Safety Assessment of Methylxanthines as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: November 9, 2018
Panel Meeting Date: December 3-4, 2018

The 2018 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya A. Cherian, Scientific Analyst/Writer.

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Cosmetic Ingredient Review

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MEMORANDUM

To: CIR Expert Panel and Liaisons
From: Priya Cherian
Scientific Analyst and Writer
Date: November 9, 2018
Subject: Safety Assessment of Methylxanthines as Used in Cosmetics

Enclosed is the Draft Final Report of the Safety Assessment of Methylxanthines (previously, Xanthine Alkaloids) as used in cosmetics [*methxa122018rep*]. The methylxanthines reviewed in this report are Caffeine, Theophylline, and Theobromine.

The Panel reviewed this document for the first time at the September 2018 Panel meeting, and issued a Tentative Report for public comment with the conclusion that these ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment. The safety of these ingredients was supported by negative carcinogenicity studies and the historically safe use of these ingredients in food products. The Panel noted the false positive genotoxicity studies observed in vitro without metabolic activation. However, any concern over these false positives was mitigated by in vitro and in vivo studies performed with metabolic activation that yielded exclusively negative results. Positive developmental and reproductive studies were also noted, but were considered negligible considering these effects were only seen at concentrations much higher than what would be used in cosmetics.

Comments received from the Council are included in the packet, and have been addressed [*methxa122018pcpc_1, methxa122018pcpc_2*]. Also included in this packet are the history [*methxa122018hist*], data profile [*methxa122018prof*], search strategy [*methxa122018strat*], flow chart [*methxa122018flow*], FDA VCRP data [*methxa122018FDA*], and transcripts from the September 2018 Panel meeting [*methxa122018min*].

The Panel should carefully review the Abstract, Discussion, and Conclusion of this safety assessment. If these are satisfactory, the Panel should issue a Final Report.

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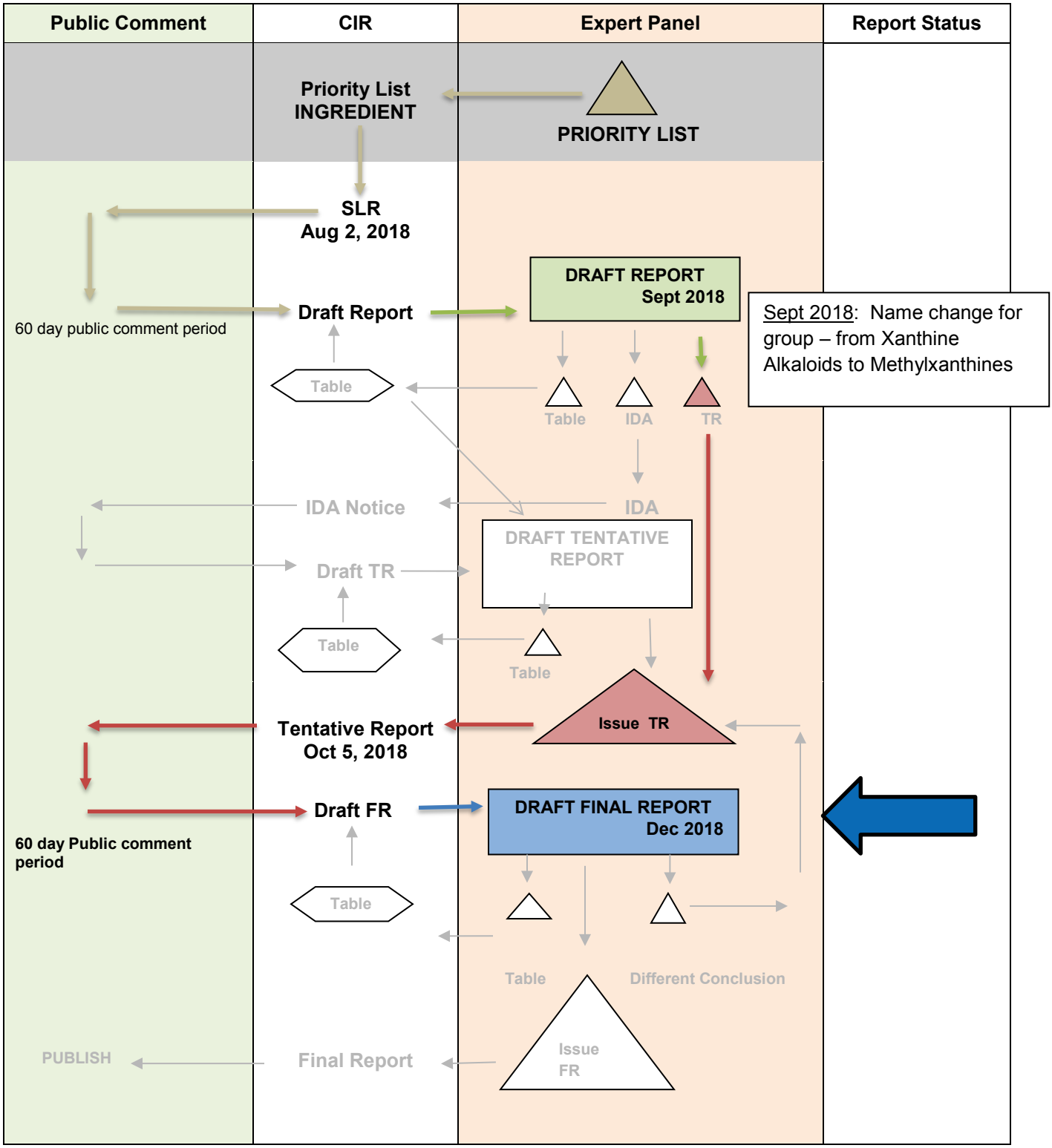
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Methylxanthines

MEETING December 2018



CIR History of:

Methylxanthines: Caffeine, Theobromine, Theophylline

August 2018: The SLR is posted for public comment

September 2018: The Panel evaluates the report, issues a tentative report for public comment; name of report changes from Xanthine Alkaloids to Methylxanthines

December 2018: The Panel evaluates the draft final report

Methylxanthines Data Profile for **December 2018**. Writer – Priya Cherian																					
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
Caffeine	X	X	X	X		X	X			X		X				x	x	X	X	X	
Theobromine	X	X					X											X	X		
Theophylline	X	X	X	X		X	X			X		X						X	X	X	

Methylxanthines

Ingredient	CAS #	InfoB	SciFin	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Caffeine	58-08-2	√	√	√	N	√	√	√	N	√	N	N	N	N	√	N	N	N	N	√
Theobromine	83-67-0	√	√	√	√	N	√	√	N	N	N	N	N	N	N	N	N	N	N	√
Theophylline	58-55-9	√	N	√	√	N	√	√	N	√	N	N	N	N	√	N	N	N	N	√

Search Strategy

Search terms: Caffeine, Theobromine, Theophylline, CAS numbers
Ingredient names with the terms dermal, toxicity, metabolism, penetration, cosmetic, development, irritation, sensitization

SciFinder, Pubmed
Caffeine
Theobromine
Theophylline
Refined for dermal, oral, inhalation toxicity; toxicokinetic; impurities; irritation; sensitization; ocular irritation; developmental and reproductive; genotoxicity; carcinogenicity; acute toxicity; dermal penetration; penetration; metabolism as well as CAS numbers

Methylxanthines Minutes

September 2018 Meeting

Day 1 – Group 1

DR. MARKS: Okay. Shall we start the afternoon? The next set of ingredients are the xanthine alkaloids. This is the first time we've seen these three ingredients. And Tom, Ron and Ron, first of all do you like the three ingredients, the grouping?

DR. SHANK: Yes.

DR. SLAGA: Yes.

DR. MARKS: Yes? Okay. And then what needs do we have for these?

DR. SLAGA: I'd like to make a comment first about the tremendous amount of genotoxicity.

DR. MARKS: Why don't you use the -- yeah, there we go. Tremendous amount of genotoxicity you said.

DR. SLAGA: Genotoxicity and carcinogenicity. There's a lot of data and to me it's a little confusing. And I'd like to summarize that part of it, okay?

DR. MARKS: Great.

DR. SLAGA: Well, first of all in AMES and even other bacteria, if there's no metabolic activation, it's a positive almost all the time. If there's metabolic activation, it's negative essentially all the time. Keep that in mind. And of course, if you were in vivo there would be metabolic activation, right.

In mammalian mutagenesis it's plus and minus. But most of those studies are not mutagenic, they're clastogenic, you know, where you induce some expression of something. Or you have DNA binding without mutagenesis. But if you got in vivo mutagenesis studies, they're all negative. And that's obviously mammals.

The IARC classifies it as inadequate evidence for carcinogenicity in animals and humans. And most of the data suggests that it really can't be classified as a carcinogen in humans. Because it's really no epidemiological data to suggest it is.

NTP, when they use sufficient animals, both in mice and rats, it comes out negative, okay. Some studies showed some positive, but these studies are related to tumor promotion. And there's very few animals and in a lot of a cases some of the tumors are spontaneous tumors where you're just enhancing, okay.

I just want to prep that before we start. I mean, to me the NTP studies override a lot of those short-term animal studies because they're well-controlled, large number of animals. And they look both at mice and rat.

DR. MARKS: Priya, do you need any clarification of that? Tom said a lot. Obviously, what he's doing is putting it into context why he's going to say it's safe?

