RISK PROFILE

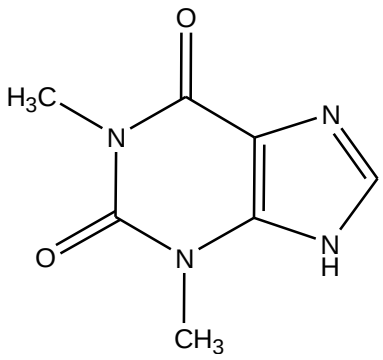
Theophylline

CAS No. 58-55-9

Date of reporting 11.06.2013

Content of document

1. Identification of substance

Chemical name (IUPAC):	1H-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl
INCI	Theophylline
Synonyms	1,3-Dimethylxanthine
CAS No.	58-55-9
EINECS No.	200-385-7
Molecular formula	$C_7H_8N_4O_2$
Chemical structure	
Molecular weight	180.16

Contents (if relevant)	
Physiochemical properties	Appearance: White crystalline powder Boiling point: Not relevant because of chemical decomposition Melting point: 270-274 °C Log $P_{ow::}$ - 0.02 Vapor pressure: 5.1X10-9 mmHg at 25°C Solubility (water): 7360 mg/L at 25 °C References: Toxline [online].

2. Uses and origin

Uses	○ **Cosmetic products:** Functions and frequency of use according to ○ *CosIng database* "Skin conditioner" - Maintains the skin in good condition – "Tonic" - Produces a feeling of well-being on skin and hair (CosIng [online]). ○ *CodeCheck*, a German online cosmetic product database (Codecheck [online)*: 2 products* "Orange peel looking skin" 1 "Skin firming for slimmer look»: 1 ○ EWG's Skin Deep [online]). *2 products* Concerns "Skin firming for slimmer look» products other than the one filed with CodeCheck ○ Internet search 12-16 May 2013: at least *20 products* (online-see references) *Claim: skin firming for slimmer look 6* *Claim: orange peel looking skin 9* *Claim: soothing: 3* *Claim: preventing stretch marks: 1* *Claim: firms the breast: 1* ○ In market control (Sainio E.L. 1997) Out of 32 "Orange peel looking skin" products on the EEA market (Finland) at the time 3 contained theophylline *Concentrations being applied* 1% (OECD SIDS 2001, Council of Europe, 2008).

	2% declared in one product claiming it helps with "orange peel looking skin" (see internet announcement mentioned in Annex) [1] Possibly, because of being somewhat less toxic the 7-methyl derivative – i.e. the caffeine molecule - has for years been much more popular as the active ingredient in the kind of products mentioned above- i.e. products some of which claim they reduce/remove the so-called cellulites or actually make the user look a bit more lean. The claim often made that the xanthine molecules theophylline, caffeine, aminophylline, theophylline, etc. "burn fat" by increasing cyclic AMP levels in tissues has not been substantiated (Epstein E *et al* 1997) – see Annex on that. So, they are practically ineffective in that respect. These substances do, however, posses an astringent property and also a dehydrating property which make them capable of firming the skin so that it looks smoother and also gives the impression of a leaner figure. Hence, the claims also often put that the product helps with *"orange peel looking skin"* and/ or *"sculpture"* the body is not in itself misleading (if can be evidenced) and are cosmetic claims. The products in question claiming like that fulfil the definition of a cosmetic product and fall within the scope of the cosmetic products regulations. There are a number of anti-cellulite/ "sculpturing" products on the market that are based on a 2:1 complex of theophylline and 1,2-diaminoethane (ethanediamine) called aminophylline. Searching on the internet for some hours only we saw 28 different products being announced. For 5 of them the marketers informs about a concentration in the range 2-3 % aminophylline. 3 % aminophylline corresponds to 2.6 % theophylline. Annex gives more information about aminophylline. In later years also some other theophylline derivatives have come more in use than theophylline itself for the same purposes – see Annex. ○ **Medicinal products/applications** Theophylline is used to prevent and treat wheezing, shortness of breath, and chest tightness caused by asthma, chronic bronchitis, emphysema, and other lung diseases. It relaxes and opens air passages in the lungs, making it easier to breathe. So it is used as a bronchodilator in the management of reversible airways obstruction. Theophylline also is a cardiac stimulant and diuretic. In many countries it is regulated as a prescription drug. In later years theophylline has mainly been used for treatment of apnea in pre-term infants. In the past it was considered first-line therapy as concerns asthma. It now plays a far less prominent role - primarily because of the modest benefits it affords, its narrow therapeutic window, and the required monitoring of drug levels (Hardman *et al.,* 1996). Studies in animals suggest that bronchodilatation is mediated by the inhibition of the phosphodiesterase isoenzymes PDE lll and PDE IV that are responsible for breaking down cyclic AMP in smooth muscle cells. Some of the adverse effects associated with theophylline appear to be mediated by inhibition of PDE lll (e.g., hypotension, tachycardia,

[1] http://www.alphabodysolutions.com/index.php?main_page=product_info&products_id=1
http://www.oxygenbotanicalsonline.com/oxygen-botanicals-slimline-celiminate-cream-cosmetic-body-product-8oz-419.html

	headache, and emesis) and adenosine receptor antagonism (e.g., alterations in cerebral blood flow). Theophylline increases the force of contraction of diaphragmatic muscles. This action appears to be due to enhancement of calcium uptake through an adenosine-mediated channel. Information about medicinal usage is provided in short by, for example, the three internet sources "drugs.com", "MedlinePlus" and "drugbank" – see online under references. ○ **Food and drinking water** Theophylline is naturally present in tea (*Camellia sinensis*), guarana (*Paullinia cupana*), coffee (*Coffea spp*.) and cocoa (*Theobroma cacao*) plants. In harvested black tea the content varies between 200 and 400 mg/kg dry weight. Green coffee beans hold approximately 5 mg/Kg. For theobroma cocoa seed these values varies from 3 200 to 4 700 mg/kg. So, the consumer is exposed regularly to theophylline through normal diets. For example a normal cup of tea (140 g) contains 1 mg of theophylline (Council of Europe 2008). Chocolate, cocoa and other beverages may also contribute to the dietary exposure. Besides 4 % of the caffeine consumed daily because of drinking of coffee, teas, and caffeinated beverages like cola, is converted immediately into theophylline in the body. This indirect exposure constitutes the dominating part of consumer's daily exposure for theophylline because of diets.
Origin Natural (exo /endo) Synthetic	Synthetic

3. Regulation

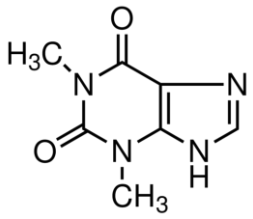
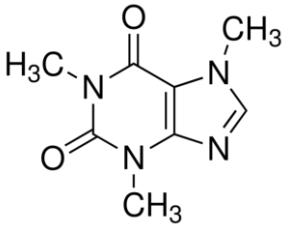
Norway	All kinds of cosmetics max 3 %. Regulation to be withdrawn 11 July 2013
EU	No regulation.
Rest of the world	Canada - Prohibited and Restricted Cosmetics Ingredients (Health Canada and regulation and cosmetic "theophylline")

4. Relevant toxicity studies

Absorption Skin GI tractus	In humans, theophylline is readily absorbed after oral intake. The absorbed fraction of a dose of approximately 7.5 mg/kg bw averaged 99%. Peak serum levels are reached within 0.5-2 h. (Council of Europe 2008). *The ability to penetrate skin by diffusion* The Council of Europe (2008) thought that since theophylline deviates only marginally from caffeine as concerns the molecular structure it also behaves approximately as do caffeine as concerns the ability to be taken up in the body over the stratum coreneum by diffusion. Caffeine has been thoroughly investigated in this respect and the skin penetration rate

usually assumed for safety assessments purpose is 20%. On that see attached monograph on caffeine as included in the Council of Europe publication from 2008 on the subject of “active ingredients” in cosmetic products. See also the reference EPA 1992 - online.

However, because of the lack of the extra methyl group at the ring position 7 theophylline is slightly less lipophilic than caffeine and, therefore, also has a lesser flux through the stratum coreneum and into the live skin tissues/body:

xanthine	Theophylline	Caffeine
Molecular structure		
Water solubility at 25 °C; mg/L (Drugbank)	7360	2170
Partitioning coefficient logK o/w (logP)	-0.02 Drugbank	0.1 EPA 1992 & Van de Standt *et al* 2004
Permeability coefficient (from water) cm/h x 1000	0.19 (Fang JY *et al* 2007)	0.87 (Shakeel F *et al* 2010)
Flux *In vitro* data / Franz cell diffusion cell studies microgram/cm^2/h (µg/cm^2/h)	2.05 (rat skin – full thickness - 25 % ethanol aqueous solution holding 0.06% theophylline[2] (Fang JY *et al* 2007)	6.82 (rat skin – full thickness - 50 % ethanol aqueous solution holding 0.4 % caffeine[3]) 2.24 (human skin – full thickness - 50 % ethanol aqueous solution holding 0.4 % caffeine[4])

[2] Skin collected from the back of Wistar rats
[3] Skin collected from the back (clipped carefully) of 4-weeks old male Sprague Dawley rats
[4] Human skin is surgical waist collected is 4 out of seven cases from the abdomen. The figure is the average result from a European ring test.

		(Van de Sandt JJM *et al* 2004)

It appears from the publication of van de Sandt *et al* that the flux values observed in these *in vitro* studies correspond to the following skin penetration rates – that also were determined on the basis of data obtained in the studies:

6.82 µg/cm^2/h (rat skin): 53.7 % (after 24 h)
2.24 µg/cm^2/h (human skin): 24.5 % (after 24 h)

The study results reported by van de Sandt *et al* cannot be compared directly with those reported by Fang *et al* since the study conditions deviate somewhat. From these results we would expect, though, that the skin penetration rate of theophylline in humans would amount to roughly 6-7 %. Actually the *in vitro* results obtained by the authors Ademola *et al* in 1992 corroborate that estimate.

Ademola JI *et al* (1992) examined the percutaneous absorption of theophylline in human skin from five sources by use of a flow-through *in vitro* diffusion system (*Franz diffusion cell).* Finds:

Percentage of applied dose that diffused trough the skin : 2.8 – 7.7
Percentage of applied dose that crosses over the Str. Cor. 3.6 – 33.4
Percentage of dose being metabolized in skin: 0.2 – 4.6
Percentage of formed metabolites that reached syst. Cir. 60

We anticipate that the trough-skin-level of 3-8 % is comparable to the 20 % usually laid to ground as concern the skin penetration rate of caffeine.

