate before and after feeding (Gardner et al. 1987).

4.11.4 Summary and discussion of reproductive toxicity

4.11.4.1 Adverse effects on sexual function and fertility

No studies were identified regarding the effects of theophylline on human fertility.

In animal studies effects of theophylline on the male reproductive system occurred as shown in the table below.

Table 31: Overview of effects on male sexual function

Species	Test and exposure	Effect on sexual function	General toxicity	Remark	reference
Mouse	Continuous breeding, 0.3% diet (500 mg/kg	Relative seminal vesicle weight decreased (19%)	Decreased body weight gain	Inconsistent with all other studies especially 14-	NTP 1985a, Morrisey, 1988

	bw/day)	epididymal sperm density decreased		week diet study	
Mouse	17 day gavage, 200 mg/kg bw/day	Mild testicular histopathological changes	None	Mild effect	Harris et al., 1992
Mouse	14 week gavage, 300 mg/kg bw/day	Reduced absolute testes weight 7%	Reduced body weight 13%	Secondary to reduced body weight	NTP 1998*
Rat	14 week gavage, 150 mg/kg bw/day	Reduced (non-significant) testis weight and uterus weight (rel.)	Reduced body weight (4%)	Secondary to reduced body weight	NTP 1998*
Rat	19 week, 0.5% diet	Testes atrophy	Mortality	Considered secondary to the general toxicity	Weinberger et al., 1978

* a discrepancy is noted in the presentation of the results of the repeated dose studies by NTP (1998) and Morrissey et al. (1988). It is noted that different statistical analyses were applied to the data. In this CLH-dossier the results as presented and evaluated by NTP (1998) have been primarily used.

In a continuous breeding study, theophylline caused reduced relative seminal vesicle weights and epididymal sperm numbers in mice at 500 mg/kg bw/day (NTP 1985a). These effects were noted in presence of a reduced bw gain in the male animals, thereby reducing the concern. Further, such effects were however not found in repeated dose studies, up to 14 week exposure (NTP 1998).

In the continuous breeding study, the number of days to deliver each litter was consistently increased after oral exposure of mice to 500 mg/kg bw/d (NTP 1985a). However, no other studies were found regarding functional effects of theophylline on animal fertility.

In repeated dose studies in female rats, a reduction in uterus weight and hypoplasia was sometimes observed in the presence of general toxicity

The results of the crossover mating trial (NTP 1985a) indicated that the reproduction in female mice was relatively more susceptible to the effects of theophylline than the males under the same exposure conditions. The effect of theophylline on female mice might be related to embryotoxicity, fetotoxicity, or to a direct effect on fertility since there was evidence of a drop in the average number of litters per pair, the litter size, adjusted live pup weight, and the proportion of pups born alive.

In conclusion, no appropriate human data are available. Several animal studies have been carried out but they indicate limited effects on sexual function and no effects on fertility. The effects on sexual function were mostly mild and/or occurred in the presence of general toxicity and were not consistent between studies in males. Taken together these effects do not meet the requirement for classification for effects on fertility.

4.11.4.2 Adverse effects on development of the offspring

Several studies were available on the potential effects of theophylline in pregnant asthmatic women. Various pregnancy outcomes were evaluated. Most of the studies were negative but it should be

noted that their statistical power was limited and their design did not allow disentanglement of the roles played by asthma itself and theophylline use (Heinonen et al., 1982; Neff and Leviton 1990; Schatz et al., 2004; Stenius-Aarniala et al., 1995). In two studies (Bracken et al. 2003; Schatz et al. 1997) use of theophylline during pregnancy was found to cause an increase in preterm deliveries.

The conclusion from the available human data for developmental effects is that they do not warrant classification for adverse effects on development.

Animal studies (with oral exposure to theophylline ranging from 124-500 mg/kg bw/day), showed reductions in the number of pups per litter in mice (NTP 1985a; Lamb et al. 1997; Morrissey et al. 1988) and rats (NTP 1985c; Lindström et al. 1990), increased percentage of resorptions in mice (NTP 1985c;Lindström et al. 1990) and reduced pup weights in mice (NTP 1985a;Lamb et al. 1997; Morrissey et al. 1988; NTP 1985b; Lindström et al. 1990) and rats (NTP 1985c; Lindström et al. 1990).

The effects in mice consisted mainly of a reduction in number of live pups per litter in the continuous breeding study (NTP 1985a; Lamb et al. 1997; Morrissey et al. 1988) and confirmed as a developmental effect (increase in resorptions) in the mouse oral prenatal developmental study (NTP 1985b; Lindström et al. 1990). No such effect was observed in the mouse oral screening study (Harris et al, 1992). This difference may have been caused by the different route of exposure as the last study used gavage whereas the other two studies used diet or drinking water. In addition, the highest dose level applied in the screening study of Harris et al. (1992) (i.e. 200 mg/kg bw/d) was below the dose level inducing an increase in resorptions in the developmental study in mice of NTP (1985b) (i.e. ≥372 mg/kg bw/d) but comparable to the lower dose level in the continuous breeding study of NTP (1985a) that induced a small but significant reduction in live pups per litter (i.e. 126 mg/kg bw/d). Differences in toxicity between diet and gavage is also shown in the two 14-week studies in mice in which exposure by gavage resulted in mortality at 300 mg/kg bw/day whereas no mortality was observed at 850 mg/kg bw/day by diet (NTP 1998). In addition, the effects in females in the 14-week diet study (NTP 1998) at the dose levels comparable to the continuous breeding study (NTP 1985a) and the mouse developmental study (1985b) of approximately 200 and 400 mg/kg bw/day were limited to reduced body weight and at the higher dose an increase in leukocytes and segmented neutrophil counts. Besides the maternal toxicity observed in the available mice studies, some information is available on the pharmacodynamic activity of theophylline. This may result in effects that are not normally observed in toxicological studies. However, theophylline could affect both the dam and the foetus. The available information does not provide clear evidence whether pharmacodynamic effects occurred to the dams that affected the foetus. Maternal toxicity in the mouse oral prenatal developmental toxicity was limited to reduced adjusted bw gain at the mid and high dose. It is considered unlikely that the observed increase in resorptions is secondary to the limited maternal toxicity at the higher dose levels.

The developmental effects in rats consist of a decrease in live pups per litter at the highest dose and a decrease in foetal body weight at the low and high dose levels (NTP 1985c). The reduced foetal body weight is an indication of retarded developmental delay and is considered not to warrant classification. A decrease in live pups per litter is however considered a severe developmental effect. At the highest dose level the developmental toxicity was observed in the presence of reduced corrected body weight gain (22%). It should be discussed whether the maternal toxicity (i.e. reduced correct body weight gain) can fully explain the observed severe developmental effects (i.e. decrease in live pups per litter) in the rat oral prenatal toxicity study. Data of feed restriction studies in Sprague-Dawley rats (Fleeman et al., 2005; Chapin et al., 1993) showed that the number of viable foetuses or the number of live pups per litter was not affected upon feed restriction despite

having reduced body weight (gain). Based on this information, it may be argued that the adverse effects on development in the rat oral prenatal developmental toxicity (NTP 1985c) are not to be a secondary non-specific consequence of maternal toxicity. However, the results of the 14-week studies point towards other general adverse effects which were not included in the evaluation in the rat oral prenatal developmental toxicity study. In the 14-week rat study, effects observed at comparable dose levels (0.2% and 0.4%) in females included an increase in segmented neutrophil counts and increases in the incidences of mesenteric and/or pancreatic periarteritis. It cannot be excluded that the observed decrease in live pups are secondary to the maternal toxicity. However, it could also be a direct effect of theophylline.

In the developmental studies in rats and mice, the administration of theophylline did not induce visceral or skeletal malformations and variations. The ip study in mice is considered not relevant as this route of exposure can induce foetal effects via direct transfer to the uterus.

In an intravenous study in rabbits (dose levels up to 60 mg/kg bw/day, corresponding to maternal plasma levels up to 106 µg theophylline/mL), cleft palate and increased incidence of skeletal variations were noted in the presence of maternal toxicity including mortality. As this study concerns a route of exposure that is not relevant for human exposure except for therapeutic treatment and the developmental effects were observed in the presence of maternal toxicity including death, this study and the observed effects are considered less relevant for classification.

4.11.4.3 Adverse effects on or via lactation

No human data were available for effects on or via lactation.

In a limited study in rats, administration in the drinking-water of 1 mg/kg bw/day (single dose level) throughout pregnancy up to lactation day 14 had no effect on maternal weight and carcass fat, the volume or composition of the milk, or on litter weight (Hart and Grimble 1990a and 1990b).

No data are available on background concentrations of theophylline in breast milk or on concentrations in breast milk in women occupationally exposed to theophylline.

Following oral or intravenous administration of theophylline to lactating women, theophylline was found in breast milk (Gardener et al. 1987; Reinhardt et al. 1983; Stec et al. 1980; Yurchak and Jusko 1976).

The data show theophylline to be excreted in breast milk but there is no information that would allow determination whether this is in amounts sufficient to cause concern for the health of the breastfed child. In conclusion, for theophylline no classification for effects on or via lactation is proposed due to lack of appropriate human and animal data.

4.11.4.4 Data on other methylxanthines

Theophylline (1,3-dimethylxanthine) is a methylxanthine derivative. Information on reproductive toxicity of related methylxanthines were retrieved and shortly summarized below.

