Health Council of the Netherlands

Theophylline

Evaluation of the effects on reproduction, recommendation for classification

2013/02

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Theophylline*
Uw kenmerk : DGV/MBO/U-932542
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Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van theofylline op de vruchtbaarheid en het nageslacht; het betreft ook effecten op de lactatie en via de moedermelk op de zuigeling. Dit advies maakt deel uit van een uitgebreide reeks waarin voor de voortplanting giftige stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld.

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

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

Met vriendelijke groet,

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

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Theophylline

Evaluation of the effects on reproduction, recommendation for classification

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

to:

the Minister of Social Affairs and Employment

No. 2013/02, The Hague, April 05, 2013

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

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

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INAHTA

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Samenvatting

In het voorliggende advies heeft de Gezondheidsraad theofylline onder de loep genomen. Theofylline, een methylderivaat van xanthine, is een luchtwegverwijder die wordt voorgeschreven bij astma en chronische obstructieve luchtwegaandoeningen. Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Subcommissie Classificatie reproductietoxische stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de Raad, hierna aangeduid als de commissie, kijkt zowel naar effecten op de vruchtbaarheid van mannen en vrouwen als naar effecten op de ontwikkeling van het nageslacht. Daarnaast worden effecten op de lactatie en via de moedermelk op de zuigeling beoordeeld.

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

- voor effecten op de fertiliteit adviseert de commissie om theofylline niet te classificeren wegens onvoldoende geschikte gegevens
- voor effecten op de ontwikkeling adviseert de commissie theofylline in categorie 1B te classificeren (*stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting*) en met H360D (*kan het ongeboren kind schaden*) te kenmerken

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

Executive summary

In the present report, the Health Council of the Netherlands reviewed theophylline. Theophylline is a methylxanthine drug and used as a bronchodilator in the therapy for respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at the request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. The Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee, evaluates the effects on male and female fertility and on the development of the progeny. Furthermore, the Committee considers the effects of a substance on lactation and on the progeny via lactation.

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

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

Chapter 1

Scope

1.1 Background

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

1.2 Committee and procedure

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

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

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

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

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

In 2012, the President of the Health Council released a draft of the report for public review. No comments were received.

1.3 Effects on or via lactation

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The guideline defines that substances which are absorbed by women and have been shown to interfere with lactation or which may be present (including metabolites) in breast milk in amounts

sufficient to cause concern for the health of a breastfed child, shall be classified and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely independent of dosage), the labelling for effects on or via lactation is based on risk characterization and therefore, it also includes consideration of the level of exposure of the breastfed child.

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

1.4 Data

Literature searches were conducted in the on-line databases Current Contents and Medline, starting from 1966 up to March 2011 and by searches on internet. A final search was performed in March 2012 in Pubmed. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted as well as several websites regarding (publications on) toxicology and health. References are divided into literature cited and literature consulted, but not cited.

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

In the assessment of the potential reproduction toxic effects of theophylline, the Committee also used data on adverse effects related to its application as a therapeutic agent.

1.5 Presentation of conclusions

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

- Lack of appropriate data preclude assessment of the compound for reproductive toxicity

- Sufficient data show that no classification for toxic to reproduction is indicated.

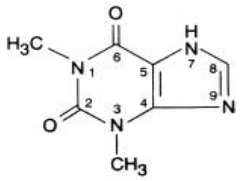
1.6 Final remark

The classification of compounds is based on hazard evaluation only (Niesink et al., 1995)[13], which is one of a series of elements guiding the risk evaluation process. The Committee emphasizes that for derivation of health-based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organizations.

Chapter 2

Theophylline

2.1 Properties

name	:	theophylline
IUPAC name	:	1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione
CAS name	:	1H-purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-
CAS registry number	:	58-55-9
EC/EINECS number	:	200-385-7
synonyms	:	1,3-dimethylxanthine
colour and physical state		white crystalline powder
molecular formula	:	$C_7H_8N_4O_2$
structural formula	:	
molecular weight	:	180.16
melting point	:	270-274°C
vapour pressure	:	$7x10^{-7}$ Pa (at 25°C; estimated)
solubility in water	:	7,360 mg/L (at 25 °C)
Log $P_{octanol/water}$	:	- 0.02 (recommended: see http://logkow.cisti.nrc.ca/logkow/search.html)

use	:	theophylline is a methylxanthine drug and used as a bronchodilator in the therapy for respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma. It is naturally found in tea and cocoa beans[2]; therapeutic doses of theophylline are in the range of 2-12 mg/kg/day, achieving 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.[18]
general toxicity	:	adverse reactions associated with theophylline are generally mild when peak serum concentrations are below 20 µg/mL, and mainly consist of transient caffeine-like adverse effects such as nausea, vomiting, headache and insomnia; however, when peak serum theophylline concentrations exceed 20 µg/mL, theophylline produces a wide range of adverse reactions including persistent vomiting, cardiac arrhythmias and intractable seizures which can be lethal.[2]
mechanism	:	theophylline has two distinct actions in the airways of patients with reversible obstruction: smooth muscle relaxation (i.e. bronchodilation) and suppression of the response of the airways to stimuli (i.e. non-bronchodilator prophylactic effects).[2]
kinetics	:	theophylline is rapidly and completely absorbed after oral administration in solution or immediate-release solid oral dosage form. Once theophylline enters the systemic circulation, about 40% is bound to plasma protein. Unbound distributes freely throughout body water, but poorly into body fat. An increase in the volume of distribution of theophylline, primarily due to reduction in plasma protein binding, occurs amongst others in women during the third trimester of pregnancy and in premature neonates. In such cases, the patient may show signs of toxicity at total (bound + unbound) serum concentrations of theophylline in the therapeutic range (10-20 µg/mL), due to elevated concentrations of the pharmacologically active unbound drug.[2] In adults and children beyond one year of age, approximately 90% of the dose is metabolized in the liver. In neonates, approximately 50% of the theophylline dose is excreted unchanged in the urine. The pharmacokinetics of theophylline varies widely among similar patients.[2] Theophylline passes freely the placenta and is excreted into breast milk.[8]