A powerful enhancer is present in fairly high concentration in a majority of the theophylline containing products that we saw on the market and for which the whole list of ingredients had been disclosed on the web.

We would think it only prudent, therefore, that the skin penetration rate premise to be applied takes into account that theophylline is formulated together with a powerful enhancer like, for example, propylene glycol. This addition helps to enhance the rate many times. In light of that we assume the conservative premise of a 10 % skin penetration rate.

The Annex provides more information about the enhancers.

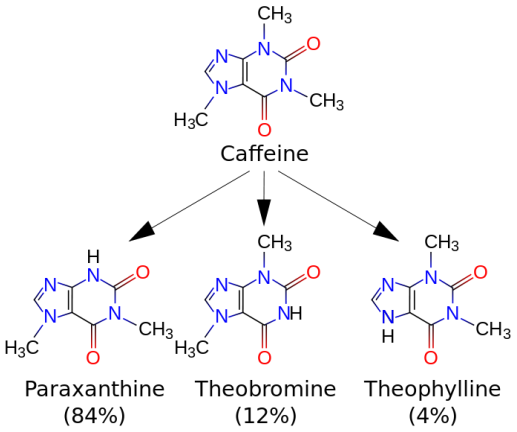
Distribution

Theophylline is rapidly distributed to all organs of rats except adipose tissue. It is distributed in erythrocytes and crosses readily the placenta barrier. It crosses the blood-brain barrier, but not in the foetal (rats). Theophylline is distributed also in breast milk. About 50% is bound reversibly to plasma proteins. (Council of Europe, 2008).

Xanthine toxicity

The four xanthines finding use in cosmetics products – i.e. caffeine, theophylline, theobromine and aminophylline – are that structurally similar they largely have same toxicity profile.

Metabolically, they are converted into one another in the liver where ca. 90-99 % of the metabolism takes place. The following figure shows the example of caffeine metabolism in humans (Wikipedia).



The metabolic pattern pertaining to theophylline and theobromine is shown below and in Annex respectively.

Metabolism differs considerably from rodents to humans. In humans the urine content of molecules with 3 methyl groups to the rings amounts to 4 %. This contrast the 40% determined in the rat urine. See Annex for details. Further, in monkeys the main metabolite is theophylline. These differences mean that some animals are questionable models as concerns some of the toxicity endpoints.[5] The different molecules are about as acutely toxic in humans as in rodents, though (LD50 oral mg/Kg bw):

Xanthine	Man	Rat	Mouse
Caffeine	150 - 200	200 - 400	185
Aminophylline		243	150
Theophylline	130 (LD low)	272	229 - 248
Theobromine	1000	1265	837

To a grater or lesser extent all these molecules are bronochodilators, CNS stimulators, adenosine agonists and diuretics. And they all are teratogenic – see below.

Compared to the one studied the most i.e. caffeine, theophylline is more relaxing to peripheral smooth muscles, especially as to respiratory smooth muscles and has a greater effect on increased drive to breathe. Theophylline is a better broncial tissue relaxant than caffeine. It has a considerably lesser ability in crossing over the blood-brain barrier (Ståhle L *et al* 1991). Nevertheless, in comparison with caffeine, theophylline exerts a more profound and potentially more dangerous stimulation of the CNS (Hardman et al., 1996).

The xanthines all have a narrow therapeutic index. Adverse effects can appear even at therapeutic doses and serum concentrations. Usually the pharmacological effects occur at lower doses than the adverse effects (EFSA 2008).

Metabolism

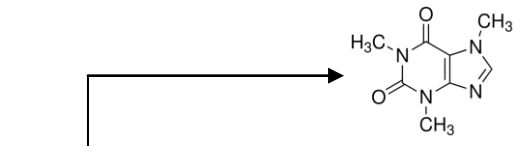
Plasma half-life is between 1-4 hours in rats and 6- 12 hours in dogs and strongly dependent on protein binding and dose. In adult humans the elimination half-time is 3-11 hours. Elimination half-time is shorter in smokers and is prolonged by the use of oral contraceptives.

[5] http://www.fetal-exposure.org/resources/index.php/1997/10/01/caffeine-and-pregnancy/

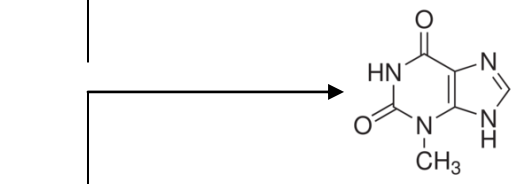
Theophylline is metabolized roughly similarly in animals and man and the same main metabolites are produced, though there are quantitative differences between species.

Following oral dosing, theophylline does not undergo any measurable first-pass elimination. In adults and children beyond one year of age, approximately 90% of the dose is metabolized in the liver. Metabolism is by ring oxidation and N-demethylation mediated by microsomal enzymes (cytochrome P-450) and is excreted by the kidney

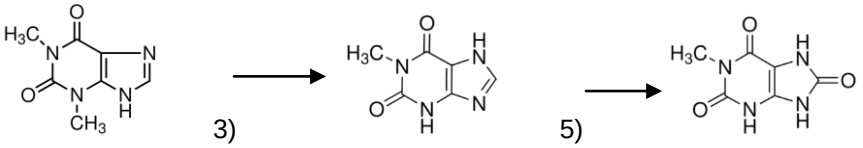
The below figure shows the metabolism of theophylline in human liver (Drug.com).



1) 6 % Caffeine



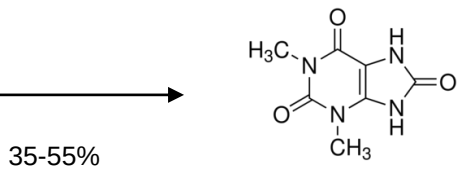
2) 9-18% 3-Methylxanthine



3) 5)

4) 1-Metylxanthine 1-Metyluric acid

3)-5) 13-26%



35-55%

1,3-Dimethyluric acid

Only 7-12% is excreted unchanged in the urine.

	In neonates, the N-demethylation pathway ((2) and (3)) is absent while the function of the hydroxylation pathway is markedly deficient. The activity of these pathways slowly increases to maximal levels by one year of age. Caffeine and 3-methylxanthine are the only metabolites with pharmacologic activity. 3-methylxanthine has approximately one tenth the pharmacologic activity of theophylline and serum concentrations in adults with normal renal function are <1 µg/ml. Caffeine concentrations are usually undetectable in adults regardless of renal function. In neonates, caffeine may accumulate to concentrations that approximate the un-metabolized theophylline concentration and thus, exert a pharmacologic effect. Both the N-demethylation and hydroxylation pathways are capacity-limited. Due to the wide inter-subject variability of the rate of theophylline metabolism, non-linearity of elimination may begin in some patients at serum theophylline concentrations > 5 µg/ml Dose-dependent pharmacokinetics is seen with plasma concentrations greater than15 µg/ml. As said. In neonates, methylation into caffeine is the predominant metabolic pathway. Methylation occurs also in adults. Elimination is modified by diet. High protein diet resulted in enhanced elimination. Studies in twins showed large inter-individual variations. (ICPS Inchem, Aranda *et al.,* 1979). Theophylline metabolism has been studied both *in vivo* and *in vitro* in rat. The quantitatively most important metabolites produced *in vivo* were 1,3-dimethyluric acid and 1-methyluric acid. A tissue survey utilising tissue slices demonstrated that the metabolism is localised only in the liver, and subcellularly to the microsomal system (Lohmann and Miech, 1976). Kinetics and metabolism of theophylline in animals have been reviewed by IARC (1991).
Excretion	The metabolites are excreted into the bile and eliminated with the urine.
Local toxic effects Irritation Sensitivity	Skin and mucous membranes irritation: In tests performed according to OECD guidelines 404 and 405 in rabbits, the undiluted substance induced mean scores of 0.6 for cornea opacity, 1.8 for conjunctival redness, and 0.6 for swelling. On day 8, one of 3 animals showed opacity grade 1 and conjunctivitis grade 2. The animal showing slight corneal opacity had also keratitis. The effects could possibly be due to mechanical irritation by the crystalline test substance. The substance in a 50% aqueous dilution was not irritating to the skin (score 0). Conclusion: The undiluted substance was not irritating to the eyes. The substance in a 50% aqueous dilution was not irritating to the skin of rabbits. (ICPS Inchem) Sensitivity No reports about a sensitization potential exist. There are no case reports in the literature.

	The European Chemicals Agency (ECHA) reports in a web-page about the toxicity of theophylline (see address under online) that the company BASF undertook a conventional LLNA study of caffeine in 2005. The test showed that caffeine was not a skin sensitizer. ECHA applying a read-across thinking assumes, therefore, that neither theophylline is a skin sensitizer. Neither caffeine nor theophylline is typical hapten molecules that bind co-valentely with endogenous macromolecules.
Systemic toxic effects Acute	<u>Acute Toxicity:</u> After oral application, the LD_{50} for rats (10 animals/group/sex) was found to be 272 mg/kg bw; as clinical symptoms of toxicity clonic convulsions, accelerated respiration and salivation (at 1 000 mg/kg bw only) were seen after oral intake. Higher doses can be tolerated when the substance is not given as a bolus. The inhalation of the substance by rats as an aerosol over the time period of 4 h resulted in an LC_{50}-value of > 6.7 mg/l. Irregular and accelerated respiration were noted in this study. The LD_{50} for dermal application was >2 000 mg/kg bw; no clinical symptoms were observed Conclusion: In animal studies theophylline showed a moderate toxicity after oral uptake and a low acute toxicity after dermal and inhalation uptake. (ICPS Inchem) *Human data* Death from theophylline intoxication is more common than from caffeine intoxication. Rapid intravenous administration of therapeutic doses of 500 mg aminophylline has resulted in death. Most toxicity is associated with long-term oral or parenteral exposure. Although seizures are rare at plasma concentrations below 40 µg/mL, convulsions and death have occurred at concentrations as low as 25 µg/mL (*Goodman and Gilman's*, 1990). NTP 1998
Repeated dose	<u>Repeated Dose Toxicity:</u> Theophylline was given by feed or by gavage to rats and mice. In rats, theophylline caused nephropathy in male rats in one study and a dose-dependent periarteritis in all treated groups. Periarteritis was not observed in mice, and in a two-year study in rats this effect only occurred in the males of the highest dose group (75 mg/kg bw/day). The particular sensitivity of rats is most probably due to their anatomical situation as compared to mice and men. Since the periarteritis is considered a rat-specific response to vasodilators it is of little, if any, relevance to humans. Furthermore, this effect has not been associated with theophylline treatment in humans. At high doses hematological parameters were changed in mice and rats, histopathological changes were not observed in mice. In these studies no histopathological changes were found in other organs including sex organs of rats or mice. The LOAEL (rat, mouse) varied from 37.5 to 225 mg/kg bw/day (ICPS Inchem)