NTP

Caffeine (1,3,7-trimethylxanthine)

NTP (1996): The potential reproductive toxicity of caffeine in Sprague-Dawley rats was evaluated using the Reproductive Assessment by Continuous Breeding (RACB) protocol. Based on decreased

body weights and feed consumption, increased water consumption, and mortality noted during Task 1, dose levels for the continuous breeding phase for this study were set at 12.5, 25, and 50 mg/kg bw/d. Male and female Sprague-Dawley rats were exposed to caffeine in deionized water by oral gavage at a dose volume of 5 ml/kg bw. Individual dose volumes were adjusted weekly. During 16 weeks of cohabitation, live pup weight adjusted for litter size was decreased by 7, 7 and 8% in the 12.5, 25, and 50 mg/kg bw/d dose groups, respectively. No differences were observed in other reproductive endpoints. A crossover mating trial (Task 3) revealed no changes on male or female fertility or in pup weight. Reproductive parameters were comparable between dose groups when naive males were mated with control or 50 mg/kg bw/d dosed females and when naive females were mated with control or 50 mg/kg bw/d dosed males.

NTP (1984a): Caffeine, a natural alkaloid drug found in tea, coffee, cocoa, and cola, and a common soft drink additive, was tested for its effects on reproduction and fertility in Swiss CD-1 mice. Caffeine was tested simultaneously at two laboratories, each using a variation on the standard RACB study design. This study used Tasks 1, 2, and 4, while the other study in mice utilized Tasks 1, 2, and 3. Caffeine was among the very first compounds run at these labs using this protocol. Data on body weights, clinical signs, and food and water consumptions were collected during the dose-range-finding phase (Task 1), and used to set exposure concentrations for Task 2 at 0.0, 0.012, 0.025, and 0.05% in drinking water. Water was chosen to mimic the route of human exposure. Water consumption was not affected by addition of caffeine. These levels of caffeine, and measured water consumption and body weights, produced calculated consumption estimates nearly equal to 22, 44, and 88 mg/kg bw/d. For the F0 animals, there were no effects on body weight. Alopecia occurred in 55% of the medium dose and 50% of the high dose animals. While there were no exposure-related changes in the number of litters/pair, viability, or adjusted pup weight, the number of live pups per litter, averaged over the 4-5 litters, dropped 15% at the medium dose and 20% for the high dose animals. No crossover mating trial was conducted, and the offspring from the last litter of control and high dose mice were reared by their dams until weaning, when they were given the same treatment as their parents until mating at 74 ± 10 days of age. At the second generation mating trial, there were no changes in any reproductive endpoint. At necropsy, at 0.05% caffeine, male body weight was reduced by 8% while male adjusted liver weight increased by 8%. No change was found in female body or organ weights, or in any sperm endpoint. In summary, a reduction in the number of live pups/litter for the F0 generation was the only reproductive effect observed in this study. This occurred in the absence of a change in body weights in the F0 parental mice.

Theobromine (3,7-dimethylxanthine)

NTP (1984b): The reproductive toxicity of theobromine was evaluated according to the Reproductive Assessment by Continuous Breeding protocol. Based on mortality noted during Task 1, dose levels for the continuous breeding phase for this study were set at 0, 0.10, 0.25, and 0.5% in the diet. Breeding pairs were housed together for 14 weeks after one week of pre-mating treatment. During this period, the following parameters were monitored: fertility, number of live and dead pups, average pup weight, and sex ratio. Theobromine treatment adversely affected at least one or more reproductive parameters in all three dose groups ($p<0.05$). Furthermore, in the 0.5% dose group, the proportion of pups born alive per fertile pair was 0.64 as compared to 0.98 in the control group suggesting that theobromine may be a fetotoxicant. The study was extended to determine the affected sex in the high dose (0.5%) group (Task 3). The crossover mating trial demonstrated that reproductive capacity was severely impaired in female mice ingesting theobromine. More specifically, the number of live pups per litter, proportions of pups born alive, and pup body weights were significantly reduced. Males ingesting 0.5% theobromine revealed a significant

increase in the incidence of abnormal sperm. The liver in both male and female mice was significantly enlarged. The testicular weight in males and brain weight in both male and female mice were depressed. No morphological changes were seen in the reproductive organs. Hormonal patterns in both male and female mice were unaffected by the theobromine treatment. Thus, under the conditions of the study, theobromine ingestion had adverse effects on various endpoints of male and female reproductive functions without significant effects on general health and growth.

EFSA (2015)

A scientific opinion on the safety of caffeine (focusing on oral route, taking into account both single doses of caffeine as well as habitual caffeine consumption) was presented by EFSA (2015). With respect to caffeine consumption during pregnancy and during lactation, the following was concluded by EFSA:

Pregnant women

There are no studies on the health effects of single doses of caffeine consumed by pregnant women prior to intense physical exercise. With regard to the different kinetics of caffeine in this population subgroup, single doses of caffeine which are of no safety concern for non-pregnant adults do not apply to pregnant women performing physical exercise.

Caffeine intakes from all sources up to 200 mg per day consumed throughout the day by pregnant women in the general population do not give rise to safety concerns for the fetus. This conclusion is based on prospective cohort studies showing a dose-dependent positive association between caffeine intakes during pregnancy and the risk of adverse birth weight-related outcomes (i.e. fetal growth retardation, small for gestational age) in the offspring. In these studies, the contribution of "energy drinks" to total caffeine intake was low (about 2 %).

Data to characterise the risk of habitual caffeine consumption in this population subgroup are scarce.

Lactating women

Single doses of caffeine up to 200 mg and habitual caffeine consumption at doses of 200 mg per day consumed by lactating women in the general population do not give rise to safety concerns for the breastfed infant. At these doses of caffeine, daily caffeine intakes by the breastfed infant would not exceed 0.3 mg/kg bw, which is 10-fold below the lowest dose of 3 mg/kg bw tested in a dose finding study and at which no adverse effects were observed in the majority of infants.

There are no data to characterise the risk of single doses of caffeine consumed by lactating women, and data on habitual caffeine consumption in this population subgroup are scarce.

4.11.5 Comparison with criteria

Fertility:

According to CLP criteria substances are classified in Category 1A for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility in humans. As concluded in paragraph 4.11.4.1, no appropriate human data are available and classification in category 1A is not appropriate.

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 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.

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 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.

Limited animal data are available that do not meet the requirement for classification for effects on fertility. Therefore, classification in category 1B or 2 is not warranted.

Developmental effects

According to CLP criteria substances are classified in Category 1A for reproductive toxicity when they are known to have produced an adverse effect on development in humans. As concluded in paragraph 4.11.4.2, the available human data are insufficient for classification. Therefore, classification in category 1A is not warranted.

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 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.

In animal studies in mice severe developmental effects (reduced no. of live pups/litter, increased no. of resorptions) occurred. It is considered unlikely that the observed increase in resorptions is secondary to the limited maternal toxicity at the higher dose levels. For the observed severe developmental effect in rats (reduced live foetuses per litter), the influence of the maternal toxicity cannot be fully excluded. The IV study in rabbits (cleft palate and increased incidence of skeletal variations) is considered less relevant. There is no information which species is more relevant to humans or information that shows that the observed effects are not relevant to humans. Based mainly on the developmental effects as seen in mice, but also taken into account the effects in rats, theophylline is proposed for classification as Repr. 1B (H360D).

Effects on/via lactation

Classification for effects on or via lactation 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.

There are no data that fulfil the first and second criterion. With respect to the third criterion, human data show theophylline to be excreted in breast milk but there is no information that would allow determination of whether this is in amounts sufficient to cause concern for the health of the breastfed child. In conclusion, for theophylline no classification for effects on or via lactation is proposed due to lack of appropriate human and animal data.

4.11.6 Conclusions on classification and labelling

Based on the developmental effects observed in mice, and taking into account the effects in rats, theophylline is proposed for classification as Repr. 1B; H360D (May damage the unborn child).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS presented 15 studies for the assessment of reproductive toxicity, including repeated dose studies (subchronic and chronic), as well as fertility and developmental toxicity studies in rodents. Thirteen of them are evaluated as Klimisch 1 or 2: one continuous breeding feeding study in mice, three reproduction/developmental screening studies in male and/or female mice (oral gavage), four 14-week studies (two in mice, two in rats, gavage and diet), one 75-week feeding study in male rats, one 19-week feeding study in male rats, and three prenatal developmental toxicity studies (one in mice, one in rats, both oral and in drinking water, and one in rabbits *i.v.*). Two Klimisch 3 prenatal developmental toxicity studies with intraperitoneal administration were also presented. In addition, four short-term 16-day feeding and gavage studies in mice and rats were presented (Klimisch 1).

Effects on sexual function and fertility

No studies were identified regarding effects of theophylline on human fertility.

Based on the animal studies, the DS concluded that the data indicate limited effects on sexual function and no effects on fertility. The effects on sexual function were mostly mild and/or occurred in the presence of general toxicity and were not consistent between studies in males. Taken together these effects do not meet the requirement for classification for effects on fertility.

Developmental toxicity

Regarding human data, several studies were available on the potential effects of theophylline in pregnant asthmatic women. Most studies evaluated were negative regarding effects on pregnancy outcome; however, the statistical power was limited, and the study design did not make it possible to distinguish between the role of theophylline and the role of asthma itself. In two studies, an increase in preterm delivery was found. The DS concluded that the human data on developmental effects did not warrant classification.