2.2 Human studies

Fertility studies

No studies were found regarding the effects of exposure to theophylline on human fertility.

Developmental toxicity studies

Schatz et al. 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. These studies were conducted at 16 centres of the Maternal Fetal Medicine Units Network of the US National Institute of Child Health and Human Development with recruitment from December 1995 through March 1999. During this time, patients with moderate asthma were first offered entry into the randomized controlled trial and if they refused, they were offered entry into the observational cohort study. The final cohort included 2,123 asthmatic participants, 1,739 from the observational study and 384 from the randomized controlled trial. No differences in perinatal outcomes were found comparing theophylline-using participants (n=273) and participants on other types of medication (n=1,850).[15]

Heinonen et al. conducted an epidemiological investigation of the 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 indentified. 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.[7]

In a prospective cohort study of 51,830 singleton pregnancies at 12 medical centres in the US between 1959 and 1966, the association between theophylline and stillbirth was evaluated. Theophylline use during pregnancy was not associated with any increase in the risk of stillbirth. This applied both to theophylline-using women who had a diagnosis of one form or another of asthma (n=392) and to those who were not so labelled (n=814; it was not clear why subjects without a status 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).[12]

In a Finnish case-control study conducted in 1982-1990, the data of 212 pregnant asthmatics with theophylline treatment were compared with findings in 292

pregnant asthmatics without theophylline treatment and 237 non-asthmatic pregnant control subjects. There were no differences between the groups as to age, height, age of onset of asthma, lung function, parity or smoking. The incidence of preeclampsia (15.6%) was higher in theophylline-treated subjects than in untreated asthmatics (10.6%) or non-asthmatic controls (6.4%). No differences were seen between the groups with regard to gestational age, birth weight, Apgar score or perinatal deaths. 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.).[20]

Schatz et al. performed a prospective study of 824 asthmatic pregnant women (receiving various types of medication) and 678 non-asthmatic pregnant women, followed between 1978 and 1989. The numbers of subjects exposed to theophylline were 292 (first trimester) and 429 (any time exposure), but exposure was not unique (if needed for the prevention of acute asthmatic episodes or symptoms interfering with sleep or normal activity, medication was administered sequentially in the following order 1) β-agonist; 2) theophylline and/or cromolyn; 3) beclomethasone, 4) prednisone). Perinatal outcomes were compared in theophylline-exposed versus unexposed individuals (with and without asthma). 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 Schatz et al., this finding may have been confounded by the presence and the severity of asthma.[16]

The effects of asthma or various asthma therapies were prospectively examined in 872 pregnant women with a diagnosis of 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 derive 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.).[1]

Lactation

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 and following four daily doses of 200 mg aminophylline (i.e. theophylline with ethylenediamine in 2:1 ratio) in two patients. 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 and 4.0 mg/L, respectively. The average milk to serum concentration was about 0.7, and 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.[21]

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 aminophylline. Serum and milk concentrations paralleled; the breast milk:serum concentration ratio was about 0.7.[19]

Reinhardt et al. investigated the kinetics of the transfer of theophylline from breast feeding mothers to their infants. Following administration of a dual dose (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 approximately 6-10 mg/L in plasma and approximately 3-7 mg/L in milk).[14]

As part of a study on the effects of pregnancy on the kinetics of theophylline throughout pregnancy and post-partum, Gardner et al. 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.[3]

2.3 Animal studies

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

Fertility studies

Theophylline was tested for its effect on fertility in Swiss CD-1 mice according to the Reproductive Assessment by Continuous Breeding (RACB) design used by the National Toxicology Program.

The RACB (Task 2) design uses 20 rodents/sex/group (controls: 40 pairs) which are exposed from one week prior to cohabitation. During a subsequent 14-week continuous exposure, the animals are housed as breeding pairs and normally four to five litters are delivered per adult pair. Theophylline was administered at 0%, 0.075%, 0.15% and 0.3% weight per volume in the feed, providing calculated consumptions of 0, 126, 260 and 500 mg/kg bw/day. After 14 weeks of treatment, male mice gained nearly 7, 6, 4 and 3% of their initial body weights, respectively, while group mean body weights of the female mice varied with the gestational phase. Alopecia occurred in both sexes of all treatment groups (20-25% in the low-dose group and >50% in the mid- and high-dose groups). Three control mice and four low-dose mice died.

In the high-dose group, the number of days to deliver each litter was consistently increased (three days longer for the first litter, five days longer for the last litter and similarly increased for all other litters).