DR. SLAGA: Well, I'm saying it's a grass substance.

DR. MARKS: Yes.

DR. SLAGA: But put that aside. I mean, the fact that there is no human epidemiological data to suggest that it's a carcinogen. And I'm drinking my coffee right now, with caffeine in it.

But I just want to put the genotoxicity in perspective. If you look at it the proper way, it comes out negative, okay. And you always have to look at the predominance of evidence. Why there is positivity genotoxicity with no metabolic activation, suggests there's something on the molecule that when it's neutralized in vivo or through metabolic activation, it becomes inactive. Okay?

DR. MARKS: Good. Thanks, Tom.

DR. SHANK: And dose.

DR. SLAGA: And dose, yes.

DR. SHANK: Dose has a big effect too. Tom said it all.

DR. MARKS: Ron Hill, so ingredients are okay. Safe?

DR. SLAGA: Safe.

DR. SHANK: Yeah. We got the sensitization data.

DR. MARKS: Right. We got that in Wave --

DR. SHANK: Six percent for caffeine. I would like to use that for read across for theobromine and theophylline. So, it's safe.

DR. SLAGA: Safe.

DR. MARKS: Safe? Okay.

DR. SHANK: Do we need all of the text on decaffeination if that's the method by which cosmetic caffeine is produced; by decaffeination of coffee beans, okay, but it has nothing to do with the cosmetic ingredient.

DR. HILL: Yes. I flagged that. And also, suggested to give careful attention to if it's being used as an extraction method to capture the caffeine. Even if it's then selling the decaf coffee that's left over, and then that makes sense to go in here. But, yeah, otherwise, how is it relevant. If it's a method for getting the ingredient, then that makes sense.

DR. MARKS: Who can answer that in terms of do we need the decaffeination in the report from a manufacturing point of view?

DR. HILL: I think there are a few places where you can see, from even the way it's written, this is actually used to extract either caffeine or theophylline or theobromine for commercial use. Whether that's relevant directly to cosmetic ingredient, it's hard to say, but almost certainly.

I'd be willing to bet that the vast majority of what's sold is coming out of plant sources as a method of preparation. I don't know how you get at that information. I think it would be near impossible.

DR. EISENMANN: I wouldn't expect production of caffeine for cosmetics to be any different than the production of caffeine for sodas or whatever.

DR. HILL: I wouldn't either. I wouldn't either in this case. I think it's a safe assumption. But if it's just pulling the caffeine out of coffee beans, that isn't relevant, I don't think. Unless anybody disagrees with me.

DR. SHANK: I agree with you.

DR. MARKS: You're going to give Priya feedback in terms of how she can change or just delete those sections?

DR. HILL: I've got some stuff here. And if that isn't enough guidance, she can just get with somebody who's been around the block a few more times.

DR. SHANK: I have it as well.

DR. MARKS: Good. Okay. So Priya, you'll get it from both Dr. Shank and Dr. Hill's notes. Okay, any other comments?

DR. SLAGA: Well, I think the genotoxicity and carcinogenicity should be extensively discussed --

DR. MARKS: Oh, yeah. That's why I mentioned --

DR. SLAGA: -- in the discussion. So that it clarifies where -- if someone looked at the report and saw all the positive, and didn't compare it the right way, I think they would get confused.

DR. MARKS: Yeah. Since this will go out as a tentative report, we'll have another look at both the discussion on why we're not concerned from a carcinogenicity point of view. And then also editing the decaffeination. Other comments? Thanks Tom, for the clarification. I'm not sure if I'd call that a prologue or not, but it was a good intro.

Tomorrow I'll move that a tentative report be issued for these three ingredients with a conclusion they're safe as used. Any other comments?

DR. HILL: I did have, and I plopped these in here so she can look at it later. For example, information about the ADME on caffeine and theophylline and so forth. Drawing from a secondary source, PharmGKB is a beautiful source for genetic information, but not everything that's in there is spot on accurate in terms of some of these pathways and so forth.

And there should be primary literature on caffeine biotransformation, theophylline, theobromine to capture in humans. Because these things have been studied pretty good. And then you can decide whether to leave the other in, but if it was me I would take it out. If you got a primary source, you draw on that, especially if it's a review article that goes back to a few key references.

DR. MARKS: Okay. Any other comments? If not, we'll move on to the next group of ingredients, which we've been waiting all day for, brown algae.

Day 1 – Group 2

DR. BELSITO: Okay, xanthine alkaloids. This is the first time we're looking at the safety assessment. Three xanthine alkaloid ingredients, comprising of caffeine and two structurally related analogs. The function is skin conditioning agents. Where are we? One question I had was what do we do with all the positive genotox studies? Paul said, negative carcinogenicity study; that's his answer.

DR. KLAASSEN: That's my answer also.

DR. LIEBLER: Yeah, I did look at Table 7, the genotox studies. And as you kind of eyeball the table you see probably about two-third negative and a third positive, roughly. The text made it seem like it was more like fifty-fifty. And so that was one point. And it's not to say that the third positive don't need to be addressed.

The other thing I noticed, from looking at the table, is the larger number of in vitro genotox

systems that are not widely used today, or have been superseded by AMES -- for example, E.coli K12. And honestly, I don't know what to do with the data from those. And I was hoping to hear actually what Tom Slaga would think about these. But I didn't review all of these individually.

DR. BELSITO: Okay. Well, Paul's comment was, "Poorly soluble, low tox profile, negative carcinogenicity studies. Theobromine has testicular affect only at high (lethal) doses. Safe as used."

Just a comment on page 17; you have under, other relevant studies, male reproduction impairment, shouldn't that go under DART?

MS. CHERIAN: We were thinking it, but we moved it. And we were wondering if it belonged there or back into DART studies. We just wanted to see where you all would prefer it.

DR. BELSITO: I thought it belonged in the DART Study. No?

DR. LIEBLER: Which one is that?

DR. BELSITO: Page 17, male reproductive impairment with theobromine and theophylline.

DR. LIEBLER: Theophylline.

DR. BELSITO: Theophylline, are under other relevant studies.

DR. LIEBLER: Yeah, reproductive.

DR. BELSITO: Rather than DART.

DR. LIEBLER: Yeah, DART.

DR. BELSITO: And then in Wave 2, we got the 6 percent data that clears caffeine for an HRIPT. So yeah, I thought safe as used.

DR. LIEBLER: I did too. And also, under the other relevant study, why couldn't we put tumorigenicity in with carcinogenicity?

MS. CHERIAN: That was another one for everyone to see it.

DR. LIEBLER: Okay. Curt, xanthine alkaloids, I guess it's used -- I was thinking of like Goodman and Gillman's, you know, pharmacology text, would refer to these as the methylxanthines.

DR. KLAASSEN: Um hmm.

DR. LIEBLER: I don't really care that much. Is there a CIR dictionary or industry-common terminology reason to use xanthine alkaloids? I think methylxanthines is sort of a more current descriptor for this class of compounds.

DR. HELDRETH: We could change it.

DR. KLAASSEN: And, you know, we know a lot about these chemicals. Some of the us have even taken them purposely today. Most of us. And it brings up a nice point in here -- I don't know if you people noticed it -- but dogs don't metabolize theobromine very well. And so there is this story out that, you know, if your neighbor's dog is always barking, you just happen to dump a bunch of Hershey bars across the fence and the dog dies.

DR. LIEBLER: I would never do that, Curt. Just remember caffeine/coffee, theophylline/tea and theobromine/chocolate.

DR. KLAASSEN: Right.

DR. LIEBLER: You got your major food groups right there.

DR. BELSITO: Okay so safe as used. Do we want to change the name of the report to methylxanthines, is that correct?

DR. KLAASSEN: Yes.

DR. LIEBLER: Yup.

DR. BELSITO: Okay. So what discussion points do we need to bring up? The genotox, the positive DART, why we're dismissing them?

DR. LIEBLER: Yeah. I'd like to have a little discussion tomorrow with Tom about the genotox, how to approach that. Because I would really appreciate his perspective on it. He's probably got the most experience with in vitro genotox.

DR. BELSITO: Okay.

DR. LIEBLER: Because it's a strikingly large number of positive studies.

DR. KLAASSEN: Yes. No question.

DR. LIEBLER: Particularly in the endpoints involving like sister chromatid exchange as oppose to mutagenesis. And I don't know what that means, but those appear to have been at the high doses for all of those. And I don't know if that's one of these things where when you get to a toxic concentration in some of those in vitro modules, you get that effect. Or you can get that effect, and it compounds the test. I'd like to hear from Tom, if he could he help us craft the discussion language for that.

DR. KLAASSEN: In general, sister chromatid exchange is very difficult to interpret, they tell

me.

DR. LIEBLER: Okay. We definitely need to talk about that. But I think the other points, obviously, are widespread frequent dietary exposure, lack of carcinogenicity; and these do nothing in skin.

DR. BELSITO: And what about the reproductive effects?

DR. KLAASSEN: That's only at a very high dose.

DR. BELSITO: Okay. But we need to point that out in the discussion.

DR. KLAASSEN: Yes.

DR. BELSITO: Anything else in the discussion? Aerosol? Does it have aerosol use? I don't remember. Incidental inhalation spray, yes. So, the aerosol boilerplate? Anything else? Okay. So, it is brown algae time.