In animal studies, the developmental effects in mice consisted mainly of a reduction in number of live pups per litter in the continuous breeding study, confirmed as a developmental effect (increase in resorptions) in the mouse oral prenatal developmental study. It was considered unlikely that the

observed increase in resorptions was secondary to the limited maternal toxicity at the higher dose levels. The developmental effects in rats consisted of a decrease in live pups per litter at the highest dose and a decrease in foetal body weight at the low and high dose levels. The reduced foetal body weight was considered as an indication of retarded development and was not considered to warrant classification by the DS. A decrease in live pups per litter was, however, considered a severe developmental effect. In the developmental studies in rats and mice, the administration of theophylline did not induce visceral or skeletal malformations and variations. The DS did not consider the *i.p.* study in mice relevant as this route of exposure can induce foetal effects via direct transfer to the uterus.

In accordance with the criteria of the CLP regulation, the DS concluded that theophylline should be classified as Repr. 1B (H360D) based on the adverse effects on development (reduced number of pups per litter and increase in resorptions) as observed in studies in mice at dose levels at which no marked maternal toxicity occurred. In addition, a reduction in live pups per litter was observed at maternally toxic dose levels in rats.

Lactation

Regarding lactation, the data show theophylline to be excreted in breast milk but there is no information that would allow determination whether this is in amounts sufficient to cause concern for the health of the breastfed child. In conclusion, no classification for effects on or via lactation was proposed due to lack of appropriate data.

Comments received during consultation

In the consultation, three Member State Competent Authorities (MSCAs) provided comments, supporting the classification as Repr. 1B (H360D) based on effects in mice (reduced numbers of live pups/litter, and increased number of resorption) and rats (reduced number of live foetuses/litter).

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

The DS included for the assessment of sexual function and fertility, one continuous breeding feeding study in mice (RACB), two reproduction/developmental screening studies in male and/or female mice (oral gavage), four 14-week studies (two in mice, two in rats, gavage and diet), one repeated dose toxicity 75-weeks feeding study in male rats with a 14-week sub-group, and one 19-week feeding study in male rats. The exposure duration ranged from 17 days up to 75 weeks.

Table*: Overview on studies for reproductive toxicity and fertility assessment*

No.	Method/Guideline	Klimisch	Reference
1	Reproductive Assessment by Continuous Breeding (RACB) NTP study design, 15 weeks + 1 week cross-over mating trial; CD-1 mice 20/sex/dose (controls: 40 pairs), oral diet: 0, 0.075, 0.15, 0.3% corresponding to 0, 126, 260, 506 mg/kg bw/d	2	NTP, 1985a / Lamb *et al.*, 1997 / Morrissey *et al.*, 1988
2	Reproduction/developmental screening assay on male fertility; 17 days, CD-1 mouse (10/group), oral gavage: 0, 20, 60, 200 mg/kg bw/d (vehicle corn oil)	2	Harris *et al.*, 1992
3	Reproduction/developmental screening assay on male and female fertility; 19 days, CD-1 mice (10/group), oral gavage: 0, 20, 60, 200 mg/kg bw/d (vehicle corn oil)	2	Harris *et al.*, 1992

4	14-week toxicity study; B6C3F1 mice, oral gavage: 75, 150, 300 mg/kg bw/d	1	NTP, 1998
5	14-week toxicity study; B6C3F1 mice, oral diet: 0, 1000, 2000, 4000 ppm, corresponding to 0, 184/229, 401/418, 793/856 (m/f) mg/kg bw/d	1	NTP, 1998
6	14-week toxicity study; F344/N rats, oral gavage: 37.5, 75, 150 mg/kg bw/d	1	NTP, 1998
7	14-week toxicity study; F344/N rats, oral diet: 0, 1000, 2000, 4000 ppm, corresponding to 0, 66/67, 129/135, 258/264 (m/f) mg/kg bw/d	1	NTP, 1998
8	75-week toxicity study; male Osborne Mendel rats, oral diet: 0 or 0.5%, corresponding to 0 or 250 mg/kg bw/d (assuming 50 g feed/kg bw/d)	2	Weinberger *et al*., 1978
9	19-week toxicity study; male Holtzman rats, oral diet: 0 or 0.5%, corresponding to 0 or 250 mg/kg bw/d (assuming 50 g feed/kg bw/d)	2	Weinberger *et al*., 1978

The main study (No. 1) for the assessment of adverse effects on sexual function and fertility was a mouse oral continuous breeding study by the US National Toxicology Program continuous breeding design (NTP, 1985a) using CD-mice exposed via diet to 0, 0.075, 0.15 and 0.3% theophylline corresponding to 0, 126, 260 and 506 mg/kg bw/d, respectively (purity >99%). The continuous breeding phase of the study with 1-week premating dosing and 14 weeks dosing during cohabitation was followed post-exposure by a cross-mating between control and high dose animals until week 19.

General toxicity: After 14 weeks continuous breeding phase treatment, dose-dependent response with alopecia was seen (20-25%: low dose; >50% of the animals mid and high dose). Seven mice died during the continuous breeding phase: three controls and four in the low dose group (reason unclear). There was no difference in daily food consumption between treatment groups and parental body weights were unchanged. Regarding weight gain, male mice in the control, 0.075, 0.15, and 0.3% theophylline groups gained nearly 7, 6, 4, and 3% of their original body weights, respectively. After the high dose cross-mating week 18, terminal body weights were increased 5% in female in the high dose ($p<0.05$), and an 11% increase in liver weight adjusted for body weight ($p<0.05$) was seen. Treated male terminal body weights were reduced by 7% vs. controls ($p<0.05$).

Reproduction: Significant reproductive effects were observed including a 19% reduction in the mean number of litters per pair for the high dose mice ($p<0.01$), fewer live pups per litter at all doses; % decrease: 22, 29, 42 for 0.075, 0.15, 0.3% groups, respectively ($p<0.01$), proportion of pups born alive significantly reduced at mid and high dose ($p<0.05$), and a 6% decrease in live pup weight adjusted for litter size in the high dose. The number of days to deliver each litter was consistently higher in the high dose group. A cross-over mating was used to identify the affected sex; there were no differences in the percent of pairs mating, or delivering a live litter. In the group cohabiting control males and 0.3% exposed females, the proportion of pups born alive was reduced by 16%, and the adjusted live pup weight was reduced by 15%, suggesting the female CD-1 mice may be more sensitive to the effects of continuous theophylline treatment. See further details under *Developmental toxicity*.

***Table**: Reproductive performance of fertile pairs during continuous breeding phase (NTP, 1985a)*

	Treatment group			
Reproductive parameters[a]	control	0.075%	0.15%	0.3%
Litters per pair	4.78±0.096 (37)[c]	4.81±0.136 (16)	4.42±0.221(19)	**3.85±0.264 (20)[d]**
Live pups per litter				
male	6.29±0.289 (37)	4.55±0.341 (16)[e]	3.99±0.364 (19)[e]	3.44±0.310 (20)[e]
female	5.57±0.196 (37)	4.71±0.439 (16)	4.48±0.408 (19)	3.44±0.324 (20)[e]
combined	**11.86±0.395 (37)**	**9.26±0.658 (16)[e]**	**8.47±0.714 (19)[e]**	**6.89±0.520 (20)[e]**
Proportion of pups born alive	**0.98±0.008 (37)**	**0.92±0.039 (16)**	**0.88±0.048 (19)[f]**	**0.90±0.031 (20)[e]**
Live pup weight (g)				
male	1.58±0.013 (37)	1.64±0.018 (16)[f]	1.66±0.025 (19)[f]	1.59±0.019 (20)
female	1.54±0.014 (37)	1.57±0.017 (16)	1.58±0.025 (19)	1.58±0.035 (20)
combined	1.56±0.013 (37)	1.61±0.018 (16)[f]	1.61±0.023 (19)[f]	1.59±0.024 (20)
Adjusted live pup weight (g)[b]				
male	1.62±0.014 (37)	1.63±0.019 (16)	1.64±0.017 (19)	1.54±0.019 (20)[e]
female	1.60±0.016 (37)	1.56±0.021 (16)	1.56±0.019 (19)	1.50±0.021 (20)[e]
combined	1.61±0.013 (37)	1.60±0.017 (16)	1.60±0.016 (19)	**1.52±0.018 (20)[e]**

a: mean±SE
b: means adjusted for total number of live and dead pups per litter by analysis of covariance
c: number of fertile pairs providing the data indicated in parenthesis
d: significantly different (p<0.01) from the control and 0.075% group
e: significantly different (p<0.01) from the control group
f: significantly different (p<0.05) from the control group

***Table**: Mean litter data during continuous breeding phase (NTP, 1985a)*

C. Cumulative days to litter

	Cumulative days to litter				
Litter:	*1st*	*2nd*	*3rd*	*4th*	*5th*
Control	21.4±0.54	43.8±1.49	64.4±1.56	83.5±1.36	103.0±0.81
0.075%	23.7±1.24	44.2±1.44	64.4±1.47	85.5±1.62	104.5±1.23
0.15%	21.3±0.30	44.2±1.23	67.7±2.98	85.5±2.60	102.92±1.08
0.3%	23.5±0.92	**50.4±2.61[a]**	**71.7±3.06[a]**	**91.2±2.88[a]**	**108.1±1.42[a]**

a: significantly different (p<0.05) compared to control value

D. No. live pups in each litter

	No. live pups in each litter				
Litter:	*1st*	*2nd*	*3rd*	*4th*	*5th*
Control	11.1±0.43	13.1±0.40	12.5±0.58	12.0±0.65	11.3±0.74
0.075%	9.6±0.89	**9.3±0.87[a]**	**9.5±0.78[a]**	**9.2±1.33[a]**	9.0±0.75
0.15%	**8.4±0.80[a]**	**8.8±1.00[a]**	**8.7±0.94[a]**	**7.7±0.99[a]**	**8.5±1.29[a]**
0.3%	**8.7±0.74[a]**	**6.3±0.70[a]**	**6.9±0.80[a]**	**6.2±0.89[a]**	**6.4±1.56**

a: significantly different (p<0.05) compared to control value

After cross-mating week 18, body-weight-adjusted seminal vesicle weight decreased by 19% and epididymal sperm density was reduced by 20% in the high dose group, these changes came along with marginal male body weight changes (~ -5%); the %-motile and the percent of abnormal morphologic forms were unchanged. There were no changes in the length of the oestrous cycle, or in the percent of time spent in the various oestrous stages.