After the last litter had been delivered, the females were evaluated for vaginal cyclicity for seven days, and then the F0 mice in the control group and the high-dose group were killed and necropsied. There was an 11% increase in relative liver weight in the high-dose females. There were no changes in the length of the

oestrous cycle or in the percent of time spent in the various oestrus stages. Treated male terminal body weights were reduced by 7%. Relative seminal vesicle weight was decreased by 19% in the treatment (0.3%) group. Epididymal sperm density was reduced by 20% in the treatment (0.3%) group. The percent motile and the percent of abnormal morphologic forms were unchanged in the treatment (0.3%) group (Lamb et al.)[9], (Morrissey et al.)[11]. This study does not allow conclusions on the effects of theophylline on reproductive performance.

Sperm morphology and vaginal cytoIogy were evaluated in F344 rats and $B6C3F_1$ mice as part of subchronic (13-week) toxicity studies of theophylline. Theophylline was administered by gavage at 0, 75, 150 and 300 mg/kg bw/day (mice) or 0, 37.6, 75 and 150 mg/kg bw/day (rats). In parallel studies, theophylline was administered in the diet at levels of 0%, 0.1%, 0.2% and 0.4%. These levels were approximately equivalent to 0, 200, 400 and 800 mg/kg bw/day (mice) or 66, 130 and 260 mg/kg bw/day (rats).

In the gavage studies, terminal body weights were decreased in male mice at 150 and 300 mg/kg bw/day, while absolute testis weights were decreased in high-dose mice (300 mg/kg bw/day). In high-dose rats (150 mg/kg bw/day), testis weights were also decreased and body weights tended to be lower. Other reproductive endpoints, including sperm number and motility, epididymis weight and oestrous cycle length, were not significantly affected.

In the parallel 13-week feeding studies, terminal body weights were decreased in mice of both sexes in all treatment groups, but not in rats. In mice, increases were noted in epididymis weights in the mid-dose and high-dose group and in cauda epididymis weights in the mid-dose group. In rats, the absolute epididymis weights were increased in the mid-dose group, whereas the cauda epididymis weights were decreased in the high-dose group. In rats, there was a dose-related increase in abnormal sperm (significant in the high-dose group only). Oestrus cycle length was not affected in any group (Morrissey et al.).[11]

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 for 17 days and then necropsied. There were no adverse changes in clinical signs, body weights or in histology of liver and kidneys. Weights of testes and epididymides, sperm density per cauda and sperm motility were not affected. 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 (Harris et al.).[4]

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 for 19 days. After seven days of dosing, these females were cohabited with male mice that were treated for five days prior to mating (until day 5 of cohabitation). After 19 days of dosing, the females were killed. There were no adverse clinical signs. 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). There were no effects on live, dead or total implants per female (Harris et al.).[4]

Developmental toxicity studies

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 above *Fertility studies*).

Significant reproductive effects were observed; fewer pups per litter were noted at all doses (reduced by 22%, 29% and 42% in the low-, mid- and high-dose group, respectively). In the high-dose group, there was a 19% reduction in the mean number of litters per pair and a 6% decrease in live pup weight adjusted for litter size.

A crossover mating trial was performed with F_0 mice to detect which sex had been affected. According to the RACB (Task 3) design, three groups are formed: control males x high-dose females, high-dose males x control females and controls x controls (20 pairs in each group). In this mating trial, there were no differences in the percent of pairs mating or delivering a live litter. However, 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% (Lamb et al.)[9], (Morrissey et al.)[11].

In summary, theophylline caused adverse reproductive effects in Swiss CD-1 mice (fewer live pups per litter at 126, 260 and 500 mg/kg bw/day, with reduced pup weight and fewer litters per pair at the high-dose level) in the absence of changes in maternal body weight.

Theophylline was administered to groups (n=20-21/group) of pregnant Sprague-Dawley (CD) rats in the feed during gestational day 6 through 15. The dietary levels were 0%, 0.15%, 0.3% or 0.4%, providing an estimated intake of 0, 124, 218 or 259 mg theophylline/kg bw/day . There were no maternal deaths. Piloerection was noted at a higher incidence in the two higher dose groups. Maternal body weight gain and maternal body weight on gestational day 20 corrected for gravid uterine weight were decreased in the high-dose group.

Maternal feed consumption was decreased in the high-dose group, and water consumption was increased in all theophylline-treated groups. Gravid uterine weights tended to be lower in the high-dose group, but the differences with the controls were not significant. There were no differences among the groups in number of implantation sites per litter, percentage pre-implantation loss, litters with resorptions or percentage resorptions per litter. The number of live foetuses per litter was decreased at the high-dose level and the average male and female foetal weight per litter was decreased in the mid- and high-dose group. The percentage of malformations per litter was not affected. External, visceral or skeletal malformations and variations were not affected by theophylline. Lindström et al. concluded that the NOAEL for maternal toxicity was 218 mg/kg bw/day and for developmental toxicity 124 mg/kg bw/day (Lindström et al.).[10]

Theophylline was administered to groups (n=23-33/group) of pregnant Swiss (CD-1) mice in the drinking water during gestational day 6 through 15. The levels in the drinking water were 0%, 0.075%, 0.15% or 0.2% providing an estimated intake of 0, 282, 372 or 396 mg theophylline/kg bw/day. There were no maternal deaths. Piloerection was noted at a higher incidence in the two higher dose group. Maternal body weight gain during gestation, maternal body weight on gestational day 17 corrected for gravid uterine weight and maternal water consumption were decreased in the mid- and high-dose group. Gravid uterine weight was decreased in the high-dose group. The percentage of resorptions per litter was increased in the mid- and high-dose group. There were no differences in number of implantation sites per litter or the percentage pre-implantation loss. The average male and female weight per litter was decreased in the mid- and high-dose group. The number of externally malformed foetuses was slightly increased in the mid-and high-dose group, but statistical significance was not obtained. Visceral or skeletal malformations and variations were not affected by theophylline.