DR. LIEBLER: It's brown algae time.

Day 2

DR. MARKS: This is the first time we've seen these three ingredients, caffeine, theobromine, and theophylline. And these are grass ingredients. Our team, reviewing all the data we had, felt that we could move with a tentative report for safe as used conclusion.

DR. BELSITO: Second.

DR. BERGFELD: Any further discussion? Could you speak into the mic, please?

MS. CHERIAN: I'd just like to clarify what verbiage you wanted to use, discussing why the positive genotox studies were negligible, or not a concern?

DR. SLAGA: I think we need to discuss the genotoxicity and carcinogenicity studies. It's kind of misleading and someone not familiar with the field could misinterpret some of the results. But, I'll just summarize it very quickly.

When they looked at NHANES or any other bacteria, without metabolic activation, there were more positives than negatives. However, when they looked at it with metabolic activation, it was always negative. The end, if they did mammalian mutagenicity in culture -- which most of these studies are clastogenic not mutagenic -- there was a mixture of positive and negative. But, in mammals in vivo it was always negative mutagenicity.

The NTP studies, which used 50 males and 50 females, both mice and rats, the carcinogenicity was negative. IARC even stated it was inadequate evidence. And their inadequate evidence was based on a couple of tumor promotion co-carcinogenicity studies where there was enhancement of a spontaneous tumor, but it was very marginal. Anyway, I think if you take the total data it is negative.

DR. BERGFELD: And that we would put in the discussion.

DR. SLAGA: Yes, we'll have that in the discussion.

MS. CHERIAN: Yesterday someone mention taking out the decaffeination techniques.

DR. SLAGA: The combination?

MS. CHERIAN: Decaffeination.

DR. SLAGA: Detoxification?

DR. MARKS: Decaffeination.

DR. SLAGA: Oh, Decaffeination.

DR. MARKS: Removing caffeine.

DR. SLAGA: Yeah. I don't remember those studies.

DR. SHANK: I was the one who mentioned that. I didn't see the relevance of that information to the use of xanthine alkaloids in cosmetics. We didn't need to have two or three paragraphs on the decaffeination of beans to -- it didn't impact at all on the safety analysis.

DR. HILL: But mixed in with there, I think, were one or two methods where they're actually using it to remove the caffeine to be able to sell the caffeine. I made comments to be sure that you sorted that out. So, if it's actually being used to secure the caffeine, you don't want to write decaffeinated, you'll write caffeine extraction for commercial production.

MS. CHERIAN: Okay.

DR. SLAGA: That would be a method of manufacture?

DR. HILL: That's a method of manufacture, yes, which is, I thought, the section it was in.

DR. BERGFELD: Any other questions? Then we'll call it to question. All those in favor of the safe conclusion? And the discussion? Thank you. Unanimous. Did you have something to say Dan?

DR. LIEBLER: Well, I was going to bring up just a relatively minor point with just the title, and I wanted to see what your take on it was, guys. These are called xanthine alkaloids in this report. I've heard that

term used frequently. But I also, more commonly have heard the term methylxanthine applied. You know, the title on the Goodman and Gilman chapter would be -- the classic pharmacology textbook -- would be methylxanthine. And I thought that maybe methylxanthine might be considered more, sort of, current and appropriate as a title for this report.

DR. BERGFELD: You want to comment on that Bart? And so, you're proposing a change in the title of the document?

DR. LIEBLER: Yes. And I might've missed it with the vote already, but anyway.

DR. BERGFELD: Bart, do we have to vote on that, or anything?

DR. SHANK: How were they listed in the dictionary?

DR. LIEBLER: By name, I think.

DR. HELDRETH: Just by name, yes. It's the panel's prerogative if you feel that the title would be --

DR. SHANK: We all call them methyl, xanthine.

DR. HELDRETH: Right.

DR. BERGFELD: Can I see a shake of heads if agree to change the title of this document? Yes, okay. We can do that by grassroots vote. Okay, any other comments? All right, moving on to the next wonderful ingredient. And Dr. Belsito you're up again for Brown Algae, lucky you.

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ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of the three methylxanthines, Caffeine, Theobromine, and Theophylline, in cosmetics. All of these ingredients are reported to function as skin-conditioning agents in cosmetic products. The Panel reviewed the data relevant to the safety of these ingredients and concluded that Caffeine, Theobromine, and Theophylline are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This is an assessment of the following 3 methylxanthine ingredients in cosmetics:

Caffeine
Theophylline
Theobromine

The ingredients in this assessment are structurally similar to one another, and in fact, are congeners in many plants in which one or more is present. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook (*wINCI; *Dictionary*), all 3 of these ingredients are reported to function as skin-conditioning agents in cosmetic products.[1] Caffeine and Theobromine are also reported to function as fragrance ingredients in cosmetics (Table 1).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Much of the information in this report was discovered in the European Chemicals Agency (ECHA) database[2-4] or was available from the Organisation for Economic Cooperation and Development (OECD) Screening Information Dataset (SIDS) reports.[5,6,] Information from these sources is cited throughout the assessment. Please note that the ECHA website and OECD SIDS documents provides summaries of information generated by industry, and when cited herein, it is those summary data that are incorporated into this safety assessment.

CHEMISTRY

Definition and Structure

Alkaloids are naturally-derived, nitrogen-containing compounds.[7] Methylxanthines are methyl-substituted alkaloid intermediates in the degradation (catabolic pathway) of adenosine monophosphate to uric acid. Caffeine, Theobromine, and Theophylline can be naturally or synthetically derived and are secondary metabolites derived from purine nucleotides.[8] The definitions and structures of the ingredients included in this report are presented in Table 1.[1] The ingredients in this group are all methylated xanthine derivatives and are in that way structurally similar. The placement of the *N*-methyl groups is the only structural difference between these three ingredients (Figure 1).

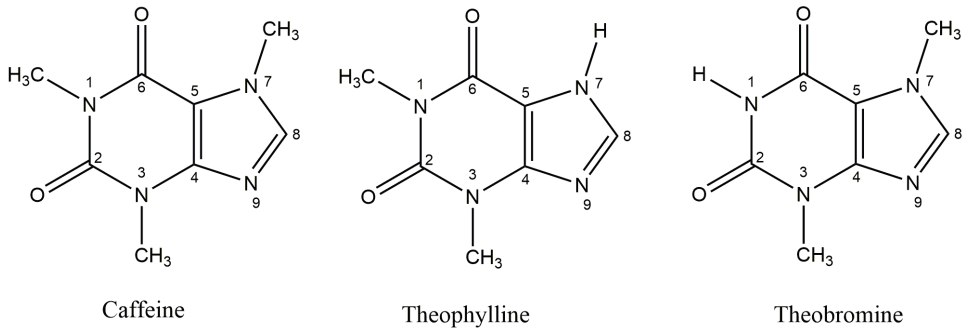


Figure 1. Methylxanthines

Physical and Chemical Properties

The placement of the *N*-methyl groups determines the pharmacological profile of each ingredient.[9] These methylxanthines, notably Theobromine, are slightly to poorly soluble in water (Table 2).[5,10-13,13] However, solubility is significantly increased in boiling water.

Method of Manufacture

Caffeine

The methods below are general to the processing of Caffeine for food or food ingredients, and it is unknown if they apply to cosmetic ingredient manufacture.

Caffeine can be extracted from plants or developed synthetically. Caffeine is most commonly extracted from green coffee beans, tea, or cola nuts.[10] Dichloromethane, ethyl acetate, edible fats and oils, supercritical carbon dioxide and acid-activated carbon can each be used in the Caffeine extraction process.

Theophylline

According to one method, the synthetic manufacturing of Theophylline comprises the reaction of dimethylurea and ethyl cyanoacetate.[10] However, Theophylline is also naturally occurring and can be found in green coffee beans (*Coffea arabica* or *Coffea canephora*), black tea (*Camellia sinensis*), cocoa (*Theobroma cacao*) cotyledon, and dried mate (*Ilex paraguariensis*).

Theobromine

Theobromine can be produced synthetically from 3-methyluric acid, but is not typically derived this way.[10] More commonly, Theobromine is obtained from botanical sources, mainly found in the cocoa bean (*Theobroma cacao*). The extraction of this ingredient typically occurs from the husks of the cocoa beans.

Impurities

Caffeine

The *Food Chemicals Codex* states that Caffeine must contain at least 98.5% pure Caffeine.[14] In addition, Caffeine must also not exceed a 0.5% or 8.5% weight loss upon drying the anhydrous form or hydrous from, respectively, and the residue on ignition must not be more than 0.1%.[14] According to the *British Pharmacopoeia*, Caffeine must not contain less than 98.5%, and not more than the equivalent of 101.5% of 1,3,7-trimethyl-3,7-dihydro-1-*H*-purine-2,6-dione (Caffeine), calculated with reference to the dry substance.[16]

Theobromine

According to the *British Pharmacopoeia*, Theobromine should be at least 99.0% and not more than 101% of 3,7-dimethyl-3,7-dihydro-1-*H*-purine-2,6-dione, calculated with reference to the dried substance.[16]

Theophylline

Specifications for Theophylline indicate that it be at least 97.0% of the active ingredient according to the United States Pharmacopeia (USP), and should not contain less than 99.0% active ingredient according to the *British Pharmacopoeia*.[15,16] Theophylline also must not exceed a 0.5% weight loss upon drying for the anhydrous form, or 7.5 - 9.5% weight loss for the monohydrate form. In addition, the residue on ignition must not be more than 0.15%.