***Table**: Organ weight (at necropsy) of CD-1 mice during cross-over mating trial with theophylline (NTP, 1985a)*

	Treatment group	
Variable[a]	control	0.3%
Females		
Body weight	36.16±0.5829 (37)[b]	**38.07±0.5569 (19)[c]**
Liver (g)	2.122±0.0549 (37)	**2.459±0.0785 (19)[c]**
Kidneys (g)	0.581±0.0113 (37)	0.606±0.0147 (19)
Males		
Body weight	**39.96±0.5991 (40)[b]**	**37.13±0.6980 (20)[c]**
Liver (g)	2.046±0.0403 (40)	**2.191±0.0563 (20)[c]**
Kidneys (g)	0.763±0.0160 (40)	0.749±0.0231 (20)
R. Epididymis (g)	0.060±0.0010 (40)	0.061±0.0014 (20)
R. Cauda (g)	0.022±0.0012 (40)	0.021±0.0009 (20)
R. Testis (g)	0.136±0.0027 (40)	0.136±0.0039 (20)
Seminal vesicles (g)	**0.656±0.0156 (40)**	**0.497±0.0206 (20)[c]**
Prostate gland (g)	0.041±0.0020 (40)	0.038±0.0033 (20)

c: significantly different ($p<0.05$) from the control group

***Table**: Summary of data of sperm evaluation (NTP, 1985a)*

	Weight (g)				Sperm motility (%)	Sperm density[a] $\times 10^6$	Abnormal sperm (%)
	bodyweight	R. Cauda	R. Epididymides	R. testis			
Control[b]	40.0±0.6[c]	0.022±0.0012	0.060±0.0010	0.136±0.0027	82.8±2.48	927±38.9	3.41±0.34
0.3%[b]	37.1±0.7	0.021±0.0009	0.061±0.0014	0.136±0.0039	85.9±2.80	**741±40.8[d]**	3.58±0.44

a: per g cauda tissue
b: number of animals: 40 for controls and 20 for 0.3% theophylline group
c: mean±SE
d: significantly different ($p<0.05$) from the control group

It was concluded that theophylline caused significant adverse reproductive effects including fewer litters/pair at the high dose, fewer live pups/litter and growth retardation based on reduced pup weight, this in absence of parental body weight changes (the impact of body weight changes during gestation phases of females cannot be fully assessed as not sufficient details are available) (see table 7 in the CLH report). NTP concluded that the significant adverse effects happened in the absence of changes in parental body weight, and although alopecia may be seen as evidence of general toxicity, it is unlikely to cause the reproductive effects in the same way that, for example, hepatic or renal toxicity may adversely impact reproductive capability. Regarding effects on sexual function, the reduced seminal vesicle weight and epididymal sperm number came along with an overall mild general toxicity expressed as a 7% decrease of male terminal body weights ($p<0.05$) and increased liver weights.

In the Reproduction/developmental screening assay on male fertility (No. 2) (Harris *et al*., 1992), male Swiss CD-1 mice (n=10/group) were exposed by gavage to 0, 20, 60 and 200 mg/kg bw/d (vehicle corn oil) for 17 days and then necropsied. No effects on the weights of testes and epididymides, sperm density per cauda and sperm motility were found. At the high dose level of 200 mg/kg bw/d, theophylline induced mild changes in the testis epithelium, consisting primarily of asynchronous germ cell development and focal loss of germ cells within individual tubules (see table 16 in the CLH report). RAC notes that the dosing was lower compared to the continuous breeding study (No 1), where effects on seminal vesicles and sperm density were reported for the high dose group of 500 mg/kg bw/d in the cross-mating trial after 18 weeks. Generally, due to the relatively small numbers of animals in the dose groups and the short duration of the study, this screening test may not conclusively provide evidence for no effects (to compare, the OECD TG 421 recommends at

least four weeks dosing with detailed testis histopathology, two weeks pre-mating and two weeks post-mating).

In the Harris *et al*. Reproduction/developmental screening assay on male and female fertility (No. 3), CD-1 mice (10/group) were dosed by gavage with 0, 20, 60, 200 mg/kg bw/d for 19 days. After seven days of dosing, these females were cohabited with male mice that had been treated for five days prior to mating (and were continued being treated until day 5 of cohabitation). After 19 days of dosing, the females were killed, and the number of live and dead foetuses and implantation sites were recorded. No adverse clinical signs were found, and one female in the high dose group was killed moribund. Pregnancy rate was non-significantly decreased in this group (6/9 vs. 9/10 in all other groups; Table 17 in the CLH report). There were no effects on the number of live or dead foetuses or the number of total implants per female. Again, RAC notes that due to the relatively small numbers of animals in the dose groups, the endpoints studied (e.g. no histopathology), and the short duration of the study, this screening test may not conclusively provide evidence for no effects (e.g. the OECD TG 421 recommends two weeks pre-mating dosing of females to cover at least two complete oestrous cycles).

In the NTP 14-week mouse repeated dose studies in B6C3F1 mice, one with dosing via gavage (No. 4) the other with dosing via the diet (No. 5), theophylline was applied in dose levels of 0, 75, 150 or 300 mg/kg bw/d (gavage vehicle: corn oil) and dietary at dose levels of 0, 184/229, 401/418, 793/856 (males/females) mg/kg bw/d. At the end of the studies, sperm samples were collected from all males for sperm morphology evaluations and vaginal samples were collected for up to 7 consecutive days prior to the end of the studies from all females for vaginal cytology evaluations. There were no biologically significant differences in sperm morphology or vaginal cytology parameters between control and dosed mice despite general toxicity. For the gavage study this included mortality in the high dose (3/10 males, 10/10 females) and reduced body weights in males mid and high dose (high dose -12%, $p<0.01$), this associated with reduced absolute testis (-7%) weights at high dose of 300 mg/kg bw/d ($p<0.01$) (see table 18 in the CLH report). The DS considered the reduced testis weights to be secondary to body weight change. It is noted by RAC that testis weights are usually quite conserved despite body weight loss and therefore absolute weights should be evaluated, testicular spermatid count is not reported, epididymal sperm concentrations are unaffected. For the feeding study, body weights were decrease at all dose levels (males $p<0.01$, females $p<0.05$). In line with the DS, RAC notes that these results are inconsistent with the continuous breeding study as the effects on sperm density and seminal vesicles weights observed in the high dose of 500 mg/kg bw/d (study No. 1) were not seen in these studies in B6C3F1 mice at comparable dose levels of 300 mg/mg bw/d (gavage) up to 800 mg/kg bw/d (diet).

In the NTP 14-week rat repeated dose studies in F344 rats, one with dosing via gavage and the other with dosing via the diet, theophylline was given at dose levels of 0, 37.5, 75 or 150 mg/kg bw/d (gavage vehicle corn oil) and dietary at dose levels of 0, 66/67, 129/135, 258/264 (m/f) mg/kg bw/d. In the gavage study (No. 6), survival, and feed consumption were not affected in either sex, and body weight not affected in males; a dose related increase in periarteritis in both sexes, that was significant in females at the high dose, was reported. No significant differences in sperm morphology or vaginal cytology parameters between control and treated rats were reported. Based on the NTP report, RAC did note changes in testis weights or histopathology. Females uterus weight, however, was apparently reduced by 30% in the high dose group (non-significant, no dose-response (weights in g): 0.595, 0.525, 0.587, 0.416 for control, 37.5, 75, 150 mg/kg bw/d, respectively), with concomitant body weight increase (necropsy body weight: 216 g in high dose females vs. 203 g

in controls; p<0.01) in the high dose. In the feeding study (No. 7) survival, body weight, and feed consumption were equally not affected, but a dose related increase in kidney nephropathy in males and an increase in periarteritis in both sexes, that was significant in females at the high dose, was seen. Mean cell volume and mean cell haemoglobin levels were increased in males at 130 and 260 mg/kg bw/d, kidney weight was increased in males and lung weight in females at 260 mg/kg bw/d. Absolute epididymis weights were increased at 130 mg/kg bw/d (but not at 260 mg/kg bw/d, the highest dose level; thus, no dose-response); the cauda epididymis weights were decreased at 260 mg/kg bw/d only (by 8%, not significant). There were no significant differences between control and exposed rats in sperm morphology or vaginal cytology parameters. The percentage of abnormal sperm was increased 1.5-fold in the high dose, but this was not significant and sperm motility was not affected. Oestrus cycle length was not affected in any group.