Lindström et al. concluded that the NOAEL for maternal and developmental toxicity was 282 mg/kg bw/day (Lindström et al.).[10]

The Committee notes that the study may have been confounded by the decreased water intake in the mid- and high-dose group.

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 for 19 days. After seven days of dosing, these females were cohabited with male mice that were treated for five days prior to mating (until day 5 of cohabitation). After 19 days of dosing, the females were killed. There were no adverse clinical signs. One female in the

high-dose group was killed moribund. Pregnancy rate was, non-significantly, decreased in the high-dose group (6/9 versus 9/10 in all other groups). There were no effects on live, dead, or total implants per female (Harris et al.).[4]

Groups of mated female Swiss CD-1 mice (n=13-15/group) were dosed by gavage with 0, 20, 60 and 200 mg theophylline/kg bw/day during organogenesis (gestational day 8-14). The dams were allowed to deliver and litters were evaluated on postnatal day 0, 1 and 4. There were no effects on the number of females littering, number of implantations per female, number of live neonates or total litter weight (Harris et al.).[4]

The foetal toxicity of theophylline and its relationship to maternal plasma levels were examined in mated rabbits (n=20/group) dosed intravenously from gestational day 6 through 18. Theophylline was injected into the auricular vein at 0, 15, 30 or 60 mg theophylline/kg bw/day. 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. The dams were sacrificed on gestational day 29.

Decreases in body weight and in feed intake and reversible toxicity (accelerated respiration, sluggish startle reactions, dilation of the auricular vessels, polyurea) were noted in the high-dose group. One animal died and four animals aborted in the high-dose group.

Foetal toxicity was observed in the high-dose group, including late foetal death and a tendency towards decreased foetal body weights. There were no differences in implantations, live foetuses or sex ratio. Cleft palate was observed in eight foetuses (two litters) of the high-dose group. Increased incidences of skeletal variations (13th rib) were noted in the high-dose group; there were no differences in the incidence of visceral or skeletal anomalies or of ossifications (Shibata et al.).[17]

Lactation

Theophylline was administered to Wistar rats (n=5 or 6/group) at 0 or 1 mg/kg bw/day via the drinking water throughout pregnancy up to lactational day 14. The dose of 1 mg/kg bw/day was stated to mimic the proportion of theophylline in tea. Theophylline had no effect on maternal weight and carcass fat during pregnancy/lactation, the volume or composition of the milk, or on litter weight.[5,6]

2.4 Conclusions

Fertility

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

In animal studies, effects of theophylline on the male reproductive system were noted. Theophylline caused reduced relative seminal vesicle weights and epididymal sperm numbers in mice at 500 mg/kg bw/day[9,11], lower absolute testis weights in mice at 300 mg/kg bw/day and in rats at 150 mg/kg bw/day[11] and increased epididymis weights in mice at 400 and 800 mg/kg bw/day[11]. These findings were accompanied by reductions in body weight. In another study in rats, the absolute cauda epididymis weights were decreased and abnormal sperm was observed at 260 mg/kg bw/day in the absence of growth retardation.[11]

In a continuous breeding study, the number of days to deliver each litter was consistently increased after oral exposure of mice to 500 mg/kg bw/day[9,11], but no other studies were found regarding functional effects of theophylline on animal fertility.

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

Developmental toxicity

Several studies were available on the potential effects of theophylline in pregnant asthmatic women. Most of the studies, that addressed various pregnancy outcomes, were negative but may not have had sufficient power or an adequate design to disentangle the roles of asthma and theophylline use.[7,12,15,20] In two studies, use of theophylline during pregnancy was found to cause an increase in preterm deliveries.[1,16]

The Committee concludes that the human data are not sufficient for classification.

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[9,11] and rats[10], increased percentage of resorptions in mice[10] and reduced pup weights in mice[9-11] and rats[10]. Some of these effects were noted in the absence of maternal growth retardation. In these studies, the administration of theophylline did not induce visceral or skeletal malformations and variations. In an intravenous study in rabbits (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.

The Committee is of the opinion that the developmental effects occurred independently from maternal toxicity. Therefore, based on the animal data the Committee recommends to classify theophylline in category 1B.

Lactation

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

In rats, administration in the drinking water of amounts of 1 mg/kg bw/day throughout pregnancy up to lactational day 14, no effects on maternal weight and carcass fat, the volume or composition of the milk, or on litter weight were observed.[5,6]

No data were found 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 to lactating women, theophylline was found in breast milk.[3,14,19,21]

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

Proposed classification for fertility

Lack of appropriate human and animal data precludes the assessment of theophylline for fertility.

Proposed classification for developmental toxicity

Category 1B; H360D

Proposed labelling for effects on or via lactation

Lack of appropriate human and animal data precludes the assessment of theophylline for effects on or via lactation.

References

1 Bracken MB, Triche EW, Belanger K, Saftlas A, Beckett WS, Leaderer BP. Asthma symptoms, severity, and drug therapy: A prospective study of effects on 2205 pregnancies. Obstet Gynecol 2003; 102: 739-752.