Mutagenicity /genotoxicity	Genotoxicity: Theophylline was not mutagenic or clastogenic in most of the standard *in vitro* tests. Positive results were found only at high, cytotoxic concentrations and without metabolic activations. Theophylline had no mutagenic or clastogenic effects *in vivo*. (ICPS Inchem)
Carcinogenicity	Carcinogenicity: Theophylline showed no carcinogenic activity in rats and mice up to the highest dose tested (75 mg/kg bw/day in rats and female mice and up to 150 mg/kg bw/day in male mice). In humans, case-control studies did not show an association between total methylxanthine intake and breast cancer. (ICPS Inchem)
Reproductive toxicity / teratogenicity	Reproduction toxicity: We collected information from different sources on this toxicity endpoint. The ones concerned are the ICPS Inchem committee, NTP, "DailyMed" which is a service of the US National Library of Medicine, the Olmsted Medical Center at the University of Minnesota /USA, and the Health Council of the Netherlands (HCN). The views of the different bodies are recited in the below. We mention firstly that the HCN undertook to unravel whether theophylline qualifies for a CMR (reprotoxic) classification within the frames of the CLP part of the EU chemicals legislation (Regulation (EC) No 1272/2008). The HCN submitted a report on that to the Netherlands Minister of Social Affairs and Employment 5 April 2013. Apparently, the HCN scrutinized the literature there are on the topic performing this task. It appears from its report that there are no newer publications than the ones we mention below and that also other scientific bodies took into considerations in order to find out about the reproduction properties of theophylline. We recite, therefore, only the conclusion of the HCN in the below text. HCN in their letter to the ministry recommends theophylline be classified as a CMR of the category 1B. Should the European commission eventually actually included theophylline in the CLP regulation as a CMR of this category, it follows from the Art 15 of the cosmetics regulation it will not be allowed in cosmetics with any functioning. In the animal tests having been conducted the pregnant dam animal was exposed for theophylline trough gestation days 5 – 16. 1. (ICPS Inchem) (2001) Theophylline was administered continuously in the feed applying the dosages 0, 124, 218, and 259 mg/kg bw/d in Sprague-Dawley (CD) **rats.** Malformed foetuses per litter occurred with an incidence of 1.38%, 0.92%, 0.33%, and 1.57% for the vehicle control, low, medium,and high dose groups, respectively. The incidence of litters with one or more external, skeletal, or visceral malformations was also unaffected by theophylline treatment. . No teratogenitic effect at any of the doses was seen (NTIS 1985b,

	Lindstroem 1990) . In another study theophylline was administered in the drinking water to pregnant CD-1 **mice** applying the dosages 282, 372, 396 mg/kg bw/d. In the treated groups there was a slight, not statistically significant trend in the proportion of litters with malformed foetuses and for the incidence of external malformations in the mid and high-dose groups (cleft palates, exencephaly). Cleft palates also occurred in the control group, while exencephaly was only observed at the low and mid dose levels. The ICPS explained however, that it is well known from the literature (Schwetz et al.1977, Beyer and Chernoff 1986 – See ICPS – document) that particularly in this species, stress and depreviation of water during gestation may induce these types of malformations in the off-springs. The authors concluded that theophylline treatment was not associated with an increase in any particular malformation or group of malformations. (Lindström, 1990) No teratogenitic effect was seen at any of the doses. A further study investigated the teratogenic and foetal toxicity of **i.v.** applications of theophylline and its relationship to maternal plasma levels in pregnant **rabbits**. Theophylline was administered i.v. to pregnant rabbits at doses of 15, 30 and 60 mg/kg bw/day using an automatic infusion pump from days 6-18 of gestation. There were no signs of maternal toxicity in the dams given 15 and 30 mg/kg bw/d. Foetuses from the group treated with 60 mg/kg bw/d exhibited developmental toxicity. Developmental toxicity was substantiated by an increased number of late deaths, decreased foetal body weights (about 10% below concurrent controls) and effects on foetal morphology. There was an increased rate of foetuses with cleft palates (8 out of 103 foetuses, in 2 of 14 litters) and with a 13th rib (63 out of 103 foetuses, number of affected litters not exactly specified). Whereas the cleft palate has to be considered a malformation, the additional rib element is assessed as a variation because it appears quite frequently in control rabbit foetuses in the strain used for this study. No substance induced signs of developmental toxicology were observed in foetuses from the 15 and 30 mg/kg bw/d group. In the 15, 30 and 60 mg/kg bw/d groups, maternal plasma concentrations (Cmax) during the treatment period were approximately 30, 56 and 106 µg/ml, respectively. These concentrations clearly exceed the effective therapeutic range of theophylline in clinical use (Shibata et al. 2000). NOAEL maternal toxicity: 30 mg/kg bw/d NOAEL foetotoxicity/teratogenicity: 30 mg/kg bw/d *Reproductive effects in humans* No association with congenital abnormalities was seen in studies with female theophylline drug users (Nelson and Forfar 1971) and women receiving theophylline during pregnancy did not deliver stillborn infants compared to controls (Neff and Leviton 1990). No effects on development of premature infants were seen (Nelson

et al., 1980, Ment *et al*. 1985). Theophylline therapy in surviving preterm children of birth weight <1501 g showed at 14 years of age significantly higher rate of cerebral palsy compared to children not exposed. In contrast children who had received theophylline achieved higher psychological test scores. There was no association between theophylline therapy and growth (Davis et al. 2000).

2. *The source NTP technological report on the toxicology and carcinogenic studies of theophylline (NTP 1998 – online)*

Under this heading we only mention information that seemingly has not been provided by ICPS Inchem. The important article of Shibata on the i.v. experimentation in rabbits was published after NTP submitted its opinion.

Theophylline may be a testicular toxicant. Feeding 0.5% theophylline to rats for 14 to 75 weeks resulted in bilateral testicular atrophy with variable atrophic changes in the epididymis, prostate gland, and seminal vesicles (Weinberger *et al*., 1978; Friedman *et al*., 1979)[6]. In continuous breeding studies, male Swiss (CD-1®) mice exposed to theophylline had reduced seminal vesicle weights and cauda epididymal sperm counts (NTP, unpublished report). Dose-related decreases in gravid uterine weight were observed in CD rats and CD-1 mice given up to 0.4% or 0.2% theophylline, respectively, in drinking water during gestation days 6 through 15 (George *et al*., 1986)[7].

Theophylline is a reported teratogen in mice. In CD-1 mice administered up to 0.2% theophylline in drinking water during gestation days 6 through 15, the percentage of resorptions per litter was increased, average foetal weight was decreased, and there were dose-related increasing trends in the percentage of litters with malformed foetuses and the percentage of malformed foetuses per litter (George *et al*., 1986).

Theophylline administered intraperitoneally at up to 225 mg/kg on day 12 of gestation produced digital defects, cleft palate, micrognathia, and hematomas in the foetuses of ICR-JCL mice (Fujii and Nishimura, 1969). Single intraperitoneal doses of up to 200 mg/kg administered on gestation days 10, 11, 12, or 13 produced cleft palates, limb anomalies (ectrodactyly, syndactyly, micromelia, polydactyly), and embryolethality in ICR mice (Tucci and Skalko, 1978).

Comments to NTP

Tucci and Skako did not involve a control group so their study has a major weakness. It has, however, considerable predictive power as to what would be NOAEL as concerns the teratogenic effect. Seemingly it is at the level of 100 mg/Kg bw/day (Survivors with cleft palate).

[6] This exposure corresponds to 300 mg/Kg bw and day using default values in the source sax's Dangerous properties of industrial material 1992

[7] These exposures corresponds to respectively 240 and 120 mg/Kg bw and day using default values in the source sax's Dangerous properties of industrial material 1992

	3. *Daily Med / **Current** medication information (Daily Med – online)* [8] In studies with mice, a single intraperitoneal dose at and above 100 mg/kg (approximately equal to the maximum recommended oral dose for adults on an mg/m^2 basis) during organogenesis produced cleft palate and digital abnormalities. Micromelia, micrognathia, clubfoot, subcutaneous hematoma, open eyelids, and embryolethality were observed at doses that are approximately 2 times the maximum recommended oral dose for adults on a mg/m^2 basis. In a study with rats dosed from conception through organogenesis, an oral dose of 150 mg/kg/day (approximately 2 times the maximum recommended oral dose for adults on a mg/m^2 basis) produced digital abnormalities. Embryo-lethality was observed with a subcutaneous dose of 200 mg/kg/day (approximately 4 times the maximum recommended oral dose for adults on a mg/m^2 basis). In a study in which pregnant rabbits were dosed throughout organogenesis, an intravenous dose of 60 mg/kg/day (approximately 2 times the maximum recommended oral dose for adults on a mg/m^2 basis), which caused the death of one doe and clinical signs in others, produced cleft palate and was embryolethal. Doses at and above 15 mg/kg/day (less than the maximum recommended oral dose for adults on a mg/m^2 basis) increased the incidence of skeletal variations. There are no adequate and well-controlled studies in pregnant women. Theophylline should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus. 4. *Olmsted Medical Center, University of Minnesota, Rochester, USA / View expressed in an article by Yawn B and Knutson M 2007* In animals, high-dose theophylline has been associated with adverse pregnancy outcomes, including congenital malformations (NAEPP 2005). In humans, the drug does not seem to increase the risk of congenital malformations, and data conflict about whether theophylline exposure is associated with an increased risk of preterm birth and preeclampsia (Gluck JC et al 2005). Theophylline is rated Pregnancy Category C because of the demonstrated risk in animal studies and the lack of well-

[8] DailyMed provides high quality information about marketed drugs. This information includes FDA labels (package inserts). This web site provides health information providers and the public with a standard, comprehensive, up-to-date, look-up and download resource of medication content and labelling as found in medication package inserts. The National Library of Medicine (NLM) provides this as a public service and does not accept advertisements. (http://dailymed.nlm.nih.gov/dailymed/about.cfm)

	designed human studies (Gluck JC *et al* 2005). 5. *The Health Council of the Netherlands (HCN) - 5. April 2013* *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], , 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. 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. 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], , 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.