NTP also included a 16-day testing in their toxicity assessment (see table 30 in the CLH report): 16-day feeding in rats and mice and 16-day gavage in rats and mice. The 16-day feeding study in F344/N rats showed increased absolute and relative testis weights and increased uterus hypoplasia at 250 and 75 mg/kg bw/d, respectively (weight gain reduced at 1000 mg/kg bw/d, the top dose). In the rat gavage study in groups dosed once daily, absolute and relative uterus weights of females receiving 100 or 200 mg/kg bw once daily were significantly less than those of the controls; uterine atrophy was observed in three females receiving 200 mg/kg bw twice daily. Body weight gain for females was reduced from low dose of 50 mg/kg bw/d and up.

The Weinberger *et al*. studies constituted of three experiments, 14 weeks, 19 weeks, and 75 weeks. In the rat oral 75-week toxicity study (No. 8) (Weinberger *et al*., 1978), Osborne-Mendel rats received one dose level theophylline via diet: 0 or 0.5%, corresponding to about 250 mg/kg bw/d. For a sub-group of 6 rats sacrificed at 14 weeks, survival and body weight were not affected; neither were testis weight or histopathology. At week 75, body weight was reduced; survival, haematology, clinical chemistry, cholesterol and sperm cell cytogenetic analysis was not affected. Relative kidney, adrenal and pituitary weights were increased. There was no effect on relative testis weight, but the incidence of testis histopathological effects was non-significantly increased: normal testis 3/6 (50%) versus 6/6 (100%) for control, atrophy 3/6 (50%), oligospermatogenesis 2/6 (33%), aspermatogenesis 1/6 (17%). In the third experiment in another rat strain, 19-week rat oral study (No. 9) in male Holtzman rats at the same dose level, relative testicular weight was equally not affected, but histopathology revealed an increase in testes atrophy and oligospermatogenesis: normal testis 1/7 (14%) versus 15/15 (100%) for control, atrophy 6/7 (86%), oligospermatogenesis 5/7 (71%), this statistically significant (p<0.05), and aspermatogenesis 1/7 (14%), non-significant. Increased mortality mainly during week 5, probably due to pulmonary disease (as stated in the CLH report), is reported. As such it may not be due to the test substance toxicity, but no further information is available to RAC. Weight gain, food intake and food efficiency were decreased according to the CLH report. For both these Weinberger studies, RAC notes the information provided by the DS being too limited, in particular it is not possible to conclude whether the effects on testis may be confounded by general toxicity of the test substance as no further information were provided on the extent of body weight, weight gain, or food intake reduction.

Summary – sexual function and fertility

Among the reproduction parameters measured in the continuous breeding study (RACB; No. 1), the fewer litters/pair at the high dose, fewer live pups/litter and growth retardation based on reduced pup weight, in the absence of parental body weight changes, are of concern. The reduced number of live pups in each litter was consistently shown, dose-dependent and in 5 consecutive litters (table 10

in the CLH report). The cross-over mating indicated females being more susceptible as treated high dose females mated with control males also showed significantly reduced number of pups born alive and reduced live pup weights. The effect of theophylline on number of live pups per litter may be a fertility or developmental effect. In this study, the number of days to deliver each litter was consistently increased in the 500 mg/kg bw/d dosing group. No other study showed functional effects on fertility.

In some studies, effects on testis have been observed. The mouse continuous breeding study (RACB, No.1), which can be considered the key study, reported reduced sperm density and seminal vesicles weight at 500 mg/kg bw/d after 18 weeks. Despite the limitation of the Weinberger studies, that only one dose level was tested (250 mg/kg bw/d), it is noted that the studies indicate that theophylline, after exposures of 19 and 75 weeks in two rat strains, may be a testicular toxicant. Other studies described above also covered doses of about 250 mg/kg bw/d, and more, but the NTP repeated dose toxicity study of 14-weeks in mice and rats, did not show a profound and consistent effect on testis. The reduced seminal vesicle weight and epididymal sperm number in the RACB study came along with an overall mild general toxicity and is considered to represent a treatment related effect of concern. The studies inconsistency in testicular effects may be attributed to the different dosing regimens, species and strains. Effects are only observed after 18-19 weeks. The study in Osborne rats show that in the same study, the 14-week sub-group showed no effects on testis weights and histopathology, while the 75-weeks sacrifice does; the RACB study exhibited its effects after the overall 18 weeks (14-week exposure with post-exposure cross-mating). The reproduction screening studies, due to the relatively small numbers of animals, the short duration of the study, and endpoints covered, may not conclusively provide evidence for effects/no effects on sexual function and fertility and thus are of limited value for the assessment of these effects. However, the 17-day study showed mild testicular effects in absence of general toxicity.

The NTP 14-week gavage study in rats and the 16-day rat studies showed some effects on uterus weights and hypoplasia, a marked reduced uterus weight (-30%), though not statistically significant, in the 14-week high dose group. No such findings were reported in the reproductive studies or in mice.

Developmental toxicity

The DS included for the assessment of developmental toxicity, the continuous breeding feeding study in mice (RACB), two reproduction/developmental screening studies in male and/or female mice (oral gavage), five prenatal developmental toxicity studies, two of them rated Klimisch 3.

***Table**: Overview on studies for developmental toxicity assessment*

No.	Method/Guideline	Klimisch	Reference
1	Reproductive Assessment by Continuous Breeding (RACB) NTP study design, 15 weeks + 1 week cross-over mating trial; CD-1 mice 20/sex/dose (controls: 40 pairs), oral diet: 0, 0.075, 0.15, 0.3% corresponding to 0, 126, 260, 506 mg/kg bw/d	2	NTP, 1985a / Lamb *et al*., 1997 / Morrissey *et al*., 1988
10	Prenatal developmental toxicity study; CD-1 mice, GD6-15 (sacrifice GD17); oral drinking water: 0, 0.075, 0.15 or 0.20%, corresponding to 0, 282, 372 or 396 mg/kg bw/d	1	NTP, 1985b / Lindström *et al*., 1990
3	Reproduction/developmental screening assay on male and female fertility; 19 days, CD-1 mice (10/group), oral gavage: 0, 20, 60, 200 mg/kg bw/d (vehicle corn oil)	2	Harris *et al*., 1992
11	Reproduction/developmental screening assay on female mice; GD8-14 (dams allowed to deliver), CD-1 mice (13-15/group), oral	1	Harris *et al*., 1992

	gavage: 0, 20, 60, 200 mg/kg bw/d (vehicle corn oil)		
12	Prenatal developmental toxicity study; ICR-JBL mice (20-26/group), GD12 single exposure, *i.p.*; 0, 175, 200, 225 mg/kg bw (purity unknown)	3	Fujii *et al*., 1969
13	Prenatal developmental toxicity study; GD10 or GD11 or GD12 or GD13 single exposure, *i.p.*; 0, 100, 150, 200, mg/kg bw (purity unknown)	3	Tucci *et al*., 1978
14	Prenatal developmental toxicity study; Sprague Dawley rats, GD6-15; oral drinking water: 0, 0.075, 0.15 or 0.20%, corresponding to 0, 124, 218 or 259 mg/kg bw/d, GD 6-15	1	NTP, 1985c / Lindström *et al*., 1990
15	Prenatal developmental toxicity study; KbI:JW rabbits (20/group), GD6-18; intravenous: 0, 15, 30, 60 mg/kg bw/d	2	Shibata *et al*., 2000

The main animal studies for the evaluation of developmental effects were the continuous mouse breeding study (No. 1), and the oral developmental toxicity studies in mice (No. 10) and rats (No. 14). Further supporting studies are discussed below.

Regarding human data, in short, most studies available on the potential effects of theophylline in pregnant asthmatic women were negative, but suffering from deficiencies including limited statistical power, and a design which did not allow to distinguish between the roles played by asthma itself and theophylline use. In two studies (Bracken *et al*., 2003; Schatz *et al*., 1997), use of theophylline during pregnancy was found to cause an increase in preterm deliveries. Overall, RAC agrees with the DS that the available human data for developmental effects do not add evidence that would warrant classification for adverse effects on development.

The mouse continuous breeding study (No. 1), described above, showed a significant decrease of the number of live pups per litter ($p<0.01$) at all three dose levels, the number of the proportion of pups born alive was significantly decreased ($p<0.05$) at the mid and high dose, while mean live pup weights when adjusted for the total number of live and dead pups per litter were significantly lower ($p<0.05$; 6% decrease) in the high dose group (0.3% theophylline). Reduced proportion of pups born alive was also seen in the crossover mating trial when cohabiting control males and high dose females.

The prenatal developmental toxicity study (No. 10) in mice seem to confirm these effects being of developmental toxicity nature as an increase in resorption was reported: CD-1 mice were exposed at GD 6-15, i.e. from implantation on during organogenesis (sacrifice day 17) orally by drinking water to 0, 0.075, 0.15 or 0.20%, corresponding to 0, 282, 372 or 396 mg/kg bw/d (purity >99%). The percentage of resorptions per litter was dose-dependently increased, being statistically significant at 0.15 and 0.2% ($p<0.05$; % increase: 10, 14, 27 and 34 at 0, 0.075, 0.15 and 0.2%, respectively). The number of litters completely resorbed was also increased, although not significant (No.(%): 1(4), 1(4), 4(12), 6(26) at 0, 0.075, 0.15 and 0.2%, respectively). There were no differences in the numbers of corpora lutea and implantation sites or the percentages of pre-implantation loss. The male and female foetal body weights were significantly reduced ($p<0.05$) at mid and high dose. External malformations were noted as a non-statistically slight trend (foetal incidences: 1/296, 2/278, 5/300, 4/197; foetal percentages: 0.3/0.7/1.6/2% at 0/0.075/0.15/0.2% groups, respectively; litter-based percentages: 4/8/10/12% for 0/0.075/0.15/0.2% groups, respectively; with cleft palate 1/0/2/4 in control/low/mid/high dose, respectively, and exencephaly for low and mid dose).