2 Drugs.com. Theophylline official FDA information, side effects and uses. 2011 [cited 2012 Aug]. available from http://www drugs com/pro/theophylline html.

3 Gardner MJ, Schatz M, Cousins L, Zeiger R, Middleton E, Jusko WJ. Longitudinal effects of pregnancy on the pharmacokinetics of theophylline. Eur J Clin Pharmacol 1987; 31: 289-295.

4 Harris MW, Chapin RE, Lockhart AC, Jokinen MP. Assessment of a short-term reproductive and developmental toxicity screen. Fundam Appl Toxicol 1992; 19: 186-196.

5 Hart AD, Grimble RF. Effect of methylxanthines on lactational performance of rats. Ann Nutr Metab 1990; 34: 297-302.

6 Hart AD, Grimble RF. The effect of methylxanthines on milk volume and composition, and growth of rat pups. Br J Nutr 1990; 64: 339-350.

7 Heinonen OP, Slone D, Shapiro S, editors. Birth defects and drugs in pregnancy. Littleton MA, USA: John Wright, PSG Inc; 1982.

8 Labovitz E, Spector S. Placental theophylline transfer in pregnant asthmatics. JAMA 1982; 247: 786-788.

9 Lamb J, Gulati D, Chambers R, Shaver S, Sabharwal P. Reproductive toxicology. Theophylline. Environ Health Perspect 1997; 105, Suppl 1: 1355-1356.

10 Lindström P, Morrissey RE, George JD, Price CJ, Marr MC, Kimmel CA, et al. The developmental toxicity of orally administered theophylline in rats and mice. Fundam Appl Toxicol 1990; 14: 167-178.

11 Morrissey RE, Collins JJ, Lamb JC, Manus AG, Gulati DK. Reproductive effects of theophylline in mice and rats. Fundam Appl Toxicol 1988; 10: 525-536.

12 Neff RK, Leviton A. Maternal theophylline consumption and the risk of stillbirth. Chest 1990; 97: 1266-1267.

13 Niessink RJM, de Vries J, Hoolinger MA. Toxicology principles and applications. Boca Raton FL, USA: CRC Press; 1995.

14 Reinhardt D, Richter O, Brandenburg G. Pharmakokinetik des Arzneimittelübergangs von stillenden Müttern auf ihre Säuglinge am Beispiel des Theophyllins. Monatsschr Kinderheilk 1983; 131: 66-70.

15 Schatz M, Dombrowski MP, Wise R, Momirova V, Landon M, Mabie W, et al. The relationship of asthma medication use to perinatal outcomes. J Allergy Clin Immunol 2004; 113: 1040-1045.

16 Schatz M, Zeiger RS, Harden K, Huffman CC, Chilingar L, Petitti D. The safety of asthma and allergy medications during pregnancy. J Allergy Clin Immunol 1997; 100: 301-306.

17 Shibata M, Wachi M, Kawaguchi M, Kojima J, Onodera K. Teratogenic and fetal toxity following intravenous theophylline administration in pregnant rabbits is related to maternal drug plasma levels. Methods Find Exp Clin Pharmacol 2000; 22: 101-107.

18 Skouroliakou M, Bacopoulou F, Markantonis SL. Caffeine versus theophylline for apnea of prematurity: A randomised controlled trial. J Paediatr Child Health 2009; 45: 587-592.

19 Stec GP, Greenberger P, Ruo TI, Henthorn T, Morita Y, Atkinson Jr AJ, et al. Kinetics of theophylline transfer to breast milk. Clin Pharmacol Ther 1980; 28: 404-408.

20 Stenius-Aarniala B, Riikonen S, Teramo K. Slow-release theophylline in pregnant asthmatics. Chest 1995; 107: 642-647.

21 Yurchak AM, Jusko WJ. Theophylline secretion into breast milk. Pediatrics 1976; 57: 518-525.

Literature consulted but not cited

Aden U. Methylxanthines during pregnancy and early postnatal life. Handb Exp Pharmacol 2011; (200): 373-389.

Bakhireva LN, Schatz M, Chambers CD. Effect of maternal asthma and gestational asthma therapy on fetal growth. J Asthma 2007; 44: 71-76.

Brandstetter Y, Kaplanski J, van CC, Ben-Zvi Z. Theophylline pharmacokinetics in pregnant and lactating rats. Res Commun Chem Pathol Pharmacol 1986; 53: 269-272.

Bucher HU, Gautschi K. Detection of caffeine, theophylline and theobromine in cord blood and in human milk. Helv Paediatr Acta 1985; 40: 163-167.

Chan SY, Tang LC, Ma HK. Stimulation of the human spermatozoal fertilizing ability by dibutyryl 3',5'-CAMP and theophylline in vitro. Arch Androl 1983; 11: 19-23.

Cornwall GA, Smyth TB, Vindivich D, Harter C, Robinson J, Chang TS. Induction and enhancement of progressive motility in hamster caput epididymal spermatozoa. Biol Reprod 1986; 35: 1065-1074.