Considerations

Several population-based studies and post-marketing adverse event reporting of theophylline use during human pregnancy have been carried out. Apparently, most of these studies were not large enough to detect a less than two fold increase in risk for congenital anomalies. So, conceivably, the majority of the studies did not demonstrate an increased risk of congenital anomalies. The HCN, summing up on the epidemiological studies mention, however, that in two studies use of theophylline during pregnancy was found to cause an increase in preterm deliveries. One is the extensive study of Schatz *et al*. showing that 6 % of the pregnant women using theophylline for their asthma experienced preterm births – whereas the figure was down to 3.6 % in non-asthmatic pregnant women (p= 0.034).

HCN also mentioned in detail the investigation of Stenius-Aarniala B *et al*, (1995) wherein the data of 212 pregnant asthmatics with theophylline treatment were compared with findings in 292 pregnant asthmatics without theophylline and 237 non-asthmatic pregnant control subjects. The outcome of this study was that three infants with malformations were born in 121patients (2.5%) treated with theophylline during the first trimester (507 +/- 180 mg theophylline orally daily) 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. HCN was of the view that this (and other studies) may not have had sufficient power or an adequate design to disentangle the roles of asthma and theophylline use. Stenius-Aamila was of the view that the safety of

	theophylline treatment during the first trimester with regard to teratogenicity remains to be determined. Mostly the malformations in foetus exposed for theophylline concerns skeletal anomaly: • The first trimester involved one case of left heart hypoplasia one case of synostosis humeroradialis (elbow dislocation) and one case of finger defects. • The 4 cases for the second and third trimester involved mostly bone defects in limbs but may also have included a case of left heart hypoplasia and a case of hypospadias. The article doesn't distinguish clearly between the asthmatic probands treated with theophylline and they who were not medicated. 7 malformation cases in 212 probands means a incidence of 3.3 % which is more than observed for the non-treated asthmatics (1.0 %) and the health control group (0.8 %) and in Finland in general (2%). Stenius-Aamiala *et al* were of the view, however it would require 10 000 probands in each group to get a sufficiently robust conclusion whether exposure for theophylline involves an increased risk for teratogenic effects in humans or not. Exposure of rodents for **caffeine** *via* oral administration has shown also this xanthine to possess teratogenic properties in experimental animals. According to an OECD SIDS evaluation as from 2001 the teratogenic effects are expressed in rats after bolus dosages (gavage) in amounts exceeding 40-60 mg/kg bw/d which involves serum concentrations in excess of 60 µg/ml. Malformations – like cleft palates and digit malformation - have been demonstrated in mice at 50-75 mg/kg bw day and at 80 mg/kg bw day in rats according to Nehlig A *et al* (1994). Such effects have also been observed in rabbits and monkeys (Brent RL 1998). The incidence for the mentioned craniofacial malformations in the mice was low; 0.9 % In one mouse study also a cranium malformation with an incidence of 2-7 % was observed. The lowest dose to produce malformation in rats - ectrodactylyl[9] - in about 6 % was 80 mg/Kg bw/day (Wilson JG et al 1984). Wilson JG *et al* in their overview article from 1984 about the teratogenic potential of caffeine in laboratory animals concluded that > It is now established that a high doses, at or near those which cause maternal toxicity, caffeine treatment during the period of organ formation may result in developmental defects in a low to moderate percentage of the offspring. Such results have been reported mainly for mice and rats, although one limited study indicates that similar results may be obtainable also in rabbits. The only one rabbit study there seems to exist this far showed that 6 out of 64 offspring of mothers administered 100 mg/kg bw caffeine on days

[9] Ectrodactyly involves the deficiency or absence of one or more central digits of the hand or foot and is also known as split hand–split foot malformation. Historical data in CrI:CD®BR Rat are low 0.001 % (Lang PL at MARTA 1993)

	1-25 of gestation, had ectrodactyly (Bertrand et al., 1970). Spontaneous occurrence of this malformation in NZW rabbits are very rare; 1 in 17 000 according to Palmer AK (1968). Tests involving healthy adult male volunteers showed that peak plasma concentrations reached 10 µg/ml after consuming 300 – 350 mg caffeine in one day 30 µg/ml after consuming 800 mg per day for a month Assuming that one cup of coffee contains 100 mg of caffeine these study results indicate that the acute toxic level (vague symptoms) is reached after approximately 8 normal cups of coffee in a day. There are, of course, large individual variations (Nordic Council report 2005). A large prospective observational cohort study for which the results were reported 13 Feb 2013 involved 59 123 healthy women giving birth to healthy off-spring. Healthy women giving birth to child with malformations had not been included in the study (Sengpiel V *et al* 2013).[10] Women's self-reporting about pre-pregnancy total caffeine intake from coffee, black tea and soft drinks ranged from 40 to 254 mg/day, average being 126 mg/day. Intake decreased over the pregnancy because of nausea. Hence, we believe that the highest plasma concentration occurring in all these women was beneath10 µg/ml. The difference between 60 µg/ml (rat studies demonstrating teratogenic effects) and 10 µg/ml is that large it cannot be expected, we think, ever to find any association between exposure for caffeine in mother-to-be and occurrence of malformations in the off-spring. So it is no surprise that, this far, epidemiology hasn't provided any indications of such associations. We then also observed the negative outcome of the hitherto most extensive study on the matter carried out by Bille C *et al* (2007) - that involved 100 000 pregnancies in Denmark. Commenting thoroughly on all published epidemiological studies up till 2009 regarding caffeine and congential malformation the authors Brent RL, Christian MS and Diener RM in an overview article as of April 2011 concluded that: > It is very unlikely that the usual or even high exposures of dietary caffeine increases the risk of birth defects for pregnant mothers exposed to caffeine Reporting in 2005 the Nordic Council group of experts expressed that > Because of the huge variation in quality of the epidemiological data available (see chapter 12), the question of whether caffeine is teratogenic or not in humans cannot be considered completely settled. However, at present, there is no evidence from epidemiological studies that high caffeine exposures cause congenital malformations in humans. This statement could easily be understood to mean that no finds of an association evidence that caffeine is not teratogenic in humans. We, on the other hand, are of the opinion that the void of finds of any association can be explained by a far too low exposure and, therefore, should not be construed to mean that the xanthates are not teratogenic in humans.

[10] The Norwegian Mother and Child Cohort Study

Apparently, the authors Brent RL *et al* (2011) think likewise. Xanthines have been found teratogenic in more mammals at high dosages; mice, rats, rabbits and monkeys. They all differ somewhat from one another as to the metabolic pattern. We would, therefore, think it probable that xanthines are teratogenic in humans as well. To our understanding no-one has ever delivered evidence these substances are *not* teratogenic in man.

The **theobromine** xanthine also is a potent teratogen. In New Zealand White rabbits a NOAEL of 21 mg/kg b.w, based on variations in skeletal development, was determined. Rabbits dosed with 75 mg per kg body weight caused serum levels between 24 and 86 µg /ml. Average serum concentrations resulting from a NOAEL dose were 12-15 µg /ml. Incompletely ossified or absent sternebrae and metacarpal bones were observed. (EFSA 2008 /IARC)[11].[12]

In adult humans 84% of caffeine taken in orally is converted metabolically into **paraxanthine**. When fed to mice paraxanthine caused a dose-related increase in total malformations, primarily cleft palate and limb malformations (York RG *et al* 1986).

Rabbits are considered better animal models than rodents as concerns teratogonicity generally (texbooks). Hence, we consider the rabbit data more relevant than other animal data.

Shibata M *et al* (2000) studying the effect of theophylline dosed i.v. in pregnant rabbits (Kb1:JW), observed the following effects as concerns skeletal variations /abnormalities

Frequency parameter relating to foetal presenting with abnormality		Dose / mg/Kg bw/day			
		0 (controls)	15	30	60
Sutural bone	Number	3	12	14	9
	% litter incidence	1.9	8.5	9.6	9.5
13th rib	% litter incidence	22.8	38.3	21.4	58.1
Cleft palate	Number	0	1	0	8
	% of live f..	0	14	0	100
Other variation	Number	1	1	0	0
Number of foetuses observed		122	122	142	103

Sutural bones – also called wormian bones - are small/medium sized,

[11] Tarka et al, representing the firm Theocorp Holding company defending safe use in foodstuff (Gras paper) thought that the teratogenic effects observed could be explained by significant reductions in food intake on gestation days 15-30, meaning they were not considered a treatment-related effect. GRAS paper 10 May 2010 (http://www.accessdata.fda.gov/scripts/fcn/gras_notices/GRN000340.pdf). Seemingly EFSA is not of the same view.

[12] Another rabbit study showed dose-dependent degeneration and necrosis of seminiferous tubules, including vacuolation of spermatids and spermatocytes to multinucleated cell formation and oligospermia or aspermia with extensive degeneration of tubule cells. Interesting therefore to note that in humans prenatal and infant exposure to theobromine appeared possibly associated with hypospadia and testicular cancer in one population study (Giannandra F 2009).

irregular-shaped bones that arise from spurious ossification centers in the membranous bone formation of the cranium. They are associated with delayed/reduced ossifications. Possibly, sutural bones arise because of intracranial pressure that widens the sutures or slows down their extinction/removal. A plausible underlying cause for this would be occasional disturbance of the fine balance between the growth of the brain and the ossification process taking place at the rim of the sutures. In healthy foetuses brain growth and the ossification are intimately interconnected and synchronic. See more about the phenomenon sutural bones in Annex.

Most authors seem to consider sutural bones a skeletal variation or a minor anomaly only. Since, however, they probably are manifestations of disturbances of the fine balance as mentioned, we are inclined to consider occurrence of sutural bones a true teratogenic effect. It has been shown that exposure for certain chemicals (drugs) enhance ossification thereby causing premature fusion of the sutures i.e. craniosynostosis (with grave consequences). We think it probable that exposure for certain chemicals can also delay/reduce ossification so that sutural bones emerge.

In the control rabbits of Shibata *et al* – Kbl :JW rabbits - the sutural bone condition occurred with a litter incidence of 1.9 %. This is close to average historical data for the Kbl:JW rabbits (Ema M *et al* 2012) (%)

Laboratory 10	Laboratory 11
1.3 (0 – 5.0)	2.96 (1.3 – 7.4)

The incidences observed at all the three dosage levels in the treated rabbits are clearly above these historical data.