***Table**: Prenatal developmental toxicity study (No. 10) in mice*

DEVELOPMENTAL TOXICITY IN SWISS (CD-1) MICE FOLLOWING MATERNAL EXPOSURE TO THEOPHYLLINE IN THE DRINKING WATER ON DAYS 6 THROUGH 15 OF GESTATION

	Theophylline (% in drinking water)			
	0	0.075	0.15	0.20
All litters (No.)[a]	26	26	33	23
Implantation sites/litter[b]	12.4 ± 0.6	12.6 ± 0.5	11.9 ± 0.6	11.2 ± 0.8
% Preimplantation loss[b,c]	7 ± 2	6 ± 2	7 ± 2	3 ± 2
% Resorptions/litter[c]	10 ± 4	14 ± 4	27 ± 5*	34 ± 9*
No. (%) litters with resorptions[f]	14 (54)	18 (69)	29[g] (88)	18 (78)
No. (%) litters totally resorbed	1 (4)	1 (4)	4 (12)	6 (26)
No. litters with live fetuses[d]	25	25	29	17
Live fetuses/litter[b]	11.8 ± 0.5	11.1 ± 0.6	10.3 ± 0.4	11.6 ± 0.5
Average male fetal body weight per litter[b,e]	1.05 ± 0.03	1.02 ± 0.02	0.92 ± 0.03*	0.88 ± 0.02*
Average female fetal body weight per litter[b,e]	1.03 ± 0.03	0.98 ± 0.02	0.90 ± 0.03*	0.86 ± 0.03*
% Fetuses malformed/litter[b,e]	0.3 ± 0.3	0.7 ± 0.5	3.0 ± 1.5	2.3 ± 1.4
No. (%) litters with malformed fetuses[e]	1 (4)	2 (8)	5 (17)	3 (18)

[a] Includes all dams with implantation sites at termination.
[b] Reported as mean ± SEM.
[c] Defined as [(No. corpora lutea − No. implantation sites) ÷ No. corpora lutea] × 100.
[d] Includes only dams with live fetuses; litter size = number live fetuses per dam.
[e] Test for linear trend ($p < 0.05$).
[f] χ^2 test ($p < 0.05$).
[g] Fisher's exact test ($p < 0.05$).
* Statistically significant ($p < 0.05$) relative to the vehicle control group.

Maternal toxicity was noted at the mid and high dose (see table 23 in the CLH report): maternal weight gain was reduced during gestation mid and high dose (p<0.05), during treatment (high dose, p<0.05) and corrected weight gain (for gravid uterine weight) for high dose (4.4 g versus 6.1 g -31%, p<0.05), showing treatment-related trend. In addition, water, but not feed consumption, was reduced for mid and high dose (p<0.05), absolute but not relative liver weights were reduced in these dose groups as well (p<0.05). Primary clinical signs associated with theophylline treatment were piloerection, rough coat and weight loss. Other signs of toxicity observed during and after treatment were lethargy, hunched back, and dehydration; however, no information on doses is provided in the CLH report. Thus, adverse effects on foetal survival noted as resorption was accompanied by maternal systemic toxicity. The malformations could be a stress- and dehydration-related phenomenon (as reported in literature; Schwetz *et al*., 1977; Beyer and Chernoff, 1986) and not a teratogenic event. It has also been suggested that water deprivation might have contributed to the effects seen in mice after treatment (Lindström *et al*., 1990).

At lower doses, no such effects on development were reported in the mouse gavage screening study with CD-1 mice (No. 3) dosed with 0, 20, 60, 200 mg/kg bw/d for 19 days. The number of dead and life foetuses and implantation sites were assessed for a group of 10 females dosed seven days prior

cohabitation with dosing continued until GD12.

In the mouse gavage screening study (No. 11), with the same strain, dosed up to 200 mg/kg bw/d during GD 8-14, with females allowed to deliver and pups evaluated on PND 0, 1, and 4, no effects were observed on number of litters, number of implantations per female, number of live born pups or litter weights. Apart from lower doses and gavage administration, it is noted that these screening studies do not cover the sensitive window of organogenesis (GD 6-15) entirely nor a minimum number of pregnancies needed for assessment of developmental toxicity in order to obtain consistency between studies.

Two further studies in mice were reported by the DS, but were of limited relevance for classification as the studies were using the *i.p.* route of administration, which is of less relevance except for therapeutic treatment. RAC however notes, that findings observed with *i.p.* treatment in a well-conducted study may be considered if confirming observations seen after oral dosing. According to the CLH report, in animals, oral absorption is virtually complete; in rats up to 34% after oral dosing was excreted unchanged in urine, and during gestation metabolism was found to be reduced with longer half-time values and transplacental transfer was demonstrated. An actual comparison of plasma blood levels of the different dosing regimens oral and *i.p.*, however, is hampered as the actual plasma levels are not reported. But assuming complete and rapid oral absorption with up to 30% unchanged parent after oral dosing, placental transfer demonstrated, lack of foetal metabolism, with developmental effects at doses >260 mg/kg bw/d (No. 1) and 372 mg/kg bw/d (No. 10), it seem not justified to exclude *i.p.* studies (with test substance directly transferred to the uterus and thus without first-pass effect). This in particular if *i.p.* doses were lower than oral doses. The toxicokinetic information after oral dosing indicates that first-pass effect may be not as prominent as to discard *i.p.* studies for assessment of developmental toxicity. In humans, a dose of 1 g in two human volunteers resulted in 10% unchanged theophylline excretion. For 7.5 mg/kg bw/d oral dose, 99% was absorbed with a peak after 0.5-2 hours (see section 4.1.2 in the CLH report). Thus, in the view of RAC, *i.p.* studies may be considered as relevant information.

The *i.p.* studies cited, Fujii *et al*., 1969 (No. 12) and Tucci *et al*., 1978 (No. 13) were both quite old and evaluated as Klimisch 3 by the DS. Only limited information is presented in the CLH report. Dosing was conducted on one particular day during gestation, GD12 and GD10/11/12/13, in the two studies, respectively. In the Fujii study, 0, 175, 200, 225 mg/kg bw were administered to ICR-JCL mice, resulting in 40% mortality in high dose, clinical signs (dyspnea and convulsions) at all dose levels (but only slight at low and mid dose). Malformations including mainly cleft palate, but also digital defects and micrognathia, were observed at all dose levels, as well as decreased foetal weights and increased subcutaneous haematoma at mid and high dose. Considering the maternal toxicity, including high dose mortality, and the limited reporting and information provided in the CLH report it is difficult to conclude on the relevance of these findings. In the Tucci *et al*. study, with dosing of 100, 150, 200 mg/kg bw, no information is available on maternal toxicity and it seems that no control group was included; thus, the study has a major deficiency. For the treatment groups, a dose dependent increase in resorptions and malformations is reported, the main malformation on all injection days and exposure groups being, again, cleft palate. Except the maximum incidences (resorptions: max. for day 13 injection, 31%; malformations: max on day 11 injection), no further dose-response information is included in the CLH report. Considering the deficiencies in reporting of the Tucci *et al*. study (control group data, dose-dependent incidences and maternal toxicity), again, it is difficult to conclude on the relevance of the reported findings including resorptions and malformations. Considering both *i.p.* studies' deficiencies, the results are of limited value for

classification and labelling.

In the prenatal developmental toxicity study in rats (No. 14) conducted by NTP, theophylline was administered to pregnant Sprague-Dawley (CD) rats via the diet at 0, 0.15, 0.3 or 0.4% on GD 6-15 (sacrifice GD 20), dose levels corresponding to 0, 124, 218 and 259 mg/kg bw/d.

Maternal toxicity: Weight gains during gestation, and corrected weight gain was statistically significantly decreased at the high dose ($p<0.05$) dams; corrected weight gain already reduced by 10% at the mid dose, although not statistically significant. Clinical signs, mainly piloerection and rough coat, were observed for mid and high dose.

Development: The number of live foetuses per litter was decreased at the high dose group ($p<0.05$) and the average male and female foetal weights per litter were decreased at mid and high dose ($p<0.05$) indicating growth retardation, however at a dose that also reduced maternal weight gain. No statistically significant differences are reported for implantation sites, pre-implantation loss, and resorptions. RAC notes however that the number of litters with resorptions was apparently increased at mid and high dose (No.(%): 8(38), 8(40), 12(57), 15(71) for control, 124, 218, 259 mg/kg bw/d, respectively). No malformations or variations were reported.