Davis PG, Doyle LW, Rickards AL, Kelly EA, Ford GW, Davis NM, et al. Methylxanthines and sensorineural outcome at 14 years in children < 1501 g birthweight. J Paediatr Child Health 2000; 36:47-50.
Dombrowski MP, Schatz M, Wise R, Thom EA, Landon M, Mabie W et al. Randomized trial of inhaled beclomethasone dipropionate versus theophylline for moderate asthma during pregnancy. Am J Obstet Gynecol 2004; 190: 737-744.
Fort DJ, Stover EL, Propst TL, Faulkner BC, Vollmuth TA, Murray FJ. Evaluation of the developmental toxicity of caffeine and caffeine metabolites using the Frog Embryo Teratogenesis Assay - Xenopus (FETAX). Food Chem Toxicol 1998; 36: 591-600.
George JD, Price CJ, Marr MC, Kimmel CA. Developmental toxicity of theophylline (THEO) in mice and rats. Teratology 1986; 33: 70C-71C.
Gilbert-Barness E, Drut RM. Association of sympathomimetic drugs with malformations. Vet Hum Toxicol 2000; 42: 168-171.
Ginsberg G, Hattis D, Russ A, Sonawane B. Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: Implications for assessing children's risks from environmental agents. J Toxicol Environ Health Part A 2004; 67:297-329.
Gonzalez-Barcena D, Kastin AJ, Schalch DS, Miller MC, Henriques-Espinoza R, Schally AV. Response to LH-RH in men infused with theophylline. Int J Fertil 1974; 19: 207-209.
Hishinuma M, Sekine J. Influence of theophylline supplementation on oocyte penetration of canine epididymal spermatozoa. J Vet Med Sci 2004; 66: 1417-1419.
Ishikawa M, Yoneyama Y, Power GG, Araki T. Maternal theophylline administration and breathing movements in late-gestation human fetuses. Obstet Gynecol 1996; 88:973-978.
Jaiswal BS, Majumder GC. Biochemical parameters regulating forward motility initiation in vitro in goat immature epididymal sperxatozoa. Reprod Fertil Dev 1998; 10:299-307.
Katz O, Sheiner E. Asthma and pregnancy: a review of two decades. Expert Rev Respir Med 2008; 2:97-107.
Kawakami E, Arai T, Nakamura U. Effects of medium containing heparin and theophylline on capacitation and metabolic enzyme activities of ejaculated spermatozoa from dogs with asthenozoospermia. Anim Reprod Sci 1999; 54: 251-259.
Kosazuma T, Kawauchi S. Effects of caffeine and related methylxanthines on fetal mouse palates cultured in vitro. J Toxicol Sci 1994; 19: 175-180.
León D, Albasanz JL, Ruíz MA, Fernández M, Martín M. Adenosine A1 receptor down-regulation in mothers and fetal brain after caffeine and theophylline treatments to pregnant rats. J Neurochem. 2002; 82: 625-634.
Loughlin KR, Agarwal A. Use of theophylline to enhance sperm function. Arch Androl 1992; 28:99-103.
Mathavan S, Premalatha Y, Christopher MS. Effects of caffeine and theophylline on the fecundity of four lepidopteran species. Exp Biol 1985; 44: 133-1385.

Park JM, Schmer V, Myers TL. Cardiovascular anomalies associated with prenatal exposure to theophylline. South Med J 1990; 83: 1487-1488.
Pollard I, Locquet O, Solvar A, Magre S. Effects of caffeine and its reactive metabolites theophylline and theobromine on the differentiating testis. Reprod Fertil Dev 2001; 13: 435-441.
Rocklin RE. Asthma, astma medications and their effects on maternal/fetal outcomes during pregnancy. Reprod Toxicol 2011; 32: 189-97.
Schatz M. Asthma treatment during pregnancy. What can be safely taken? Drug Saf 1997; 16: 342-50
Sinha MP, Sinha AK, Singh BK. Effect of methylxanthines on motility and fertility of frozen-thawed goat semen. Theriogenology 1995; 44: 907-914.
Stenius-Aarniala B, Piirilä P, Teramo K. Asthma and pregnancy: a prospective study of 198 pregnancies. Thorax 1988; 43: 12-18.
Ullmer E, Solèr M, Perruchoud AP. Ätiologie, Diagnostik und Therapie der COPD. Ther Umsch 1999; 56: 125-130.
VanderPloeg LC, Wolfrom DM, Rao AR, Braselton WE, Welsch CW. Caffeine, theophylline, theobromine, and developmental growth of the mouse mammary gland. J Environ Pathol Toxicol Oncol 1992; 11: 177-189.
Wasunna A. Effects of theophylline administration and intracranial abnormalities on protective head turning response in preterm infants. East Afr Med J 2003; 80: 204-206.
Yoshioka K, Suzuki C, Itoh S, Kikuchi K, Iwamura S, Rodriguez-Martinez H. Production of piglets derived from in vitro-produced blastocysts fertilized and cultured in chemically defined media: effects of theophylline, adenosine, and cysteine during in vitro fertilization. Biol Reprod 2003; 69: 2092-2099.
Zanardo V, Trevisanuto D, Cagdas S, Grella P, Cantarutti F. Prenatal theophylline and necrotizing enterocolitis in premature newborn infants. Pediatr Med Chir 1996; 19: 153-156.

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

Annexes

Annex **A**

The Committee

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The first draft of the present document was prepared by Dr. B.A.R. Lina from TNO Triskelion BV, Zeist, the Netherlands, by contract with the Ministry of Social Affairs and Employment.