Also the incidence for the 13th rib effect in the control rabbits (22.8 %) is well within the range of the historical control data: 0- 46 % (Ema M *et al* 2012).

Shibata *et al* reported that as concerns the sutural bone condition the incidence at the dosages 30 and 60 mg/Kg bw/day is significantly grater than the incidence in the controls. As concerns the "13th rib effect" the incidence at the highest dosage is significantly grater that that for the controls. Shibata *et al* thought, however, that a dose-dependency as to these effects had not been demonstrated and so these authors considered them not rug-related. Shibata *et al* considered only the cleft palate effect rug-related.

We observe that as concerns the sutural bone incidence the one for the lowest dosage (15 mg/Kg bw/day) is only marginally lower than the incidences of the two other higher doses – and like these ca. 5 times higher: In relative figures:

0 (controls)	15	30	60
1	4.7	5.05	5.0

The reason why the incidence stay at the same level in the treated animals when increasing the dosage may perhaps be that the mechanism mediating the up-coming of sutural bones get saturated at a dose level of around 15 mg/Kg bw/day - and so cannot effectuate further enhancement. At present this is a hypothesis only. However, it cannot be excluded that a saturation effect prevents increase of the response with

	increasing dosage. In Annex is shown another example on a sutural bone effect caused by a potent teratogenic chemical where the incidence at two out of three dose levels is considerably higher than that for the control level there, however, being no dose-response relationship. Hence, we think it probable that all three skeletal effects, the sutural bone, the 13th rib and the cleft palate effect are anomalies caused by the exposure for theophylline in these animals. On this background we are of the opinion that 15 mg/Kg bw/ day should be considered a NOAEL – even though it could also be argued it is more like a LOAEL. We note that the medicinal court, the *Daily Med,* expresses that: > Doses at and above 15 mg/kg/day (less than the maximum recommended oral dose for adults on a mg/m^2 basis) increased the incidence of skeletal variations. So, the impression is this body consider the dosage 15 mg/Kg bw/ day a LOAEL value. In the above mentioned study of Stenius-Aarniala B *et al*, (1995), the pregnant asthmatics in question consumed on average ca.8 mg theophylline /Kg bw/day. We note that this intake is not very much below the determined NOAEL/LOAEL. So we are left with a strengthened suspicion the increased risk for malformations as seen in the study of Stenius-Aamiala is actually due to the exposure for theophylline during the pregnancy and not due to other causes. Exposure much less than 8 mg/Kgbw /day may possibly cause effects in off-spring other than clearly visible craniofacial defects or limb deformations that also are clearly seen. This may concern effects stemming from imbalances causing reduced cranial ossification during the pregnancy and that are not easily detected. Examples may possibly be hypotonia and minor connective tissue defects. Theopylline treatment for asthma during pregnancy increases the risk for preterm delivery. And theophylline therapy in surviving preterm children of birth weight <1501 g showed at 14 years of age significantly higher rate of cerebral palsy compared to children not exposed (Davis et al 2000). So, apparently, exposure for theophylline in new-borns has a certain harmful effect on brain's motoric centres. Occurrence of hypotonia also may be referred to such brain damage.

5. Exposure estimate and critical NOAEL

NOAEL critical	Establishing a NOAEL on the basis of animal experimentation we observe the repeated dose toxicity LOAEL (rat, mouse) that varied from 37.5 to 225 mg/kg bw/day. The effects involved were nephropathy in male rats in one study and a dose-dependent periarteritis in all treated groups. The mechanism of the nephropathy effect (LOAEL: 75 mg/kg bw/day)

	may relate to the fact that xanthines acts as a potent vasoconstrictor in the kidney. The relevance of this finding is considered unclear because the effect were observed even in the controls to a slight extent – and were not found in other repeated dose studies with theophylline. Periarteritis (LOAEL 37.5 mg/kg bw/day) is considered a rat-specific response to vasodilators being of little, if any, relevance to humans. Furthermore, this effect has not been associated with theophylline treatment in humans. In light of these considerations we would think the teratogenic effect the critical one; NOAEL of 15 mg/kg bw/d. As explained in the above we assume that theophylline is teratogenic in humans. Apparently, this view is shared by the Health Council of the Netherlands.
Exposure cosmetic products	**Systemic exposure dose (SED) for theophylline in humans:** The bulk of cosmetic products containing theophylline are stay-on products that regularly are put on daily for a longer period of time. This concerns the so-called anti-cellulite products and the so-called "sculpturing" products. Obviously, it is also only these products wherein theophylline is used to any extent. This is because the effect claimed requires an efficient active ingredient. Several of the marketers placing these products on the market convey in their announcements (see Annex) that the product should be applied liberally 2 times a day. Some use-instructions collected are: • Apply to skin every day wherever cellulite-prone skin is a problem until results are seen, usually 4 to 6 weeks. Apply 1 to 2 times a week thereafter for maintenance. It is preferable to apply after bath or shower • can be used on the stomach, buttocks, thighs, arms, or anywhere you may need to spot … • targets hips, thighs and buttocks • Use products for "problem zones" (hips, buttocks, belly) • Dispense a generous amount into hands and briskly massage into entire thigh and buttock area. Maximum results achieved when performed twice daily Apparently, the products are generally meant to be applied on stomach, buttocks, thighs, hips. According to the Council of Europe (2008) the anti-cellulite products are meant to be used on the back of thighs and buttocks two times daily for a considerable time period. Making use of the so-called Wallace's "Rule of nine[13]" it is estimated that the skin area of

[13] Wallace's "Rule of nine": In lack of more specified data it is customary to resort to the so-called Wallace's "Rule of nine" when estimating the area of specific body parts. See Annex and http://www.google.no/search?q=%22Wallace%E2%80%99s+%E2%80%9CRule+of+nine%22&lr=&hl=no&as_qdr=all&tbm=isch&tbo=u&source=univ&sa=X&ei=sl-rUdmmKYre4QScq4DQDQ&ved=0CDsQsAQ&biw=1150&bih=538

	• stomach + hips + buttock + back of thighs all together constitute an area of (17 500[14] x 0.36 =) ca. 6300 cm^2 As concerns the amount smeared out on each unit of the area we assume the default value of the SCCS of 1 mg product per cm^2. This is probably a conservative estimate. Within the frames of the work with caffeine in the Council of Europe industry informed that as concerns the anti-cellulite products the usual application is 1-2 mg /cm^2. So estimating the SED we apply the following premises ➢ amount of product applied twice a day (6300 x 1 x 2 =) 12,600 mg ➢ Concentration of theophylline in product: 2 % (reference is made to announcements on the web also as concerns anti-cellulite products relying on aminophylline) ➢ Skin penetration rate: 10 % (considered conservative , *inter alia*) ➢ Body weight: 60 Kg (SCCS default value) SED = 12600 x 0.02 x 0.10/60 = 0.42 mg/Kg bw/day
Margin of Safety (MoS)	MoS = NOAEL/ SED *MoS for exposure only because of use of cosmetic products* $MoS_{cosmetics}$ = 15/0.42 = 36 *MoS for total exposure* In order to establish a realistic picture of the risk the consumer faces because of exposure to theophylline not only is it necessary to take into account usage of cosmetic products but also the daily exposure due to diets. The foodstuffs then to take into account would bee coffee, tea and caffeinated beverages (cola). These foodstuffs are usually not considered necessities, but most people in western countries takes in considerable quantities on a daily basis. It seems only prudent, therefore, to assume that the exposure for theophylline that some women experience because of use of the mentioned anti-ageing products come on top of the daily exposure for theophylline because of diets. So MoS_{total} = NOAEL / ($SED_{cosmetic}$ + SED_{diets}) Within the frames of the above mentioned recent extensive Norwegian prospective observational cohort study (Sengpiel V *et al* 2013) it on the basis of state of the art intake studies was laid to ground that the average daily intake of caffeine in Norwegian pre-pregnant women amounts in average to 126 mg/day. Some took in as much as 254 mg/day. It has been established that 4 % of the caffeine consumed is immediately metabolised into theophylline (*inter alia*). So the SED_{diets} then would be on average (126 x 0.04/60 =) 0.084 mg/Kg bw/day. Applying the maximum intake of 254 mg/day SED_{diets} increases to 0.17 mg/Kg bw/day. Besides, there is a contribution from free theophylline in the tea – but this is vanishingly small because there are only trace amounts of theophylline in normal black tea. A normal cup of tea

[14] SCCS default value for entire skin surface.

contains only 1 mg theophylline (Council of Europe 2008).

These SED_{diets} figures are for Norwegian women and may possibly differ somewhat from SED_{diets} estimates for most other countries within the European single market.

Information as to the frequency of use of coffee in certain western countries can be gained from the many epidemiology studies which have been carried out over the years. Two such studies of *pregnant* women - one Danish (Olsen J *et al* 1991) and one French (Ou Shu X *et al* 1995) strongly indicate that a rather large part of the population in at least these two EEA countries take in more than 400 mg caffeine per day:

Danish study			French study		
Caffeine daily intake due to coffee alone (mg)	number of women	percentage	Caffeine daily intake due to coffee alone (mg)	number of women	percentage
800 and above	850	7	above 800	15	3
400 - 700	3 243	28	400- 800	69	16
0 - 300	7 510	65	below 400	356	81
SUM	11 603		sum	440	
Average intake	307 mg		Average intake	280 mg	

The current World Health Organization (WHO) guidelines recommend a caffeine intake below 300 mg/day during pregnancy, while the American College of Obstetricians and Gynecologists and the Norwegian Food Safety Authority concur with the Nordic Nutrition Recommendations (NNR), recommending a maximum caffeine intake of 200 mg/day (Sengpiel *et al* 2013). UK Food Standards Agency in 2008 reduced its maximum recommended daily caffeine intake during pregnancy from 300 to 200 mg.[15] In France the "Haute Authorité de Santé" in 2005 only advised pregnant women to moderate their intake of caffeine (La consommation de ces boissons pendant la grossesse doit être modérée.)[16]

In 2003 the earlier EUs Scientific Committee on food (SCF) came out with the view that it is questionable whether a daily intake above 300 mg is safe in relation to the unborn life. Since then the EU scientific committee for the foodstuffs (EFSA) has not produced any opinion on this subject. We have no further information as would be recommendations currently put by health authorities around Europe.

On this background we for the purpose of this safety assessment choose the figure 250 mg/day as a rough approximate average value for caffeine intake of the adult European female citizen early in pregnancy. Roughly, it corresponds to 2 ½ normal cups of coffee per day.