Natural Occurrence

Caffeine

Caffeine can be found naturally in many plants.[10] The most common sources include coffee (*Coffea canephora* and *Coffea arabica*), cocoa beans, tea leaves, and guarana (*Paullinia cupana*). Coffee beans contain, on average, 1.1% Caffeine in green arabica coffee beans (*Coffea arabica*), and 2.2% in green robusta (*Coffea canephora*) beans. Tea plants can contain up to 5% Caffeine, but levels are dependent on seasonal variation, origin, and fertilizers.[10]

Theophylline

Theophylline is commonly found in black tea (*Camellia sinensis*), green coffee beans (*Coffea arabica*), dried mate (*Ilex paraguariensis*), and cacao (*Theobroma cacao*).[10]

Theobromine

Theobromine is also found in the same sources as identified above, but according to one textual authority, is primarily sourced commercially from the cocoa plant (*Theobroma cacao*).[10]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2018 VCRP data, Caffeine is reported to be used in 1033 formulations, 882 of which are leave-on products and 151 rinse-off (Table 3).[17] Theobromine and Theophylline are reported to have much smaller frequencies of use at 5 formulations each. The results of the concentration of use survey conducted by the Council indicate Caffeine also has the highest concentration of use in a leave-on formulation; it is used at up to 6% in non-spray body and hand products.[18] .

Cosmetic products containing Caffeine and Theobromine are applied near the eyes (e.g., at maximum concentrations of 1.5% and 0.0025%, respectively, in eye lotions), and Caffeine is used in products that can result in incidental ingestion (e.g., at 0.2% in lipstick). Caffeine is also used in sprays (e.g., up to 0.2% in face and neck sprays) and powders (e.g., up to 2% in face powders), and these products can result in incidental inhalation. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles < 10 µm compared with pump sprays.[19,20] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[21,22] Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the air.[23-25]

The methylxanthines named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[26]

Non-Cosmetic

Caffeine

Caffeine is most commonly used in beverages, such as coffee, tea and soft drinks. It can also be used as a flavoring agent in baked goods, desserts, and candy.[27] The US FDA has categorized Caffeine as "generally recognized as safe" (GRAS) when included in cola-type beverages at concentrations that are equal to or less than 0.02% [21CFR182.1180]. Dietary Caffeine intake was studied in 37,602 subjects in the US.[28] The 90th percentile intake was 380 mg/d.

A smaller percentage of Caffeine is used in over-the-counter (OTC), FDA-approved, drug products, prescription drug products, and dietary supplements.[27] Caffeine is frequently used in analgesic products, as it may thereby augment the relief of headaches and pain associated with migraines or menstruation. Caffeine is also used in some medications to treat bronchopulmonary dysplasia and apnea in preterm infants. The US FDA has determined that Caffeine is safe as an active ingredient in OTC weight control drug products and when used as an active ingredient in stimulant drug products at dosage limits of 100 - 200 mg every 3 - 4 hours for adults and children ages 12 and up. [21CFR182.1180]

Theobromine

Theobromine is mainly used in the production of Caffeine.[10] The majority of consumed Theobromine is through chocolate/cocoa products. Theobromine is also used as a bronchodilator, diuretic, and vasodilator.

Theophylline

Theophylline is a bronchodilator and can be used to treat lung diseases such as bronchitis, asthma, and emphysema.[10] It can also be used for the relief of biliary colic, and in diuretics.

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

Caffeine

The follicular penetration of Caffeine was studied using a combination of the Franz diffusion cell (FDC) technique, and the follicle closing technique (FCT).[29] Caffeine, 12.5 mg (study design 1) or 2500 mg (study design 2), was dissolved in 100 ml Dulbecco's phosphate-buffered saline (DPBS). Three types of skin samples were used as test barriers: a human reconstituted model, a human heat-separated model, and full-thickness human skin. Study design 1 involved the study of the permeability

of both the reconstituted human epidermis model and the heat-separated human epidermis. The skin samples had a high, low, or no follicular density. These samples were exposed to the test substance for 24 hours. DPBS was used as the receptor medium. Samples taken from the receptor chamber were examined using high-performance liquid chromatography (HPLC). After 24 hours, the skin sample that had a normal follicular density had the lowest absorption of the three types of samples (36.1 ± 9.85%). The skin sample without follicles had absorption of 43.4 ± 9.73%, and the high follicular density sample had the highest percentage of absorption (47.1 ± 9.10%).

In study design 2, the full-thickness skin membranes were mounted in the diffusion cell and subsequently exposed to the FCT. Shunts in the closed follicular pathway samples were blocked with a varnish-wax mixture. The varnish-wax mixture was also applied to the open follicular pathway samples, but only near the follicles preventing shunt blockage. Breast skin was more penetrable than abdominal skin in both types of skin samples (blocked or open follicular orifices). For example, in skin samples with artificially blocked follicular orifices, 3.6 ± 0.59% of the test substance penetrated in the epidermis of the breast skin, while 2.5 ± 0.94% penetrated into the epidermis of the abdominal skin. In both abdominal and breast skin samples, test substance penetration was higher in samples with open follicular orifices. In breast skin with open follicular orifices, 7.9 ± 0.56% of the test substance penetrated into the epidermis, while 3.6 ± 0.59% penetrated into the epidermis of blocked follicular orifice samples.

A solution containing 4 mg/mL Caffeine was applied at a dose of 50 µL/cm^2 to mounted human skin.[30] Six hours after application, skin samples were cleaned with soap and isotonic water. Permeation was measured for 42 hours. Caffeine permeation was reported to be 24 % in cells that were not washed, and 8% in cells that were washed.

Studies have also been done to examine the effect of skin thickness and occlusion on the absorption of Caffeine.[31,32] Human abdominal skin samples were mounted on Dianorm Teflon macro 1 cells or Franz diffusion cells. Occlusion did not have an effect on the dermal absorption of Caffeine. When Caffeine in saline was applied to skin samples varying in thickness, it was observed that the maximum flux of Caffeine was increased with decreasing thickness, but these increases were considered to be non-significant. The amount of Caffeine in the skin membrane was not affected by skin thickness.

Theophylline

Human skin was used to examine the metabolism and absorption of 98% Theophylline.[6] Absorption varied among skin samples. The lowest reported absorption was 3.6%, while the highest was 33.4%. Diffusion ranged from 2.2 - 7.7%. Approximately 0.2 - 4.6% of the applied substance was metabolized, and more than 60% of the metabolites diffused through skin samples. Reported metabolites were 1,3,7-trimethyluric acid, 1,3-dimethyluric acid, and 3-methylxanthine. The amount of metabolites varied per skin sample.[6]

A flow-through in vitro diffusion system was used to study percutaneous absorption of 8-[^{14}C]-Theophylline (radiolabeled) through 5 different samples of excised human skin.[33] Donors of the skin samples differed by age, sex, and ethnicity. Eagle's medium containing gentamicin sulfate and 10% fetal bovine serum was continuously perfused along the well. Receptor fluid was aerated with 95% O_2; 5% CO_2 and pumped underneath the skin's surface at a rate of 3 mL/h. The diffusion area was 1.0 cm^2. Skin cells were exposed to a solution of Theophylline (6.8 and 306.8 µg/cm^2) and receptor fluid was collected hourly for 20 hours. The percentage of the applied dose that diffused through the skin was similar between all skin samples, with a range of 2.8 - 7.8%. The percentage of the applied dose that was absorbed varied greatly between skin samples (3.6 - 33.4%). The metabolites were estimated by thin-layer chromatography. Between 0.2 ± 0.1% - 4.6 ± 0.2% of the applied doses were metabolized, and over 60% of the total formed metabolites penetrated through the skin. The metabolites that were observed were 1,3,7-trimethyluric acid, 1,4-dimethyluric acid, and 3-methylxanthine.

Absorption, Distribution, Metabolism, and Excretion (ADME)

Caffeine

Caffeine is readily absorbed through body membranes. The typical half-life of Caffeine in humans, after oral consumption, is 2.5 - 4.5 hours, but the time is increased during the third trimester of pregnancy and in women taking oral contraceptives.[5] Caffeine is widely distributed throughout body tissues, and is metabolized by hepatic enzyme systems only. The majority of Caffeine, 70 - 80%, is metabolized through 3-*N*-demethylation to paraxanthine, carried out by the liver enzyme CYP1A2.[34] Approximately 7 - 8% of Caffeine is metabolized by 1-*N*-demethylation to Theobromine, while 7-*N*-demethylation to Theophylline also accounts for about 7 - 8% of Caffeine metabolism products.[34] The remaining Caffeine is metabolized through C-8 hydroxylation, resulting in the formation of 1,3,7-trimethyluric acid. However, outside of the liver, no significant metabolism of Caffeine occurs in other organs. The majority of Caffeine is excreted via urine (> 95% in humans).[5]

Theobromine

Theobromine can be seen in the body as a result of Caffeine metabolism. In humans, Theobromine is metabolized in the liver into xanthine (i.e. fully demethylated), and further metabolized into methyluric acid, facilitated by CYP1A2 and CYP2E1.[35] Some mammals, such as cats and dogs, metabolize Theobromine at a slower pace. The lethal dose for Theobromine in dogs is reported to be 100-500 mg/kg bw. In humans, Theobromine is metabolized at a faster rate. The approximate half-life of Theobromine is 7.1 ± 0.7 hours in humans, and approximately 18 hours in dogs.[36] An estimated 10% of Theobromine is excreted via urine unchanged, while the rest is excreted as metabolites.