The Health Council and interests

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

Annex **B**

The submission letter (in English)

Subject : Submission of the advisory report *Theophylline*
Your reference : DGV/MBO/U-932342
Our reference : U-7650/HS/fs/543-M13
Enclosed : 1
Date : April 5, 2013

Dear Minister,

I hereby submit the advisory report on the effects of theophylline on fertility and on the development of the progeny; it also concerns effects on lactation and on the progeny via lactation. This advisory report is part of an extensive series in which reproductive toxic substances are classified in accordance with European guidelines. This involves substances to which people can be exposed while pursuing their occupation.

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

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

Yours sincerely,

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

Annex **C**

Regulation (EC) 1272/2008 of the European Community

3.7. Reproductive toxicity

3.7.1. *Definitions and general considerations*

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

In this classification system, reproductive toxicity is subdivided under two main headings:
(a) adverse effects on sexual function and fertility;
(b) adverse effects on development of the offspring.

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

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

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

3.7.1.3. *Adverse effects on sexual function and fertility*

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

3.7.1.4. *Adverse effects on development of the offspring*

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

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

3.7.2. ***Classification criteria for substances***

3.7.2.1. *Hazard categories*

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

Table 3.7.1(a)

Hazard categories for reproductive toxicants

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

Table 3.7.1(b)

Hazard category for lactation effects.

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

3.7.2.2. *Basis of classification*

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

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

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

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

3.7.2.3. *Weight of evidence*

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

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

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

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

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

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

3.7.2.4. *Maternal toxicity*

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

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

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

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

Maternal mortality:

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

Mating index

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

Fertility index

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

Gestation length

(if allowed to deliver)

Body weight and body weight change:

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

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

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

Food and water consumption (if relevant):

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

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

Post-mortem data:

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

3.7.2.5. *Animal and experimental data*

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

3.7.2.5.2. Results obtained from Screening Tests (e.g. OECD Guidelines 421 — Reproduction/ Developmental Toxicity Screening Test, and 422 — Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test) can also be used to justify classification,

although it is recognised that the quality of this evidence is less reliable than that obtained through full studies.

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

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

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

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

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

3.7.2.5.8. In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics

information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Please also refer to the section on maternal toxicity (3.7.2.4) for further guidance in this area.

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

3.7.3. *Classification criteria for mixtures*

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

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

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

Table 3.7.2

Generic concentration limits of ingredients of a mixture classified as reproduction toxicants or for effects on or via lactation that trigger classification of the mixture

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

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

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

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

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

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

3.7.4. *Hazard Communication*

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

Table 3.7.3
Label elements for reproductive toxicity

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

Precautionary Statement Response	P308 + P313	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405	
Precautionary Statement Disposal	P501	P501	

Annex **D**

Additional considerations to Regulation (EC) 1272/2008

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

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

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

* Organisation for Economic Cooperation and Development.

Annex **E**

Fertility and developmental toxicity studies

Table 1 Fertility studies in laboratory animals.

authors	species	experimental period/ design	dose	general toxicity	effects on reproductive organs/ effects on reproduction
Morrissey et al. (1988) Lamb et al. (1997)	Swiss CD-1 mice	tested according to the Reproductive Assessment by Continuous Breeding (RACB) design used by the National Toxicology Program; In Task 2 of this RACB design, 20 animals/sex/ group (controls: 40 pairs) 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.	0, 0.075, 0.15, 0.3%; diet (equivalent to 0, 126, 260, 500 mg/kg bw/d	alopecia in both sexes of all treatment groups (low dose: 20-25%; mid/high dose: >50%; mortality in 3 control and 4 low-dose mice; 11% increase in relative liver weight in high-dose females; 7% reduction in terminal bw and increased absolute liver weight in high-dose males	no changes in length of oestrous cycle or in percent of time spent in various oestrus stages'; decreased relative seminal vesicle weight (by 19%) in high-dose group; reduced epididymal sperm density (by 20%) in high-dose group; no changes in percent motile and percent abnormal morphologic forms in high-dose group

Morrissey et al. (1988)	F344 rats (n=10/ group); B6C3F1 mice (n=10/group)	sperm morphology and vaginal cytoIogy evaluations	rat: 0, 37.6, 75, 150 mg/kg bw/d mice: 0, 0.75, 150, 300 mg/kg bw/d; gavage 0, 0.1, 0.2, 0.4%; diet (equivalent to ca. 66, 130, 260 mg/kg bw/d in rats, and 0, 200, 400, 800 mg/kg bw/d in mice)	gavage: decreased terminal bw in male mice at 150 and 300 mg/kg bw/d; diet: decreased terminal bw in male and female mice in all treatment groups (no effect in rats)	gavage: decreased absolute testes weight in high-dose rats and mice; no stat. sign. effects on other reproductive endpoints, including sperm number, sperm motility, epididymides weight, oestrus cycle length diet: rats: increased epididymidis weights in mid-dose group; decreased cauda epididymidis weights in high-dose group; dose-related increase in abnormal sperm (stat. sign. in high-dose group only); no effect on oestrus cycle length mice: increased epididymidis weights in mid-dose and high-dose group; increased cauda epididymidis weights in mid-dose group.
Harris et al. (1992)	male Swiss CD-1 mice (n=10/group)	mice exposed for 17 d; then necropsied.	0, 20, 60, 200 mg/kg bw/d; gavage	no adverse changes in clinical signs, bw, or in liver and kidney histology	high-dose: mild changes in testis epithelium consisting primarily of asynchronous germ cell development and focal loss of germ cells within individual tubules.
Harris et al. (1992)	Swiss CD-1 mice (n=10/group)	female mice treated for 19 d, then killed;. after 7 d of treatment, cohabitation with male mice that were treated for 5 d prior to mating (until d 5 of cohabitation).	0, 20, 60, 200 mg/ kg bw/d	no adverse clinical signs; 1 female in high-dose group killed moribund.	high dose: decreased pregnancy rate (6/9 vs. 9/10 in all other groups; n.s.). no stat. sign. effect on number of live-, dead-, or total implants per female

bw=body weight; d=day(s); n.s.=not statistically significant; stat. sign.=statistically significant