***Table**: Prenatal developmental toxicity study (No. 14) in rats*

DEVELOPMENTAL TOXICITY IN SPRAGUE–DAWLEY (CD) RATS FOLLOWING MATERNAL EXPOSURE TO DIETARY THEOPHYLLINE ON DAYS 6 THROUGH 15 OF GESTATION

	Theophylline (% in feed)			
	0	0.15	0.30	0.40
All litters (No.)[a]	21	20	21	21
Implantation sites/litter[b]	13.7 ± 0.7	13.4 ± 0.6	14.6 ± 0.7	13.8 ± 0.4
% Preimplantation loss[b,c]	3 ± 1	10 ± 4	4 ± 2	7 ± 2
% Resorptions/litter[b]	8 ± 5	4 ± 1	11 ± 5	11 ± 2
No. (%) litters with resorptions	8 (38)	8 (40)	12 (57)	15 (71)
No. (%) litters totally resorbed	1 (5)	0 (0)	1 (5)	0 (0)
No. litters with live fetuses[d]	20	20	20	21
Live fetuses/litter[b]	13.8 ± 0.3	12.9 ± 0.6	14.2 ± 0.4	12.0 ± 0.4*
Average male fetal body weight per litter[b,e]	3.6 ± 0.1	3.6 ± 0.1	3.3 ± 0.1*	3.2 ± 0.1*
Average female fetal body weight per litter[b,e]	3.5 ± 0.0	3.5 ± 0.1	3.1 ± 0.1*	3.0 ± 0.0*
% Fetuses malformed/litter[b]	1.4 ± 0.6	0.9 ± 0.7	0.3 ± 0.3	1.6 ± 0.7
No. (%) litters with malformed fetuses	4 (20)	2 (10)	1 (5)	4 (19)

[a] Includes all dams with implantation sites at termination.
[b] Reported as mean ± SEM.
[c] Defined as [(No. corpora lutea − No. implantation sites) ÷ No. corpora lutea] × 100.
[d] Includes only dams with live fetuses; litter size = No. live fetuses per dam.
[e] Test for linear trend ($p < 0.05$).
* Statistically significant ($p < 0.05$) relative to the vehicle control group.

In the developmental study in rabbits (No. 15), theophylline was administered intravenously to mated KbI:JW rabbits at 0, 15, 30, 60 mg/kg bw/d on GD 6-18 from the day of implantation during

organogenesis (sacrifice GD 29). According to the CLH report, maternal toxicity was evident with decreased body weights and feed intake, clinical signs, and one death (no reason stated), and also, four abortions seen in the high dose. Foetal toxicity was evident based on an increase in late foetal deaths at the high dose, while no differences in number of implantations and life foetuses was reported. Cleft palate was observed in eight foetuses (two litters) at the high dose but not in control animals, as well as an increase in 13th rib (skeletal variation); otherwise no differences in the incidences of visceral or skeletal anomalies or ossifications. As these effects were reported only for the high dose group, which induced severe maternal toxicity (including death), these findings are not considered for classification purpose.

Summary – developmental toxicity

Available human data for developmental effects do not indicate adverse effects that would warrant classification for adverse effects on development.

In mice, a reduction in number of live pups per litter in the continuous breeding study (No. 1) is the main effect. The reduced number of live pups in each litter was consistently shown, dose-dependent, in five consecutive litters and confirmed in the cross-over mating with untreated males for the high dose females. These effects were confirmed as a developmental effect (increase in resorptions) in the mouse oral prenatal developmental study (No. 10). No such effects however were observed in the mouse oral screening studies (No. 3, 11). This study inconsistency may be related to different route of administration and differences in toxicity after diet and gavage dosing (the latter being gavage studies and not diet), or the fact that the screening studies did not cover the entire period of organogenesis and had a lower number of dams.

In addition, the highest dose level applied in the screening study (No. 3, 11: 200 mg/kg bw/d) was below the dose level inducing an increase in resorptions in the developmental study in mice (No. 10; i.e. ≥372 mg/kg bw/d), but comparable to the lower dose level in the continuous breeding study (No. 1), inducing a small but significant reduction in live pups per litter (i.e. 126 mg/kg bw/d). The *i.p.* studies suffer from major deficiencies in reporting. The developmental effects in rats consist of a decrease in live pups per litter at the highest dose and a decrease in foetal body weight at the low and high dose levels (No. 14). At this highest dose level, a 22% reduction in corrected weight gain is reported and it is questionable that this can fully explain the offspring effects. As noted by the DS, feed restriction studies in SD rats (Fleeman *et al*., 2005; Chapin *et al*., 1993) showed that the number of viable foetuses or the number of live pups per litter were not affected upon feed restriction despite having reduced body weight. While toxicity in 14-week gavage studies also comprised segmented neutrophil counts and increases in the incidences of mesenteric and/or pancreatic periarteritis (rat specific phenomenon) at comparable dose levels, RAC considers the offspring effects as being a direct test substance related effect, and a severe adverse effect on foetal development. The effects observed in rabbits are not considered by RAC, as severe maternal toxicity including death and abortion accompanied the effects in offspring.

Effects via / on lactation

The DS included for the assessment of effect of lactation one study (Hart and Grimble, 1990a/b) in female Wistar rats with theophylline administration in the drinking water of 0 or 1 mg/kg bw/d (single dose level), throughout pregnancy and up to lactation day 14, to groups of 5 or 6 pregnant females. The dose levels were chosen to mimic the theophylline intake that occurs when drinking tea. Bodyweight and milk samples were measured. No effect on maternal weight and carcass fat nor volume or composition of the breast milk were observed during pregnancy and lactation. No effects

on litter weight were observed.

In relation to human information, the DS included four studies mostly on the kinetic of theophylline transfer to breast milk following oral or intravenous administration.

The transfer of theophylline to breast milk following single oral doses of theophylline of 4.25 mg/kg bw in three asthmatic patients was investigated, and in two patients after four daily doses of 200 mg aminophylline (i.e. theophylline with ethylenediamine in 2:1 ratio). The average milk to serum concentration was about 0.7; milk concentration paralleled the time-course of serum concentrations. Irritability and fretful sleeping were observed in one infant only on days when the mother was taking theophylline, while no such effects were seen in the other infant (Yurchak and Jusko, 1976).

Another study investigated the kinetics of transfer to breast milk in three nursing patients following single intravenous doses of 3-5 mg/kg bw of theophylline. The breast milk:serum concentration ratio was about 0.7 (Stec *et al*., 1980).

A third study investigated the kinetics of the transfer of theophylline from breast-feeding mothers to their infants following administration of two oral doses (300 mg followed by 200 mg after four hours) of theophylline to 12 lactating mothers. Breast milk:plasma ratios between 0.6-0.9 were calculated. The mean levels obtained within one to ten hours after the first dose were in the range of 6-10 mg/L in plasma and in the range of 3-7 mg/L in milk (Reinhardt *et al*., 1983).

The last study investigated the kinetics of theophylline in 11 asthmatics throughout pregnancy and post-partum. On four occasions, they determined theophylline concentrations in breast milk samples collected prior to treatment and at three time points after treatment, as well as in infant plasma samples obtained prior and after feeding. Concentrations in milk roughly paralleled those in plasma. The breast milk:plasma concentration ratios varied between 0.54 and 1.08. According to the authors, characterisation of the theophylline acquisition by the nursing neonates was hampered by an inadequate number of neonatal plasma samples. In all cases, however, detectable levels of theophylline were present in the neonate before and after feeding (Gardner *et al*., 1987).

Comparison with the criteria

Sexual function and fertility

As concluded in paragraph 4.11.4.1, no appropriate human data are available and classification in category 1A is not appropriate.

A range of studies on theophylline investigating sexual function and fertility and repeated dose toxicity is available. However, based on study design only study (No. 1) may provide conclusive results regarding sexual function and fertility. In this study, effects on sexual function was observed as testicular effects, which were rather mild. Overall, it is suggested theophylline may be a testicular toxicant, as some effects were also observed in other studies after 19-75 weeks, although in presence of general toxicity. These effects are inconsistent between studies as in particular the 14-week NTP studies in rats and mice did not confirm these effects. The adverse and consistent effects on females in the RACB study may be developmental toxicity or a fertility effect, but the developmental toxicity studies (described below) confirm a developmental effect based on an increased incidence of resorptions. Thus, overall, no fertility effects are identified that meet the

requirements for classification. The effects on sexual function (testicular effects) are rather mild and inconsistent between studies. RAC concludes in line with the DS, that these limited data do not meet the requirement for classification for effects on fertility. Therefore, **classification for adverse effects on sexual function or fertility is not warranted.**

Development

According to CLP criteria, substances are classified in Category 1A for reproductive toxicity when they are known to have produced an adverse effect on development in humans. As concluded in paragraph 4.11.4.2, the available human data are insufficient for classification. Therefore, classification in category 1A is not warranted.

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 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.

RAC considers the reduction in number of live pups per litter in the continuous breeding study (No. 1), consistently shown, dose-dependent, in 5 consecutive litters, and confirmed in the cross-over mating with untreated males for the high dose females, as the most relevant finding for classification. These effects were confirmed as a developmental effect as an increase in resorptions were seen in the mouse oral prenatal developmental study (No. 10). These findings are treatment-related and severe developmental effects, although the evidence is not clear whether pharmacodynamic effects occurred directly to the foetus or to the dams that affected the foetus. The parental toxicity however was mild in the continuous breeding study. Maternal toxicity in the mouse oral prenatal developmental toxicity was limited to reduced adjusted bw gain at the mid and high dose. In line with the DS, it is considered unlikely that the observed increase in resorptions is secondary to the limited maternal toxicity at the higher dose levels. In rats, the reduced foetal body weight is an indication of the developmental delay and not sufficiently severe to warrant classification. RAC agrees with the DS that the decrease in live pups per litter in rats is a severe developmental effect and consistent with the effects observed in mice studies. There is no information which species is more relevant to humans or information that shows that the observed effects are not relevant to humans.