Theophylline

Theophylline is extensively metabolized in the liver (up to 90% in adults). This ingredient undergoes *N*-demethylation and 8-hydroxylation via cytochrome P450 1A2 into 1-methlxanthine, 3-methylxanthine, and 1,3-dimethyluric acid.[37] Metabolites are excreted into bile and eliminated via urine.[6] Only 7 - 12% of orally ingested Theophylline is excreted unchanged in urine. After review of the kinetics and metabolism of Theophylline in rats, the International Agency for Research on Cancer (IARC) concluded that Theophylline is quickly and completely absorbed from the digestive tract, readily crosses placental boundaries, can be distributed in breast milk, and is dispersed throughout all organs, with the exception of adipose tissue.[10] In plasma, about 50% of the ingested Theophylline is bound to plasma proteins. The elimination half-time of Theophylline is approximately 3 - 11 hours in adults, which can be shortened or prolonged by certain medications and behaviors, such as smoking and oral contraceptives.

Animal

Oral

Theobromine

Theobromine was given to rabbits orally in doses of 1 and 5 mg/kg bw.[10] Rabbits given these doses showed complete gastrointestinal absorption. Rabbits given high doses (10 - 100 mg/kg bw) displayed a reduction in absorption rate. Despite the reduced absorption rate, absolute bioavailability of the given Theobromine reached 100%.

A single dose of 15 - 50 mg/kg bw of Theobromine was given to dogs orally.[10] Peak plasma concentrations were observed within 3 hours of dosing. Dogs given a high dose (150 mg/kg bw) had peak plasma concentrations 14 - 16 hours after dosing, implying slowed intestinal absorption.

Human

Dermal

Caffeine

A non-occlusive 2.5 cm^2 patch of 4 µg Caffeine in acetone was applied to the forearms of people from the age of 22 - 40 or 65 - 86.[30] After 24 hours, the site was washed and urine was collected. For the 22 – 40 age group, the dose recovered in urine was 32.1 ± 4.2%. For the 65 - 86 age group, the dose recovered was 61.8 ± 5.4%.

The role of hair follicles in the dermal absorption of Caffeine was studied.[38] A solution that contained Caffeine (2.5%), ethanol, and propylene glycol was applied to the skin of six male subjects who had not ingested Caffeine for at least 48 hours before testing. The average hair follicle density was 20 - 32 follicles/cm^2. Hair was clipped down to 0.5 mm in the 25 cm^2 application area of the chests of volunteers. For the first test, a microdrop of varnix-wax mixture was applied beside each hair follicle orifice, and 2 mg/cm^2 of the Caffeine solution were applied to the test area of each subject for 8 h (formulation allowed to evaporate); subjects were not allowed to shower or bathe for 72 hours. A blood sample was taken at 5, 10, 20, and 30 minutes, and 1, 2, 5, 8, 24, and 72 hours after application. The same procedure was repeated in the same test areas after 3 more days of a Caffeine-free diet, but with each hair follicle orifice blocked with a microdrop of the varnish-wax mixture. Caffeine was extracted from the serum samples with dichloromethane. An aliquot of each serum sample was measured by a surface ionization mass spectrometry (SI/MS) technique.

When hair follicles were left open, the average Caffeine levels in the blood 5 minutes after application were 3.75 ng/ml, and when hair follicle orifices were blocked, Caffeine was not detected in the blood until 20 minutes after application. After 20 minutes, the average amount of Caffeine detected in the blood in the blocked follicle group was 2.45 ng/ml, compared to an average blood concentration of 7.57 ng/ml in the open follicle group. The highest concentration in the open follicle group was approximately 11.75 ng/ml an hour after application, while the highest concentration in the blocked follicle group was 6.65 ng/ml at 2 hours after application. After 72 hours, no Caffeine was detected in the blood serum in either test group.

Oral

Caffeine

An absorption study was performed using four male subjects given 0, 1, 5 and 10 mg/kg per oral. Plasma peak was reached at 47 ± 5 minutes. The mean plasma concentration was 8.3 ± 0.1 µg/mL, with an apportion rate constant of 6.3 ± 1.9 per hour and elimination rate constant of 0.11 ± 0.02 per hour. Ninety-nine percent of the given dose was absorbed. The metabolites 3,7-dimethylxanthine, 1,3-dimethylxanthine, and 1,7-dimethylxanthine were measured in the plasma. Approximately 85% of the administered dose was recovered in the urine within 48 hours. The main metabolites excreted were 1-methyluric acid, 7-methylxanthine, and 1,7-dimethylxanthine.[5]

Nine pregnant and 4 post-partum women were exposed to Caffeine.[5] No other study details were provided. Results showed a significant prolongation of Caffeine elimination in the pregnant women. Post-partum women showed normal rates of Caffeine elimination.

Thirteen males, 9 females not on oral contraceptive steroids (OCS), and 9 females taking OCS were examined for the disposition and elimination of Caffeine.[5] The elimination half time of Caffeine was significantly longer in women taking OCS. The average elimination half time for women taking OCS was 10.7 hours, versus 6.2 hours in women not taking OCS. Women on OCS had a total plasma clearance of 0.79 mml/min/kg, and free clearance of 1.12 ml/min/kg. Women not taking OCS had a total plasma clearance of 1.3 mml/min/kg and free clearance of 1.97 ml/min/kg. Kinetic parameters were similar in men and women, with the exception of volume of distribution, which was significantly higher in women.

A similar study was done using 9 females who had been taking a low-dose estrogen-containing oral pill for at least 3 months, compared to 9 females who did not take OCS.[5] Each subject was given an oral dose of 162 mg of Caffeine. In subjects who took the low-dose estrogen-containing oral pill, the elimination half-life of Caffeine was 7.88 hours, the plasma clearance was 1.05 ml/min/kg, and peak plasma concentration was 3.99 µg/ml. In subjects who did not take OCS, the elimination half-life was 5.37 hours, plasma clearance was 1.75 ml/min/kg, and the peak plasma concentration was 4.09 µg/ml.

Theophylline

The distribution of Theophylline into breast milk was studied in five women. On average, less than 10 percent of the mother's Theophylline intake was distributed into breast milk.[6]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute toxicity studies summarized below are described in Table 4.

Acute dermal toxicity studies were performed in which both Caffeine and Theophylline, in olive oil, were applied under a semi-occlusive patch for 24 h to rats. The LD_{50} was > 2000 mg/kg bw for both test substances.[5,6]

Numerous acute oral toxicity studies were performed with Caffeine. The lowest reported LD_{50}s in mice and rats are 127 mg/kg bw (vehicle not specified) and 192 mg/kg bw aqueous (aq.), respectively.[5] In other species, LD_{50}s of Caffeine were 230 mg/kg bw (guinea pigs and hamsters), 224 mg/kg bw (rabbits), and 240 mg/kg bw (dogs). For Theobromine, the reported LD_{50}s in rats and dogs (vehicle not specified) were 950 and 300 mg/kg bw, respectively.[10] For Theophylline, the lowest LD_{50}s reported for mice, rats, guinea pigs, and rabbits are 235, 225, 183, and 350 mg/kg bw, respectively.[6]

Acute inhalation studies were performed in rats on Caffeine and Theophylline mixed with a hydrophobic fumed silica.[5,6] The LC_{50} following aerosol exposure or Caffeine mixed with 2% of a hydrophobic fumed silica was 4.94 mg/L. Following a dust aerosol exposure to Theophylline mixed with 1% and 2% silica, an LC_{50} of > 6.7 mg/L was established.

Short-Term, Subchronic, and Chronic Toxicity Studies

Details of the short-term, subchronic, and chronic toxicity studies are provided in Table 5.

Repeated dose oral toxicity studies were performed with the methylxanthines. In a study in which rats were given diets containing 0.5% Caffeine or 0.8% Theobromine for 7 or 8 weeks, treated rats of both groups displayed statistically significant decreases in thymus weights and vacuolar degeneration of spermatogenic cells; the effects were more severe with Theobromine.[39] In 90-day studies in which mice and rats were dosed with Caffeine in drinking water, the no-observable-adverse effect-levels (NOAELs) were 167 and 179 mg/kg bw/day in male and female mice, respectively, and 151 and 174

mg/kg bw/day in male and female rats, respectively; the highest doses administered in these studies were 167 and 179 mg/kg bw/day to male and female mice, respectively, and ~272 and 287 mg/kg bw/day in male and female rats, respectively.[10]

In a study in which immature and mature rabbits were fed a diet containing ≤ 1.5% Theobromine for 20 or 120 days, respectively, only 25% of the immature rabbits survived until study termination in each group, including controls.[40] Rabbits placed in groups 0, 1, 2, and 3 were given doses of 0, 0.5, 1, and 1.5%, respectively. In mature rabbits, on day 30 of treatment, group 1 gained 6% of their original body weight, while groups 2 and 3 lost an average of 5.9 and 18.1%, respectively. By day 30, 0/8 animals died in group 1, 5/8 animals died in group 2, and 4/8 animals died in group 3. By day 120, 4/8, 7/8, and 7/8 animals died in groups 1, 2 and 3, respectively. In immature rabbits, mortality was clearly dose-dependent. Lesions were apparent in the thymus in both immature and mature rabbits. Mature rabbits displayed severe pulmonary congestion and slight to moderate hydropericardium. Slight ascites were also present in the liver, as well as kidney congestion and redness of the gastro-intestinal mucosa. In immature rabbits, lesions were similar to mature rabbits. In addition, edema of the thymus and extensive hemorrhaging was present.