Hence, SED_{diets} = (250 x 0.04 / 60 =) 0.17 mg /Kg bw/day

[15] http://www.nhs.uk/news/2008/11November/Pages/Newcaffeineadviceinpregnancy.aspx
[16] sante.fr/portail/upload/docs/application/pdf/femmes_enceintes_recos.pdf

	$MoS_{total} = 15 / (0.42 + 0.17) = 26$ We are of the view that this total margin of safety is only a fourth of the one required to protect pregnant women early in a pregnancy or women planning a pregnancy from giving birth to malformed off-spring.

6. Other sources of exposure than cosmetic products

Food stuffs	See above
Pharmaceuticals	Theophylline is still used somewhat both in the prophylaxis of chronic asthma and COAD, and as emergency treatment in acute severe asthma. A rough guide to total daily dosage is 10-15 mg/kg in adults (higher in children) in 2 divided doses for sustained release preparations. (Priory Medical Journals). 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 (Skouroliakou M *et al* 2009). Theophylline is a prescription drug for asthma and is available as tablets (250 – 350 mg). Normal daily dose is 7.7 mg/Kg bw which cause a serum peak level of ca. 8 µg/mL It is contraindicated in pregnancy and lactation unless there is a clear need.
Other sources	Data not retrieved.
Adverse side effects - from uses other than cosmetics	One of the factors that limit the usefulness of theophylline is the high incidence of side effects within the therapeutic range and the narrow therapeutic index. As plasma levels exceed 15 mg/l (normal therapeutic range 10-20 µg/mL), the frequency of side effects increases, the most common being a sinus tachycardia, nausea, tremor and indigestion. Patients may also complain of central stimulatory effects such as anxiety, nervousness and insomnia. Deaths associated with theophylline toxicity have been reported. These may be due to cardiac toxicity leading to life threatening dysrhythmias, especially in association with anaesthetic agents such as pancuronium and halothane and sympathomimetics. Most deaths are associated with neurotoxicity, and the mortality from theophylline related seizures approaches 30 %. These seizures are often initially focal progressing to generalized tonic-clonic convulsions and an encephalopathic picture. There is no close relationship between the plasma level of theophylline and the onset of seizures as this may be also influenced by the presence of hypoxia, hypercapnia and acidosis. Other clinical features of acute theophylline over-dosage include nausea, vomiting, metabolic acidosis, hypokalaemia. gastrointestinal bleeding, rhabdomyolysis. (Priory Medical Journals)

	The use of theophylline is further complicated by its interaction with various drugs, chiefly cimetidin and phenytoin. Its toxicity is increased by erythromycin, cimetidine anf fluoroquinolones such as ciprofloxacin. It can reach toxic levels when taken with fatty meals, an effect called dose dumping. Theophylline toxicity can be treated with beta blockers. In addition to seizures, tachyarrhythmias are a major concern (Wikipedia) The pharmacokinetics of theophylline varies widely among similar patients and cannot be predicted by age, sex, body weight, or other demographic characteristics. In addition, certain concurrent illnesses and alterations in normal physiology can significantly alter the pharmacokinetic characteristics of theophylline.

7. Assessment

The Health Council of the Netherlands recently recommended that theophylline be classified a CMR (toxic to reproduction) substance of the category 1b. Already in 2007 the Canadian health authorities (Health Canada) banned theophylline and found it – and its complex with 1,2-diaminoethane the aminophylline - unsafe for use in cosmetic products.

For years theophylline was employed as an approved remedy for asthma. It was considered first-line therapy in more countries. In more recent years it has, however, gone more or less out of use because it successively became clear that the therapeutic benefits do not sufficiently outweigh the many side effects like hypotension and the risk for cardiac problems.

In two studies, use of theophylline during pregnancy was found to cause an increase in preterm deliveries. There may be a risk for skeletal and heart malformations entailing therapeutic usage.

Theophylline is clearly teratogenic in rodents and rabbits. There are indications it is teratogenic also in monkeys. All xanthines used medicinally - and in cosmetics - are teratogenic in named mammalian animals. The probability is high they all are teratogenic in humans as well.

In the marketplace theophylline and its complex with 1,2-diaminoethane – that release theophylline when used – are often used in concentration up till 2- 3 %. This concern primarily stay-on anti-age products meant to be used on larger part of body for a firming/skin smoothening purpose – confer so-called anti-cellulite and “sculpturing” products – and also so-called body creams as well as creams meant to firm the skin in the woman breast. Theophylline is easily taken up in body over the skin. In a majority of the products it is formulated together with vehicles that enhance the flux across the skin many times.

A margin of safety of 100 is considered sufficient in relation to malformation effects observed in rabbits. The following maximum concentrations comply with this margin.

- Stay-on anti-age products meant to be used on larger part of body for a firming/skin smoothening purpose – confer so-called anti-cellulite and “sculpturing” products: 0.7 %
- Body lotion: 1 %
- Face cream: 6 %

These maximum concentrations also are derived assuming a total exposure which includes a contribution from the diets that amounts to 0.17 mg/Kg bw per day. The exposure for theophylline because of use of cosmetic products alone is about equal to the dietary contribution; 0.15 mg/Kg

bw/day. The total exposure, i.e. 0.32 mg/Kg per day, we consider a safe exposure in relation to unborn life. .

8. Conclusion

We propose the following maximum usage limits for theophylline in cosmetic product wherein it seems to find employment

- Stay-on anti-age products meant to be used on larger part of body for a firming/skin smoothening purpose – confer so-called anti-cellulite and “sculpturing” products: 0.7 %
- Body lotion: 1 %
- Face cream: 6 %

The products concerns must carry the following warning sentences:

Must not be used in combination with medication for asthma based on theophylline
Must not be used in combination with antibiotics

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Claim: firming of the skin for slimmer look

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- *http://www.sigmaconesthetics.ca/products/circadia/firming-and-shaping-gel.php*
- *http://www.circadia.com/docs/body%20care/professional-firming-and-shaping-gel.pdf*
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- *http://nanowellus.com/eshop/inx_detail.asp?tcPageKind=detail&tcModeCode=1096*
- *http://melissabeauty.co.uk/shop/carole-franck/*

Claim: orange peel looking skin

- *http://www.puraderma.it/eng/dett_celludren_body_lotion.asp?index=0*
- *http://www.stellarinskin.com/ingredients/*
- *http://www.alphabodysolutions.com/index.php?main_page=product_info&products_id=1*
- *http://www.bronsonvitamins.com/613/body-contours-anti-cellulite-cream*
- *http://www.amazon.com/gp/product/B000GAOAKA?ie=UTF8&tag=buyeguid-20&linkCode=as2&camp=1789&creative=390957&creativeASIN=B000GAOAKA (http://www.buyersguide.com/Cellulite/MWTIncCellulean.php=)*
- *http://www.awb-engineering.com/CosmeticBodyCelluliteSerum.html*
- *http://www.triderma.com/cellulite-slimmer-reduction-and-toning-cream.html*
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Claim: Soothing

- *http://www.specialchem4cosmetics.com/product-directory/anti-inflammatories_0_14138/skin-care-facial-care-facial-cleansing-body-care-baby-care_5_14595/s-theophylline/index.aspx?did=0&d=1*
- *http://www.pevonia.co.uk/buy/smooth-tone-body-cream*
- *http://www.puraderma.it/eng/dett_celludren_body_lotion.asp?index=0*

Claim: preventing stretch marks

- *http://www.polstore.com/eris-pharmaceris-m-foliacti-stretch-marks-preventing-cream/*

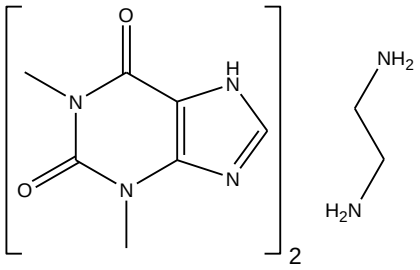
Claim: Firms the breast

- *http://skinequality.com/shop/index.php/botanical-soothing-cleanser-4232.html*

10. Annexes

➢ AMINOPHYLLINE

A number of the anti-cellulite products on the market are based on the following 2:1 complex between theophylline and 1,2-diaminoethane. It is called aminophylline.



Aminophylline, CAS No 317-34-0, MW: 420

Also aminophylline find use as an asthma remedy.

There is a monograph on aminophylline in the Council of Europe document on active ingredients in cosmetics from 2008. In carrying out a safety assessment on the use of this complex in cosmetics the Council treated the two components per se conferring to the separate monograph for theophylline. When being used aminophylline releases theophylline.

The council informs that aminophylline works against local water retention and that the use concentration is: 3-10%.

Searching on the internet 1 June 2013 the following 5 aminophylline containing cosmetics said to contain 2 – 3 % of the ingredient were identified:

• http://www.amazon.com/Fat-Fader-Toning-Aminophylline-Raspberry-Ketones/dp/B007XOAKK6	Said to contain 2.5 % aminophylline.

• http://cellulitecreamreport.org/procellix-aminophylline-cellulite-cream/ • http://1procellix.com/Landing.aspx?CampaignID=10&aff=affW4Pft&sub=10045	In the latter address it is said to contain 2 % aminophylline
• http://www.amazon.com/s/url=search-alias%3Daps&field-keywords=aminophylline%20cream%202/?_encoding=UTF8&tag=stans08-20&linkCode=ur2&camp=1789&creative=9325 • http://facetreatments.com/facetreatments/tag/aminophylline/	Said to contain 2 % aminophylline
• http://www.greenbeautyproduct.com/weight-loss-cream.htm	Said to contain 2 % aminophylline
• http://www.acnedoctor.com/cellulite_treatment.html	Said to contain 3 % aminophylline

Other cosmetics containing aminophylline being offered for sale on the web that day were

Firstly, 16 different skin care products mentioned by “Goodguide” at http://www.goodguide.com/ingredients/67762-aminophylline

And then at least the following 7 anti-cellulite products.