RAC acknowledges the fact that theophylline is a known medication used in humans and has been extensively used during decades.

No human data have been made available to RAC that would provide evidence for developmental effects relevant for classification according to CLP. According to the DS, data on theophylline use in human medicine was considered in the CLH dossier based on a search on the European Medicines Agency public website, which did not give any results containing information on the reproductive toxicity of theophylline. The human data included in the CLH dossier by the DS on the potential effects of theophylline in pregnant asthmatic women were negative. However, the data had several deficiencies, including limited statistical power and deficiencies in their design, which did not make it possible to distinguish between the roles played by asthma itself and theophylline use. In two studies (Bracken *et al*., 2003; Schatz *et al*., 1997), use of theophylline during pregnancy was found to cause an increase in preterm deliveries.

A typical dosing for humans is around 600 mg/d. Therapeutic dose levels should envisage 8-20 µg/L plasma concentration. Dosing is individual, and for adults a daily dose in the range of 11-13 mg/kg

bw/d is recommended. Animal findings reported in the before described studies start at 126 mg/kg bw/d (the low dose of the mouse continuous breeding study). A direct comparison of the dosing regimens, and effects observed in human and animal studies would require robust human data and toxicokinetic information for humans and animals (including plasma levels, AUC). However, this is not available to RAC and based on the poorly available human data (not providing robust evidence for no effects), it is not possible to derive a threshold level for developmental effects, nor the conclusion that a lack of evidence in humans at the therapeutic dose levels would be in contradiction to the animal data.

RAC takes note of the recommendation for theophylline as bronchodilator of choice for asthma and chronic obstructive pulmonary disease in the pregnant patient (e.g. Briggs *et al*., Drugs in pregnancy and Lactation, 2011, 9th ed). In general, medical treatment includes a risk benefit evaluation and it might be necessary to treat women for asthma, although they are pregnant. In that case, the choice will be for the least adverse treatment. A recommendation for theophylline as the preferred medication for pregnant women does not necessarily mean that there is no adverse effect. It is evident that human data on developmental toxicity are challenging and have some drawbacks, not only due to the size and exposure of the population but also due to limited information on whether exposure took place in the critical time window. In fact, theophylline is advised against in the first trimester of pregnancy; the theophylline medication package insert advices against the treatment of women in the first three months of pregnancy, due to insufficient data for this time window. In the following two trimesters of the pregnancy, theophylline should only be used after strict risk-benefit evaluation and only if absolutely necessary. At the end of pregnancy, theophylline was shown to inhibit the contractions.

The CLP classification criteria are independent on how a chemical is used, and classification of theophylline will apply to its industrial use(s). The conclusion for classification according to the CLP criteria is not in disagreement with the recommendation of theophylline as bronchodilator of choice for pregnant patients.

In agreement with the DS, based mainly on the developmental effects as seen in mice, but also taking into account the effects in rats, **RAC concluded that classification of theophylline as Repr. 1B (H360D) is warranted.**

Effects on/via Lactation

Classification for effects on or via lactation can be assigned based on 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.

RAC agrees with the DS's conclusion that there are no data that fulfil the first and second criterion. Concerning human data that show theophylline to be excreted in breast milk, there is no information that would allow determination of whether these amounts would be sufficient to cause concern for the health of the breastfed child.

The limited data from the Wistar rat study (Hart and Grimble, 1990a/b), with doses equivalent to a daily cup of tea of course, is insufficient for assessing the potential for effects on the litter. The human data shows theophylline to be excreted in breast milk with a breast milk:plasma ratio between 0.54 to 1.08.

The medication package insert of theophylline advices for breastfeeding women that theophylline should only be used if absolutely necessary, at as low as possible doses, as theophylline is transferred to milk. The neonate should be carefully monitored for theophylline effects as therapeutic serum concentrations maybe reached. If higher therapeutic doses are needed, women are advised to stop the nursing. RAC notes, that no human information on adverse effects on the neonate via breastfeeding has been made available in the CLH report.

RAC proposes no classification for effects on or via lactation for theophylline, due to lack of appropriate human and animal data.

Supplemental information - In depth analyses by RAC

The DS submitted a summary of the toxicity of two other methylxanthine derivatives including caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine). The information on reproductive toxicity of caffeine was summarised from two NTP studies (NTP, 1996 and NTP, 1984a) and for theobromine in one NTP study (NTP, 1984b). A scientific opinion on the safety of caffeine was presented by EFSA (2015) and is also summarised.

A very recent publication from CIR (Cosmetic Ingredient Review) was published in August 2018 and includes an assessment of safety of the three xanthine alkaloids caffeine, theophylline and theobromine. According to the Dictionary, caffeine, theobromine and theophylline function as skin-conditioning agents, and theobromine also functions as a fragrance ingredient. This publication was not submitted by the DS in the CLH report or during the consultation and will therefore not be summarised here. The link to the publication can be found here: https://www.cir-safety.org/panelbook/safety-assessment-xanthine-alkaloids-used-cosmetics.

Caffeine: The potential reproductive toxicity of caffeine in Sprague-Dawley rats was evaluated using the RACB design (NTP, 1996). Dose levels were 12.5, 25, and 50 mg/kg bw/d. No differences were observed in reproductive endpoints. A crossover mating trial revealed no changes on male or female fertility or in pup weight. Reproductive parameters were comparable between dose groups when naïve males were mated with control or 50 mg/kg bw/d dosed females and when naïve females were mated with control or 50 mg/kg bw/d dosed males.

Reproductive toxicity was also tested in Swiss CD-1 mice with caffeine (NTP, 1984a) with a RACB design with doses estimated equal to 22, 44, and 88 mg/kg bw/d. While there were no exposure-related changes in the number of litters/pair, viability, or adjusted pup weight, the number of live pups per litter, averaged over the 4-5 litters, dropped 15% at the medium dose and 20% for the high dose animals. No crossover mating trial was conducted, and the offspring from the last litter of control and high dose mice were reared by their dams until weaning, when they were given the same treatment as their parents until mating at 74±10 days of age. At the second generation mating trial, there were no changes in any reproductive endpoints. At necropsy, at 0.05% caffeine, male body weight was reduced by 8% while male adjusted liver weight increased by 8%. No change was found in female body or organ weights, or in any sperm endpoint. In summary, a reduction in the number of live pups/litter for the F0 generation was the only reproductive effect observed in this study. This occurred in the absence of a change in body weights in the F0 parental mice.

EFSA (2015)

A scientific opinion on the safety of caffeine (focusing on oral route, taking into account both single doses of caffeine as well as habitual caffeine consumption) was presented by EFSA (2015). With respect to caffeine consumption during pregnancy and during lactation, the following was concluded

by EFSA:

Pregnancy

There are no studies on the health effects of single doses of caffeine consumed by pregnant women prior to intense physical exercise. Regarding the different kinetics of caffeine in this population subgroup, single doses of caffeine which are of no safety concern for non-pregnant adults do not apply to pregnant women performing physical exercise.

Caffeine intakes from all sources up to 200 mg per day consumed throughout the day by pregnant women in the general population do not give rise to safety concerns for the foetus. This conclusion is based on prospective cohort studies showing a dose-dependent positive association between caffeine intakes during pregnancy and the risk of adverse birth weight-related outcomes (i.e. foetal growth retardation, small for gestational age) in the offspring. In these studies, the contribution of "energy drinks" to total caffeine intake was low (about 2%).

Data to characterise the risk of habitual caffeine consumption in this population subgroup are scarce.

Lactating women

Single doses of caffeine up to 200 mg and habitual caffeine consumption at doses of 200 mg per day consumed by lactating women in the general population do not give rise to safety concerns for the breastfed infant. At these doses of caffeine, daily caffeine intakes by the breastfed infant would not exceed 0.3 mg/kg bw, which is 10-fold below the lowest dose of 3 mg/kg bw tested in a dose finding study and at which no adverse effects were observed in the majority of infants.

There are no data to characterise the risk of single doses of caffeine consumed by lactating women, and data on habitual caffeine consumption in this population subgroup are scarce.

Theobromine: The reproductive toxicity of theobromine was evaluated in CD-1 mice according to the RACB design with doses levels of 0, 0.10, 0.25, and 0.5% in the diet, corresponding to approx. 0, 126, 335, and 630 mg/kg bw/d. Theobromine treatment adversely affected at least one or more reproductive parameters in all three dose groups ($p<0.05$). Furthermore, in the 0.5% dose group, the proportion of pups born alive per fertile pair was 0.64 as compared to 0.98 in the control group suggesting that theobromine may be a foetotoxicant. The study was extended to determine the affected sex in the high dose (0.5%) group (Task 3). The crossover mating trial demonstrated that reproductive capacity was severely impaired in female mice ingesting theobromine. More specifically, the number of live pups per litter, proportions of pups born alive, and pup body weights were significantly reduced. Males ingesting 0.5% theobromine revealed a significant increase in the incidence of abnormal sperm. The liver in both male and female mice was significantly enlarged. The testicular weight in males and brain weight in both male and female mice were depressed. No morphological changes were seen in the reproductive organs. Hormonal patterns in both male and female mice were unaffected by the theobromine treatment. Thus, under the conditions of the study, theobromine ingestion had adverse effects on various endpoints of male and female reproductive functions without significant effects on general health and growth.

4.12 Other effects

Not relevant for this CLH-report.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this CLH-report.

6 OTHER INFORMATION

Not relevant for this CLH-report.

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