A 16-day gavage study was performed using mice given Theophylline in corn oil at doses as high as 400 mg/kg bw once daily.[41] All females (5/5) and 3/5 males dosed with 400 mg/kg bw died on day 1. A similar study was performed using rats. Animals were given up to 400 mg/kg Theophylline in corn oil via gavage once per day for 16 days; all male and females died after exposure of 400 mg/kg Theophylline given once daily and 9/10 animals died after exposure to 200 mg/kg Theophylline given twice a day. A 16-day feed study was also performed using groups of 5 rats/sex. Rats were given Theophylline at concentrations of up to 8000 ppm. All rats survived; the final mean body weight was statistically decreased in rats given 8000 ppm. In a study in which Theophylline was given to 10 mice/group/sex via diet at a maximum concentration of 4000 ppm for 14 weeks, statistically significant decreases in mean body weights and increases in leukocyte, segmented neutrophil, and lymphocyte counts were recorded at the 2000 and 4000 ppm levels. In a study in which 10 mice/group/sex were given Theophylline in corn oil for 14 weeks via gavage at a maximum dose of 300 mg/kg bw, a statistically significant decrease in mean body weights of male mice given 150 or 300 mg/kg bw was apparent.

Two-year studies were also performed.[41] Theophylline in corn oil, at up to 150 mg/kg bw, was given to 50 mice/group/sex for 2 years via gavage. Administration resulted in decreases in survival, and decreases of body weights of male mice dosed with 150 mg/kg and female mice dosed with 25 mg/kg. Final body weights of female mice dosed with 75 mg/kg were significantly less than the control groups. In a 2-year gavage study using 50 rats/sex/group, animals were dosed with up to 75 mg/kg bw Theophylline in corn oil. No statistically significant differences in the survival between treated and control groups were seen. Dosed rats had a statistically significant decrease in final mean body weights compared to the control group. In addition, chronic inflammation of the mesenteric arteries was increased in male rats given 75 mg/kg bw.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Details of the developmental and reproductive toxicity studies summarized below are provided in Table 6.

Studies were performed evaluating the developmental toxicity of Caffeine in mice. Mice dosed with up to 39 mg/kg/d of Caffeine in drinking water did not display any consistent dose-related effects on fertility, litter size, offspring weight, sex ratio, or fetal abnormalities.[42] Details of this study were not provided. When mice were given up to 350 mg/kg Caffeine on days 8 - 18 of gestation via gavage, animals did not display differences in embryonic resorption, growth, skeletal development, or terata, compared to control groups. No other details of this study were provided. [5] In different studies, mice displayed reduced maternal body weight gain (100 - 400 mg/kg/d Caffeine; gestation day (GD) 6 - 15) a reduction of live male pups/litter, female body weight, and adjusted seminal vesicle weight (up to 88 mg/kg/d; 21 - 35 weeks). When male mice were dosed with up to 1000 mg/kg/d Caffeine for 100 or 140 days (before mating) via drinking water and mated with untreated females, an increase of preimplantation loss and resorptions were seen.

Multiple reproductive toxicity studies were performed using rats. Dose-dependent maternal weight gain decreases were reported when rats were given Caffeine in doses of 10 - 40 mg/kg/d (GD 1 – 20; 12 females/group; oral administration), 40 - 80 mg/kg/d (GD 1 – 19; 20 females/group; oral administration), 10 - 100 mg/kg/d (GD 6 – 20; administered as single bolus or 4 divided doses), 100 mg/kg/d (GD 7 - 19, 7 - 16, 16 – 19, or day 19; oral administration), and 70 – 2000 mg/kg (GD 0 – 20; 61 females/group; drinking water).[5] In a study in which 61 Osborne-Mendel rats were treated with up to 125 mg/kg Caffeine via gavage on GD 0 - 19, 6/61 females died at the highest dose level. At the 80 mg/kg dose level, 2 litters were resorbed, and at the 125 mg/kg dose level, 4 litters were resorbed. No other details regarding this study were given. Resorption was also noted at concentrations of 1500 and 2000 ppm in a different study involving 61 female rats given Caffeine in drinking water on days 0 -20 of gestation. At these doses, decreased implantation efficiency, and a decreased number of viable fetuses was also noted.

A 23% average sperm radius decrease, as well as a decrease in sperm motility and velocity was observed in 8 rats given 25 mg/kg Caffeine via gavage for 17 weeks.[5] No teratogenic effects were observed when rabbits were given up to 125 mg/kg of Caffeine via gavage on gestation days 6 -16. When 40 pregnant monkeys (*Macaca fascicularis*) were given Caffeine (10 -

15, 25 - 30, 25 - 30 mg/kg/d) in drinking water eight weeks before pregnancy to several months after pregnancy, a dose-related increase in stillbirths, decreased maternal weight, and miscarriages were present.

Female Sprague-Dawley rats were fed diets containing up to 99 mg/kg bw/d Theobromine during GD 6 – 19.[10] No maternal toxicity was reported, but a slight decrease in fetal body weight as well as an increase in skeletal variation frequency was apparent. Male rats were fed Theobromine in the diet for 28 days at concentrations of 0.2 - 1.0%.[10] At the 0.8% level, rats displayed severe testicular atrophy. At the 0.6% level, rats exhibited seminiferous tubular-cell degeneration. Testicular changes occurred only at lethal concentrations. A similar study was performed using a concentration of 0.6% Theobromine in the diet for 28 days. No testicular atrophy was reported.

Rabbits given up to 63 mg/kg bw Theobromine via feed displayed little to no maternal toxicity. Details regarding dosing procedures were not provided. In a different study, female rabbits were given up to 200 mg/kg bw Theobromine via gavage on GD 6 - 29. At the 200 mg/kg dose level, 40% of the dams died, but little to no maternal toxicity was reported in rabbits given 25 - 125 mg/kg.

Theobromine was fed to male dogs at doses of 25, 50, 100, or 150 mg/kg/d for one year.[10] No testicular atrophy was seen at any dose level.

Up to 300 mg/kg bw Theophylline was given to male B6C3F1 mice via gavage for 13 weeks.[10] Mice that were dosed with 300 mg/kg bw/d displayed a slight but statistically significant decrease in testicular weight. When dosed with 150 mg/kg bw/d or less, no effects were observed.

CD1 mice given 0.2% Theophylline on GD 6 - 15 via drinking water displayed an increased percentage of resorptions/litter and a reduced number of live fetuses.[41] Dose-related decreases in the number of live pups/litter was also reported in a different study in which mice (sex not stated) were given 0.3% Theophylline in feed for one week before mating and during 13 weeks of cohabitation.[10] A statistically significant decrease in testicular weight was observed at the 300 mg/kg bw dose level in male mice dosed for 13 weeks via gavage or diet. No effects were reported at the 150 mg/kg bw dose level. The group treated with 0.4% Theophylline via drinking water on GD 6 – 15 displayed decreases in litter size and fetal weight.[41]

Male Holtzman rats were given Theophylline at a concentration of 0.5% for 19 weeks (method of administration not provided).[10] Eighty-six percent of the treated rats displayed testicular atrophy. A similar study was performed in male rats given 0.5% Theophylline for 14 to 75 weeks (method of administration not provided).[41] Bilateral testicular atrophy and atrophic changes in the epididymis, prostate gland, and seminal vesicles was noted.

Male Fischer 344 rats were given up to 300 mg/kg/d Theophylline for 13 weeks.[10] A statistically significant decrease in testicular weight was reported after dosing by gavage with 150 mg/kg bw/d, but no effects were reported when animals were dosed with 75 mg/kg bw/d or less.

GENOTOXICITY

Details of the genotoxicity studies summarized below are provided in Table 7.

In Vitro

Caffeine

Multiple tests were available regarding the genotoxicity of Caffeine. Ames tests performed without metabolic activation, at concentrations as low as 1000 µg/mL, yielded positive results.[5] In Ames tests performed in *Salmonella typhimurium* with metabolic activation at doses up to 6000 µg/plate, only negative results were reported. However in a different study, positive results were observed in *Escherichia coli* at concentrations as low as 6000 µg/well. In a different study, *S. typhimurium* cells were exposed to Caffeine with and without metabolic activation at concentrations as high as 20 mM.[45] No mutations were observed. Bacterial gene mutation assays performed on Caffeine without metabolic activation yielded negative results in concentrations as high as 20,100 µg/plate (*S. typhimurium*). All chromosomal aberration studies performed without metabolic activation yielded positive results at concentrations as low as 194 µg/mL (Chinese hamster cell line (CHL)). Sister chromatid exchange (SCE) assays performed on Caffeine with metabolic activation yielded both positive and negative results. Positive results were apparent with concentrations as low as 19 µg/mL (human xeroderma pigmentosum cell lines) and negative results were seen with doses as high as 400 mg/kg bw (Chinese hamster cells).[43] DNA damage and repair assays performed without metabolic activation yielded negative results in Chinese hamster lung fibroblast V79 (V79) cells at up to 5826 µg/mL and positive results in *E. coli* at concentrations as low as 1550 µg/mL.[5] A DNA damage and repair assay performed with metabolic activation on *E. coli* resulted in bacterial growth and a minimal inhibitory concentration range of 187 - 1125 µg/plate. All micronucleus tests performed on Caffeine without metabolic activation, concentrations as low as 5 µg/mL in human hepatoma cells, resulted in positive results.