1. http://www.sexproductswholesale.net/Anti-Cellulite-Aminophylline-Slimming-Cream-for-Toning-and-Firming-wholesale-454.html
2. http://www.luckyvitamin.com/p-156019-ideal-marketing-concepts-amilean-advanced-cellulite-slimming-lotion-with-aminophylline-8-oz
3. http://www.shopwiki.co.uk/l/Anti-Cellulite-Aminophylline-Cream
4. http://cellulite-redux001.info/aminophylline-cellulite-gel/
5. http://www.officialhcg.com/aminophylline-cream-50-units/
6. http://urbancargo.com/tag/ab-firming-gel/
7. http://www.nutritionexpress.com/health+concerns/skin+health/cleansers+moisturizers+oils/body+moisturizers+oils/body+lotions+creams/amilean+amilean+advanced+cellulite+slimming+lotion+8+fluid+ounce.aspx

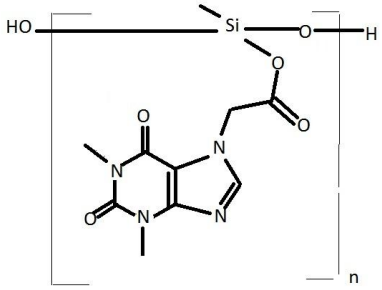
The ethanediamine constituent is a potent allergenic compound and a liver toxicant. The Council of Europe (2008) estimated a Margin of Safety for the use of ethandiamine alone in the anti-cellulite product being down to the very low level of 9. Canada has prohibited the use of aminophylline as well in cosmetics. In spite of these unfavourable conditions aminophylline seems more popular in today’s marketplace than is theophylline alone.

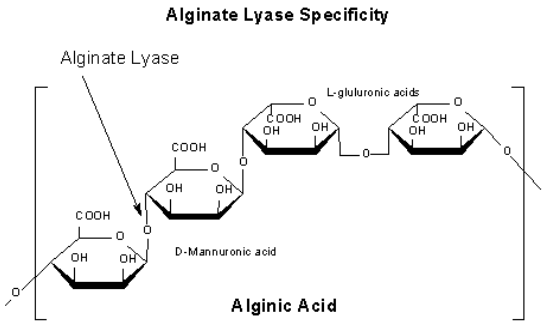
➢ DERIVATIVES SUBSTITUTING THEOPYLLINE

In later years two theophylline derivatives have come more in use than theophylline itself for the same purposes. Firstly, this concerns a compound with the INCI name

- Methylsilanol Carboxymethyl Theophylline Alginate

The Monaco based producer EXSYMOL, in an e-mail 17 May 2013 to the Norwegian Food Safety Authority (by Hans Jørgen Talberg), informed about the following molecular structure





Siloxanes and Silicones, Me (1,2,3,6-tetrahydro-2,6-dioxo-7H-purin yl)acetyloxy, hydroxy-terminated

CAS No 128973-73-9

CAS No 9005-32-7

1,3-dimethyl--7-

EXSYMOL, using the trade name *" theophyllisilane C"* for this compound, explain that it goes about an aqueous solution containing 1.3% of METHYLSILANOL CARBOXYMETHYL THEOPHYLLINE ALGINATE. According to another source [17] the solution also contains 0.1 % alginic acid.

According to the source the "Handbook of Green Chemicals" (Michael and Irene Ash 2004) the use level of *"theophyllisilane C"* is 5-6%

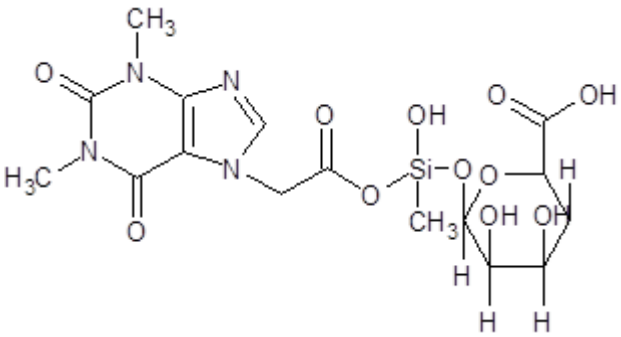
Hence, this theophylline derivative is present in a concentration amounting to (6 x 0.013 =) 0.078 % which is 25 times less than the concentration of theophylline (2%) finding use in the different anti-age products mentioned. Supposedly, it works better than theophylline in relation to claimed effect. It is contained in 11 products mentioned in the CodeCheck and 17 products in the Good guide database.[18]

Secondly, there is the following compound having the INCI name

- Acefylline methylsilanol mannuronate

On the internet according to the firm SAAEL[19] and other sources [20] the molecular structure is as follows:

[17] http://www.biosiltech.com/sites/default/files/Theophyllisilane%20C%20-%20A3%20brochure.pdf
[18] http://www.goodguide.com/ingredients/273251-methylsilanol-carboxymethyl-theophylline-alginate
[19] http://www.saael.com/en/saa/?type=detail&id=1858
[20] http://www.guidechem.com/cas-162/162030-43-5.html



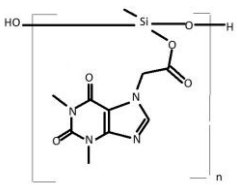
6-(2-(1,3-Dimethylxanthen-7-yl)-1-oxoethyloxy)methylhydroxysilyloxy)-3,4,5-trihydroxytetrahydromannopyran-2-carboxylic acid

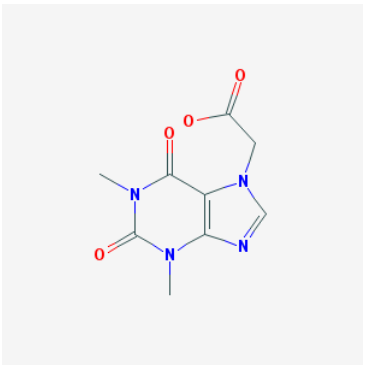
CAS No: 162030-43-5

According to the source ”guidechem” [21] this is also the molecule that the firm EXSYMOL call “Xantalgosil C”.

A very limited internet search 18 May 2013 revealed 12 products of the “slimming” type, and two anti-wrinkle products containing this ingredient. Apparently also this derivative finds much more use than do theophylline it self.

The SAAEL firm mention the compound is easily hydrolysed. We would think it probable that both the *theophyllisilane C* and the *xantalgosil C* compound liberate acefylline when taken up in the live skin tissue



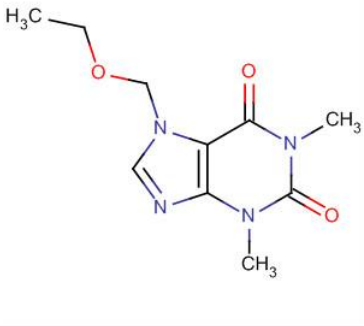
--->  + HO-(-Si(Me)(OH)-O-)-H

Theophylline acetic acid
7-(Carboxymethyl)theophylline
7-Theophyllineacetic acid
Acefylline
CAS No 837-27-4

In the body acefylline behaves approximately as do theophylline, caffeine and the other well known xanthines.

[21] http://www.guidechem.com/cas-162/162030-43-5.html

Sloan and Bodor reportedly synthesized 7-acyloxymethyl derivatives of theophylline that diffuse through the skin far more efficiently than theophylline itself but are bio-transformed rapidly to theophylline (Gennaro AR). This concerns, for example, the following molecule:



CAS No: 64210-70-4

➢ METABOLISM OF THEOBROMINE IN MAMALS (EFSA 2008)

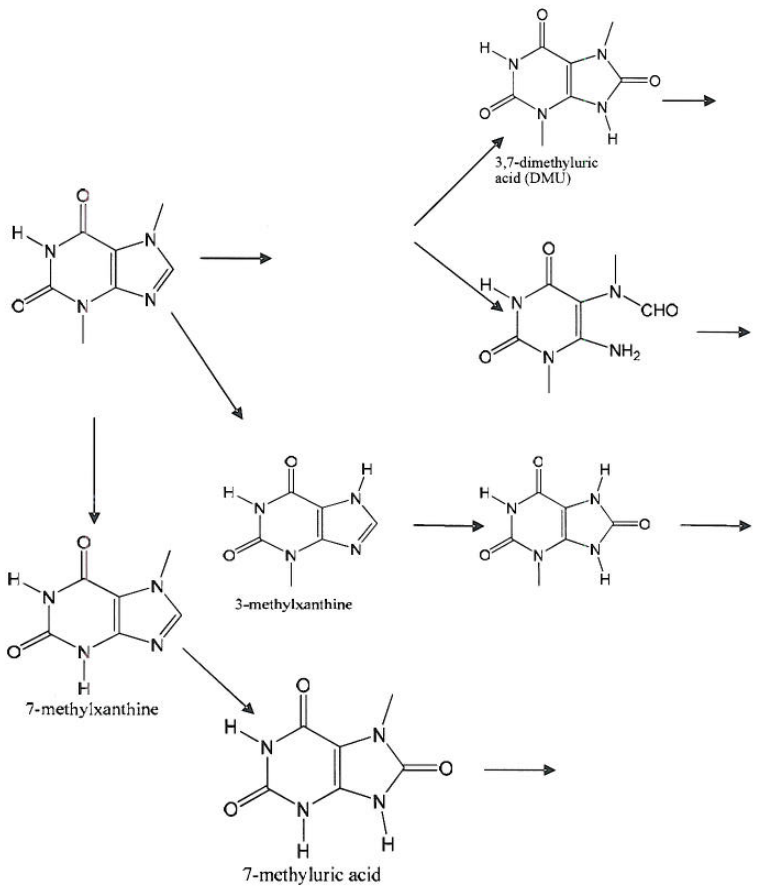


Figure 3. Biotransformation of theobromine in mammals

➢ METABOLIC DIFFERENCES BETWEEN HUMANS AND RODENTS

Metabolite in urine /ingestion of caffeine	Percentage in urine		
	Man	Mouse	rat
Unchanged caffeine	2	2.2	3.1
1-methyluric acid	28	9.8	6.5
1-methylxanthine	21	7.2	5.8
1,7-dimethyluric acid	9	7.0	4.1
1,3-dimetyluric acid	3	7.9	5.3
1,3-dimetylxanthine (theophylline)	8	0.8	8.1
1,7-dimetylxanthine (theobromine)		16.9	18.2
3-methylxanthine	3	-	-
5-acetylamino-6-amino-3-methyluracil	13	-	-
a diaminouracil metabolite	5	-	-
amino 1,3,7-trimethyl uracil derivative	-	11.6	22.6
Trimethyluriv acid	-	5.6	9.7
Trimethylallantion	-	< 0.5	5.4
amino 1,7-dimethyl uracil derivative	-	1.7	3.0
POLAR metabolites		28.7	7.9

➢ THE EFFECT OF VEHICLES

Ademola Ji *et al* concluded that their study indicated that a high level of absorption enhancement will be required before transdermal theophylline preparations could produce **therapeutic** plasma concentrations. The aim at that time was to develop efficient dermal deliverance systems in a medicinal context particularly as concerns management of asthma and bronchitis. Due to dermal absorption benefits and its indication in infants, many studies have since been performed to enhance the permeation of theophylline through skin.