Table 2a Developmental toxicity studies in laboratory animals with theophylline: oral administration

authors	species	experimental period/ design	dose	general toxicity	developmental toxicity
Morrissey et al. (1988) Lamb et al. (1997)	Swiss CD-1 mice	tested according to RACB (Task 2) design (see above Table 1); and crossover mating trial with F0 mice to detect which sex had been affected; according to the RACB (Task 3) design, three groups are formed: control males x high-dose females, high-dose males x control females, and controls x controls (20 pairs in each group).	0, 0.075, 0.15, 0.3%; diet (equivalent to 0, 126, 260, 500 mg/kg bw/d)	see above Table 1.	fewer pups/ litter at all doses (reduced by 22%, 29%, 42% at the low, mid, high dose, respectively). high dose: 19% reduction in the mean number of litters/pair; 6% decrease in live pup weight adjusted for litter size; consistently greater number of days to deliver each litter (three d longer for the first litter, 5 d for the last litter, and similarly increased for all other litters) in the group cohabiting control males and high-dose females: reduced proportion of pups born alive (by 16%); reduced adjusted pup weight (by 15%) no differences in percent of pairs mating or delivering a live litter.
Lindström et al. (1990)	Swiss (CD-1) mice (n=23-33/group)	gd 6-15	0, 0.075, 0.15, 0.2%; drinking water (equivalent to 0, 282, 372, 396 mg/kg bw/d	no maternal deaths decreased water consumption at mid and high dose higher incidence of piloerection at mid and high dose. decreased maternal bw gain during gestation and bw on gd 17 corrected for gravid uterine weight at the mid and high dose decreased maternal feed consumption at the high dose	decreased gravid uterine weights at the high dose (n.s.) decreased number of live foetuses/litter at the high dose decreased male and female foetal weight/litter at the mid- and high dose no stat. sign. differences in number of implantation sites/ litter, percentage preimplantation loss, litters with resorptions, percentage resorptions/litter

					no effect on percentage of malformations/ litter or on incidences of external, visceral or skeletal malformations and variations
Lindström et al. (1990)	Swiss (CD-1) mice (n=23-33/group)	gd 6-15.	0, 0.075, 0.15, 0.2%; drinking water (equivalent to 0, 282, 372, 396 mg/kg bw/d	no maternal deaths decreased water consumption at mid and high dose higher incidence of piloerection at mid and high dose. decreased maternal bw gain during gestation and bw on gd 17 corrected for gravid uterine weight at the mid and high dose	decreased gravid uterine weight at the high dose increased percentage of resorptions/litter at the mid and high dose decreased male and female foetal weight/litter at the mid and high dose slightly increased number of externally malformed foetuses at the mid and high dose (n.s.) no stat.sign. differences in number of implantation sites/ litter or percentage of preimplantation loss or in incidence of visceral or skeletal malformations and variations the study may have been confounded by the decreased water intake in the mid- and high-dose group.
Harris et al. (1992)	Swiss CD-1 mice (n=10/group)	see above Table 1	0, 20, 60, 200 mg/kg bw/d; gavage	see above Table 1	decreased pregnancy rate (6/9 vs. 9/10 in all other groups; n.s.) no stat. sign. effect on number of live-, dead-, or total implants/female
Harris et al. (1992)	Swiss CD-1 mice (n=13-15/group)	gd 8-14; dams were allowed to deliver and litters were evaluated on pnd 0, 1, 4.	0, 20, 60, 200 mg/kg bw/d; gavage	not reported	no stat. sign. effects on number of females littering, number of implantations/ female, number of live neonates, total litter weight

bw=body weight; d=day(s); gd=gestational day(s); n.s.=not statistically significant; pnd=postnatal day(s); stat. sign.=statistically significant

Table 2b Developmental toxicity studies in laboratory animals with theophylline: intravenous injection

author	species	experimental period/design	dose	general toxicity	developmental toxicity
Shibata et al. (2000)	Kbl:JW rabbits (n= 20/group)	gd 6-18; sacrifice on gd 29	0, 15, 30, 60 mg/kg bw/d; iv C_{max} of theophylline similar on gd 6 and gd 18, viz. 30, 56, 106 µg/mL at low, mid, high dose, respectively	high dose: 1 animal died, 4 animals aborted; decreased maternal bw and feed; reversible toxicity (accelerated respiration, sluggish startle reactions, dilation of the auricular vessels, polyurea)	high dose: foetal toxicity including late foetal death and tendency towards decreased foetal bw; cleft palate in 8 foetuses (2 litters); increased incidence of skeletal variations (13th rib) no stat. sign. differences in implantations, live foetuses or sex ratio, incidences of visceral or skeletal anomalies or ossifications.

bw=body weight; d=day(s); gd=gestational day(s); iv=intravenous; stat. sign.=statistically significant.

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