The majority of cytogenetic tests performed using Caffeine yielded positive results at concentrations as low as 0.05 µg/mL in both human peripheral blood leukocytes and human embryonic fibroblasts.[5] However, negative results were seen in studies at concentrations as high as 160 µg/mL (rat MCT1cells). A cytogenetic test performed with metabolic activation resulted in an increase in chromosome breaks at a concentration of 2.0 mg/mL in the presence of S-9 (human diploid fibroblasts). In

cytogenetic studies in which the use of metabolic activation was not noted, positive and negative results were seen (HeLa cells). In one study, Caffeine yielded positive results at a concentration of 4850 µg/mL (CHL). Caffeine did not promote breaks or growth effects in two studies using immortalized human cervical cancer cells (HeLa) cells at doses as high as 5826 µg/mL.[44] Multiple mammalian cell gene mutation assays performed without metabolic activation yielded negative results in concentrations as high as 194 µg/mL in V79 cells. An unscheduled DNA synthesis assay performed without metabolic activation on human lymphocytes obtained from both healthy donors and patients with systemic lupus erythematosus (SLE), yielded negative results at concentrations as high as 583 µg/mL. No inhibition of DNA repair in normal lymphocytes or reduction of DNA repair in SLE cells were reported. In a human lymphoblast mutation assay, Caffeine was considered to be non-mutagenic when dosed with concentrations as high as 20 mM.[45]

Theobromine

S. typhimurium (strains not specified) cells were treated with Theobromine at concentrations of 0.5 - 5000 µg/plate in an Ames test performed with and without metabolic activation.[46] Results were negative. Negative results were also obtained in a chromosomal aberration assay (Chinese hamster ovary (CHO) cells; 0-1000µg/mL Theobromine) performed with and without metabolic activation. When CHO cells and cultured lymphocyte cells were used in a sister chromatid exchange assay performed without metabolic activation (up to 1000 µg/mL Theobromine), results were positive. When tested with metabolic activation, results were equivocal and not dose-related.

Theophylline

Negative and positive results were also seen in genotoxicity studies involving Theophylline. In one study involving hamster V79 cells and human cells, positive results were only obtained when testing was done without metabolic activation.[47] No DNA damage was observed when human cells were dosed with up to 20 mg/mL Theophylline. When V79 cells were exposed to up to 20 mg/mL Theophylline without metabolic activation, weak mutagenic effects were present. However, in the presence of metabolic activation, negative results were yielded. An Ames test using *E. coli* in concentrations as low as 150 µg/mL produced negative results.[6] Another bacterial gene mutation assay using *E. coli* also yielded negative results, however information on dosing was not provided. Cytogenetic assays performed without metabolic activation also had conflicting results, with positive results at concentrations as low as 500 µg/mL in human lymphocytes and negative results at concentrations as high as 1800 µg/mL in human lymphocytes. A hypoxanthine-guanine phosphoribosyltransferase (HGPRT) assay performed with and without metabolic activation produced negative results (V79 cells; up to 9 µg/mL). A mouse lymphoma assay also yielded negative results when L5178Y *tk* +/- cells were dosed with up to 5 mg/mL. SCE assays in Chinese hamster Don-6, human diploid fibroblast (dose not stated) and CHO cells (18-360 µg/mL) yielded positive results. Negative results were apparent in a SCE assay performed without metabolic activation using human lymphocytes at concentrations up to 100 µg/mL.

In Vivo

Caffeine

Assays testing the cytogenetic potential of Caffeine were performed using rats as well as human volunteers. When 30 rats were rats dosed with 46 mg/kg/d via feed for 117 weeks, there were no statistically significant differences compared to control rats.[5] In a different cytogenetic assay involving humans, 9 volunteers were given 800 mg of Caffeine in tablet form each day. No significant increase in chromosome damage was seen.

Multiple dominant lethal assays performed on mice yielded negative results at doses as high as 200 mg/kg/d Caffeine.[5] However, positive results were seen when a micronucleus assay was performed on Chinese hamsters.[5] Induction was apparent at the 300 mg/kg/d dose level.

An SCE assay was performed in Chinese hamsters given a single dose of up to 300 mg/kg Caffeine.[5] Bromodeoxyuridine (BrdU) tablets were implanted two hours before Caffeine dosing. A slight increase in SCEs was apparent at the 150 mg/kg dose level and higher. A similar SCE assay was performed using mice given up to 1000 mg/kg Caffeine each day for 5, 10, or 15 days. The frequency of SCEs increased in a time-dependent manner.

Theophylline

The majority of in vivo genotoxicity studies involving Theophylline yielded negative results.[6] However, when hamsters were given up to 600 mg/kg in an SCE assay, positive results were noted. In a micronucleus assay in which mice were given up to 150 mg/kg Theophylline for 14 weeks via gavage, no increase in micronucleated cells were seen. Similar results were observed in a micronucleus assay in which mice were given up to 850 mg/kg bw/d Theophylline in the diet for 14 weeks. Negative results were also seen in a cytogenetic assay in which rats were given up to 230 mg/kg bw/d Theophylline via oral feed for 75 weeks.

CARCINOGENICITY STUDIES

IARC concluded there is inadequate evidence for the carcinogenicity of Caffeine in experimental animals and in humans; IARC had an overall evaluation that Caffeine is not classifiable as to its carcinogenicity to humans.[10]

Details of the carcinogenicity studies summarized below are provided in Table 8.

Sprague-Dawley rats (50 rats/sex) were given Caffeine (up to 2000 ppm) for 104 weeks via drinking water.[5] No statistically significant difference between the incidences of tumors in control and treated rats were apparent except for mammary fibroadenomas. The incidence of mammary fibroadenomas showed a significant inverse dose-response relationship. Fifty percent of the control animals displayed mammary fibroadenomas, while 26% rats dosed with the highest concentration showed mammary fibroadenomas.

Forty female Wistar rats were given a 0.2% (2000 mg/L) Caffeine solution as their drinking fluid for 12 months.[48] Twenty-two out of the 40 treated rats had pituitary adenomas, while 9 out of the 30 untreated rats had pituitary adenomas. Pituitary hyperplasia was seen in 5/40 treated rats, and in 1/30 untreated rats.

Three groups each of 50 male and 50 female Wistar rats were maintained on a basal diet and given either tap-water (controls), a 0.1% solution of synthetic Caffeine (purity 100%), or a 0.2% Caffeine solution as the drinking fluid for 78 weeks.[10] Rats that survived were then given tap-water only for 26 more weeks. A total of 65/96 untreated rats had developed tumors. In the 0.1% solution group, 75/88 rats were tumor-bearing, and in the 0.2% group, 55/94 rats were tumor-bearing.

National Toxicology Program (NTP) studies regarding the carcinogenic potential of Theophylline were found. Theophylline was not carcinogenic in rats and mice when administered at up to 150 mg/kg bw/d in male B6C3F1 mice and up to 75 mg/kg in male and female Fischer 344 rats.[41] Authors of an NTP study concluded there was no evidence of carcinogenic activity based on 2 year gavage studies performed on F344/N rats and B6C3F1 mice.

No information regarding the carcinogenicity of Theobromine was found in the published literature.

Co-Carcinogenicity

Osborne-Mendel rats were given a diet consisting of 0.5% Caffeine, Theobromine, or Theophylline alone or with sodium nitrite.[49] In the group fed both nitrite and the methylxanthines, the mortality rate was significantly increased. Food intake was decreased in Caffeine-treated rats, and a further reduction of food intake was noted in rats treated with both Caffeine and sodium nitrite. Food intake was not affected in rats treated with Theobromine alone, but rats treated with both Theobromine and sodium nitrite displayed significant decreases in intake. There was no effect on feed consumption in rats treated with sodium nitrite only. Terminal mean body weights were decreased in all treated rats. The addition of sodium nitrite created a slight increase in the reduction of body weight. No neoplastic or pre-neoplastic lesions were observed.

In a different study, groups of 50 rats were given 100 mg/kg *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN).[5] After administration of BBN, rats were given 110 mg/kg/d Caffeine for 21 months via drinking water. Groups received either Caffeine-treated water only, Caffeine-treated water and phenacetin incorporated into their diet, or phenacetin alone. No carcinogenic potential was seen in rats given only Caffeine treated water, but an increase in tumor incidences was seen in rats given Caffeine and phenacetin combined. The increase in tumor incidences was greater in rats treated with both Caffeine and phenacetin versus rats treated with phenacetin alone.

Groups of 12 - 20 Fischer 344 rats were given one 200 mg/kg diethylnitrosamine (DEN) intraperitoneally.[5] After a 2-wk period, the animals were then given 90 mg/kg/d Caffeine via drinking water for 6 weeks. Control rats were given a DEN injection only. Carcinogenetic potential was evaluated by comparing the number and area of glutathione-*S*-transferase placental form positive (GST-P+) foci in the liver of treated rats with control rats. There was no increase in the number and area of GST-P+-foci in treated animals.