Already in 1992 Touitou E *et al* detected that combining theophylline with a polyethylene-glycol-enhancer-base a very high permeation could be obtained. In 2007 Fang JY et al demonstrated that addition of some terpenes to theophylline in a 25 % ethanol aqueous solution increased the flux of theophylline trough rat skin 16 – 23 times:

Terpene (3 %)	Flux μg/cm^2/h	Degree of flux enhancement
None (control)	2.05	-
Alpha terpineol	42	20
Linalool	45	23
1,8-cineole	38	19
Nerolidol	32	16

In 2010 Shakeel F *et al* showed that by the use of water-in-oil nano-emulsions the flux could be enhanced **17 times**.

We looked up on the internet the freely available EDETOX DATABASE of the University of Newcastle /UK[22] that provide data on *in vitro and in vivo* determined flux and skin penetration rates for numerous chemicals. Seemingly the provided overview as to published data on this is comprehensive and up till date.[23]

The following table show the EDETOX recorded *in vitro (*Franz diffusion cell) flux data obtained in rat skin studies of theophylline. In all the cases the skin used is dorsal dermatomed to 0.45mm. This is taken from the publication Twist JN et al 1989.

Theophylline in the following solutes	Flux μg/cm^2/h
Water – three studies – average value	17
Ethanol	52.3
Methanol	117.1
1-propanol	27.8
Propylene glycol	7.9
Glycerin	2.5
PEG 400	2.0

Out of the mentioned solutes that mimic a relevant cosmetic product formulation the most are the glycerine and PEG 400 solutions. Propylene glycol is the vehicle used the most in cosmetic products. As seen it may enhance the flux many times.

So, the enhancing power can be quite substantial. Another such vehicle is polysorbate 20. Akhtar N *et al* (2011) recently showed that with the help it the flux though skin of ascorbic acid could be increased from 0.63 to 3.17 microgram/cm^2/h – i.e. a 5 fold increase.

Other outstanding examples on how vehicles can enhance the ability of drugs to penetrate the skin barrier are:

Drug	MW g/mol	Flux nanomol /(cm^2 x min)		
		Level necessary to reach therapeutic steady state blood level [24] (Roy SD 1997)	In vitro human cadaver Aqueous solution Flynn GL 1990 Flynn database	In vivo human / In vitro h. cadaver + vehicle other source
Scopolamine *	303,4	0,00073	0,015	0, 36 (*Lechitin*?)[25]
Estradiol	272,4	0,0017 – 0,0025	0,01	0,49 (*micro emulsion*)[26]

[22] http://edetox.ncl.ac.uk/searchinvitro.aspx

[23] There is also mention in this database of an in vivo study in man that involved a dose put on the forearm and left uncovered for the whole exposure time. It concerned an ethanol water solution holding 0.06 % theophylline. The systemic exposure had been determined simply by estimation the remains of theophylline on the skin. The authors determined a skin penetration rate of high 16.9 %. This we think a biased result since some of the dose not only was absorbed into the body but also in the clothes and bedding (Wester RC et al 1998)

[24] Determined by the equation: Flux = Therapeutic steady state blood level (ng/L) x total body clearance (L/h) / 1 cm^2

[25] Chandrasekaran SK 1978, Wilimann H 1992

Testosterone	288,4		0,009	0, 05 (pig skin extra.)[27]
Clonidine *	230,1	0,00019 – 0,0019		
Nitroglycerin	227,1	0,09 – 0,78	0,19	1,6[28]
Nicotine *	162,3	0,08 – 0,24	80	

Among the many powerful enhancers having come in use in later years also as concerns the anti-ageing cosmetic products there are the following remedies

- Propylene glycol
- Butylene glycol
- Polysorbate 20
- Polysorbate 80
- Paraffin liquid
- Ethanol
- Liposomes of different kinds

One or the other of these enhancers are present in fairly high concentration in a majority of the theophylline containing products that we saw being sold on the market and for which the whole list of ingredients had been disclosed on the web.

We would think it only prudent that the skin penetration rate premise to be applied in this safety assessment take into account that theophylline is formulated together with a powerful enhancer like, for example propylene glycol. This addition helps to enhance the rate many times. In light of that we chose the conservative premise of a 10 % skin penetration rate.

➢ CALCULATION OF THE SKIN SURFACE (based on "the rule of nine")

The rule of nine is a way to calculate the percentage of the body surface that is affected and is often used to calculate the burn percentage.

Each leg constitutes 18% (front = 9%, back = 9%)
Each arm = 9% (front = 4.5%, back = 4.5%)

➢ NO EVIDENCE FOR CLAIMED LIPOLYTIC EFFECT

One efficiency study in humans showed no significant effect (Epstein E *et al* 1997)

Abstract

Numerous manufacturers are marketing topical creams, claiming that they improve or eliminate unwanted fat or cellulite in a short period of time. The active ingredient in most of these creams is theophylline, and claims have been made that it initiates lipolysis by binding to adipocyte beta-adrenergic receptors. The creams are applied with vigorous massage to facilitate absorption and apply mechanical stress to the fat cells. The efficacy of these creams is largely untested. This prospective randomized study was conducted to determine whether there is scientific evidence that application of these creams alone can eliminate unwanted fat or cellulite. Eleven women with normal body weight as defined by insurance tables applied either Skinny Dip(TM) or a placebo to one thigh and one half of the abdomen for 8 weeks. Each subject was examined, photographed, weighed, and measured by a study monitor on a weekly basis. There were no statistically significant differences in appearance, abdominal

[26] Peltola S et al, 2003
[27] Morgan RW 1998
[28] Ghosh T el al 1997

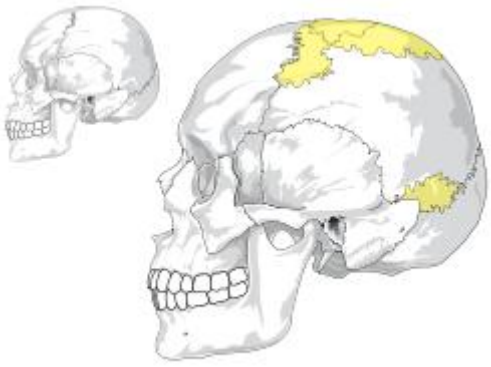
circumference, thigh circumference, or skin fold measurements among subjects using the active agent (Skinny Dip(TM)) or the placebo. This study failed to support the efficacy of topically applied lipolytic creams in eliminating unwanted fat manifesting as a localized bulge or cellulite presenting as a dimpling of the skin.

➢ THEORIES ABOUT OCCURRENCE OF SUTURAL BONE

Although unusual, sutural bone is not rare in humans – or other mammals (ca. 1-3 % in rabbits). They occur more frequently in disorders that have reduced cranial ossification, hypotonia or decreased movement (Hypotonia is a state of low muscle tone). Sanchez-Lara PA *et al* 2007 found 3.5 greater odds of developing a sutural bone with premature suture closure ($P < 0.001$); i.e. with craniosynostosis. Further, these authors obtained results that suggest that sutural bones may arise as a consequence of mechanical factors that spread sutures apart and affect dural strain within sutures and fontanelles.

The incidence of sutural bones is grater than that for the much more rare condition craniosynostosis: 1:2,500 in live human births (Johnson D *et al* 2011). As pointed out by Grau N *et al* (2006) craniosynostosis in addition to craniofacial anomalies, can manifest as impaired cerebral flow, airway obstruction, impaired vision and hearing, learning difficulties, and adverse psychological effects.

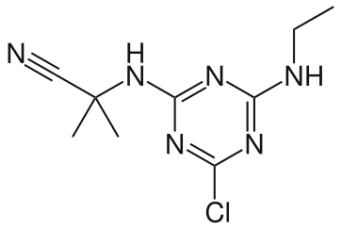
Sutural bones are a marker for various diseases and important in the primary diagnosis of Brittle Bone Disease (osteogenesis imperfecta) that is a congenital bone disorder. People with OI are born with defective connective tissue, or without the ability to make it (Wikipedia).



Sutural bone in yellow.

➢ OTHER CASES CONCERNING RABBITS BEING DOSED WITH A CHEMICAL AND INVESTIGATED AS TO WHETHER THE DOSINING CAUSES A SUTURAL BONE EFFECT

1. Mated New Zealand White rabbits given orally a herbicide called Bladex (based on the chemical cyanazine) (EPA 1983):



Cyanazine (CAS No 21725-46-2) is a recognized teratogen of moderate toxicity (Wikipedia)

Results

Type of anomaly	Dose / mg/Kg bw/day			
	0 (controls)	1.0	2.0	3.0
Sutural bone incidence (%)	3.3 (3 /95)	4.0 (4 /98)	5.6 (3 /54)	3.1 (2/64)
Number of foetus with 13th rib Incidence %	37 39%	47 48%	56 60%	64 100%
Number of foetuses observed	95	98	94	64

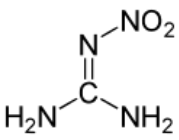
Historical data for the sutural bone effect in New Zealand White rabbits is 0.47 % (0-1.90 %) according to Ema *et al* (2012) and 3.7 % according to Palmer AK (1968)

As to the 13th rib effect the authors reported that the percentage of foetuses with more than 12 pair of ribs slightly increased in a dose- response relationship. The historical data for this effect is: 57.5 % (range 0 – 87%) (Ema *et al* 2012).

The conclusion of the study was that

> Bladex is foetotoxic at 2 mg/Kg bw/day and above. A dose-response increase was noted in the mean number of postimplantation losses and live foetuses per dam. A biological significant increase was noted in the array of skeletal variations noted at this dose level and above (i.e. absence of tibial tarsal bones and presence of additional rib no 13)

2. Mated New Zealand White rabbits given orally nitroguanidine by gestation (US Army Medical Research and Development Command 1988[29]):



Nitroguanidine (CAS No 556-88-7) is used as an extremely low sensitivity explosive

[29] http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA200472

Results

Type of anomaly	Dose / mg/Kg bw/day			
	0 (controls)	100	316	1000
Sutural bone incidence (%)	0	0	0.5 (1 /131	1.1 (1/87)
Number of foetuses observed	121	135	131	87

Nitroguanidine was found not to be a teratogen chemical.