Committee for Risk Assessment

RAC

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

Adopted

17 September 2020

17 September 2020

CLH-O-0000006848-58-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

EC Number: **200-385-7**

CAS Number: **58-55-9**

The proposal was submitted by **The Netherlands** and received by RAC on **16 July 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **14 October 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 December 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Peter Hammer Sørensen**

Co-Rapporteur, appointed by RAC: **Ruth Moeller**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **17 September 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	theophylline; 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione	200-385-7	58-55-9	Repr. 1B	H360D	GHS08 Dgr	H360D			
RAC opinion	TBD	theophylline; 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione	200-385-7	58-55-9	Repr. 1B	H360D	GHS08 Dgr	H360D			
Resulting Annex VI entry if agreed by COM	TBD	theophylline; 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione	200-385-7	58-55-9	Repr. 1B	H360D	GHS08 Dgr	H360D			

GROUNDS FOR ADOPTION OF THE OPINION

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).

HUMAN HEALTH HAZARD EVALUATION

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

No.	Method/Guideline	Klimisch	Reference
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)*

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

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

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 gavage: 0, 20, 60, 200 mg/kg bw/d (vehicle corn oil)	1	Harris *et al*., 1992
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 <u>developmental study in rabbits</u> (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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).