Tumor Promotion

Groups of mice were given an injection of 7,12-dimethylbenz[a]anthracene (DMBA) once a week for 6 weeks.[50] The test substance (250 and 500 mg Caffeine/L of drinking water) was then given to groups of 54 - 55BD2F1 or 37 – 42 C3H mice, one week after the end of DMBA injections. In BD2F1 mice, the low dose of the test substance revealed a 20% increase of mammary carcinoma multiplicity, while the high dose revealed a 40% increase. C3H mice had an increase of mammary carcinomas of 13% at 250 mg/L Caffeine, and an increase of 117% at 500 mg/L Caffeine.

DERMAL IRRITATION AND SENSITIZATION

Irritation

Animal

Caffeine

A study was performed in which 500 mg of a test substance containing 50% Caffeine was applied to the skin of three White Vienna rabbits under a semi-occlusive patch for 4 hours.[5] The irritation index was 0.

Theophylline

Doses of 2 g/kg bw Theophylline in olive oil were applied to Wistar rats via a semi-occlusive patch for 24 hours.[6] After patch removal, sites were washed. No deaths or substance-related effects were reported.

Semi-occlusive patches containing 0.5 g of 50% aqueous Theophylline were applied to two White Vienna rabbits.[6] Patches remained on the skin for 4 hours. After removal of patches, sites were washed and scored. Four hours after patch removal, very slight reddening of the skin was present in both rabbits. No other signs of irritation were reported. The reported irritation index was 0.0.

One male and two female White Vienna rabbits were given a 0.5 g dose of a test substance consisting of a 50% aqueous solution of Theophylline under a semi-occlusive patch.[6] Patches were kept on for four hours. After patch removal, the exposed area was washed. Sites were scored 4, 24, 48, and 72 hours after removal. The female rabbits had slight redness four hours after patch removal. No other signs of irritation were reported.

Sensitization

Animal

A local lymph node assay was performed (LLNA) using concentrations of 0, 0.5, 2, and 5% Caffeine in an ethanol:water (70:30) vehicle.[51] The assay was performed according to OECD guideline 429. Four female mice (CBA) per dose level were used. No other study details were provided. No adverse effects were observed.

Human

A human repeated insult patch test (HRIPT) was performed on 105 test subjects.[52] The test substance (a body product containing 6% Caffeine) was applied (20 µL) to the backs of the subjects under an occlusive patch. Applications occurred 9 times over a period of 3 weeks during the induction phase. After a 2 week rest period, the test substance was applied to the original test site and to a previously untreated test site, under an occlusive patch. No allergic reactions were noted during the induction or challenge phases.

OCULAR IRRITATION STUDIES

Caffeine

Undiluted Caffeine (0.1 mL) was instilled into the eyes of three rabbits.[5] Average irritation indices were 0.9 (corneal opacity), 0 (iritis), 1.6 (conjunctival erythema) and 0.6 (conjunctival edema). Irritation was observed in all three animals within the first 24 hours, and only 1 animal showed minimal corneal and conjunctival irritation by day 8. The test substance was considered to be non-irritating.

Theophylline

Fifty-one mg of undiluted Theophylline (0.1 mL) was instilled into one eye of 3 male White Vienna rabbits.[6] Eyes were not rinsed. Tested eyes were examined 1, 24, 48, and 72 hours and 8 days after application. Minimal corneal opacity was reported for 1 - 2 rabbits each day, for up to 8 days. Minimal to well-defined redness and swelling was observed in the conjunctiva in all tested rabbits for up to 3 days. By day 8, only one animal showed conjunctival redness (grade 2), corneal opacity (grade 1) and keratitis. The irises of test animals were unaffected. Mean irritation indices for corneal opacity, iritis, conjunctival redness, and conjunctival swelling were 0.6, 0.0, 1.8, and 0.6 respectively. The test substance was considered to be non-irritating.

EPIDEMIOLOGICAL STUDIES

A summary of epidemiological studies can be found in Table 9. Case-control studies regarding the carcinogenic potential of Caffeine through the intake of coffee (≥ 7 cups/day) provided no evidence of a potential breast cancer risk.[5,10,53] Multiple studies confirmed this result. Different studies showed no or irregular association between Caffeine intake via beverages and cancer in the reproductive organs or pancreas.[46] Cohort studies showed no correlation between risks for bladder cancer and

Caffeine consumption through beverages, while a number of case-control studies showed a weak positive association between bladder cancer and coffee intake.[53]

SUMMARY

The safety of three methylxanthines as used in cosmetics is reviewed in this CIR safety assessment. According to the *Dictionary*, Caffeine, Theobromine, and Theophylline are reported to function as skin-conditioning agents, and Caffeine and Theobromine also are reported to function as a fragrance ingredient.

According to 2018 VCRP survey data, Caffeine is reported to be used in 1033 formulations, 882 of which are leave-on products and 151 are rinse-off. Theobromine and Theophylline are reported to have a much smaller frequency of use of 5 formulations each. The results of the concentration of use survey conducted by the Council indicate Caffeine also has the highest concentration of use in a leave-on formulation; it is used at up to 6% in non-spray body and hand products.

Follicular penetration of Caffeine was studied using a combination of the Franz diffusion cell and follicle closing techniques; the skin sample without follicles had absorption of 43.4 ± 9.73%, and the high follicular density sample had the highest percentage of absorption (47.1 ± 9.10%). When different areas of the body were tested for Caffeine penetration, breast skin was more penetrable than abdominal skin. The effect of washing skin on dermal Caffeine penetration was tested. A 24% permeation rate was reported for cells that were not washed, and a 8% permeation rate was reported for cells that were washed. In another study, it was observed that skin thickness did not have a significant effect on Caffeine penetration.

Human skin subjected to 98% Theophylline displayed a diffusion range of 2.2 - 7.7%. Approximately 0.2 - 4.6% of applied substance was metabolized, and more than 60% of the metabolites diffused through skin samples. Reported metabolites were 1,3,7-trimethyluric acid, 1,3-dimethyluric acid, and 3-methylxanthine. The amount of metabolites varied per skin sample. Theophylline absorption was tested among various skin samples. Theophylline absorption ranged from 3.6-33.4%, while diffusion ranged from 2.2-7.7%. In a different study, 8-[^{14}C]-Theophylline (radiolabeled) was diffused through excised human skin. Between 0.2 ± 0.1% - 4.6 ± 0.2% of the applied doses were metabolized, and over 60% of the total formed metabolites penetrated through the skin.

Caffeine is readily absorbed through bodily membranes and is distributed throughout the body. Theobromine and Theophylline are both metabolites of Caffeine, and make up 14% of Caffeine's metabolism, combined. Factors such as pregnancy, oral contraceptives, and age affect the metabolism of Caffeine. All three methylxanthines are metabolized by the hepatic enzymes.

Theobromine can be present in the body as a result of Caffeine metabolism. Theobromine is metabolized in the liver into xanthine (i.e. fully demethylated), and further metabolized into methyluric acid, facilitated by CYP1A2 and CYP2E1. Theophylline is metabolized by ring oxidation and *N*-demethylation facilitated by microsomal enzymes in the liver (cytochrome P-450). After a review of the kinetics and metabolism of Theophylline in rats, IARC concluded that Theophylline is quickly and completely absorbed from the digestive tract, readily crosses placental boundaries, can be distributed in breast milk, and is dispersed throughout all organs, with the exception of adipose tissue.

Theobromine was given to rabbits, and the absolute bioavailability of the given Theobromine reached 100%. Peak plasma concentrations were reached within 3 hours when dogs were given a single dose of 15-50 mg/kg bw Theobromine.

Different age groups were dermally dosed with 4 µg Caffeine in acetone. The age group of 65-86 displayed the highest dose recovery (61.8 ± 5.4%). The role of hair follicles in the dermal absorption of Caffeine (2.5%) was studied. The highest concentration in the open follicle group was approximately 11.75 ng/ml an hour after application, while the highest concentration in the blocked follicle group was 6.65 ng/ml at 2 hours after application.

Four men were treated with up to 10 mg/kg Caffeine orally. Ninety-nine percent of the dose was absorbed, with 85% of the given dose excreted in the urine. Significant prolongation of Caffeine elimination was observed in pregnant women compared to post-partum women. The elimination half time of Caffeine between women taking OCS and women not taking OCS was examined. Women on OCS had an average elimination half time of 10.7 hours, while women not taking OCS had an average elimination half time of 6.2 hours. In a different study, the elimination half times of women taking OCS and not taking OCS were 7.88 and 5.37 hours, respectively. The distribution of Theophylline in breast milk was studied in 5 women. It was observed that less than 10% of the mother's Theophylline intake was distributed into the breast milk.

Studies involving acute dermal, oral and inhalation toxicity of the relevant ingredients reported low toxicity levels. The reported dermal LD_{50} for Caffeine and Theophylline were > 2000 mg/kg bw when test substances were applied via a semi-occlusive patch. The lowest reported LD_{50}s in mice and rats are 127 mg/kg bw (vehicle not specified) and 192 mg/kg bw (aq.), respectively. In other species, LD_{50}s of Caffeine were 230 mg/kg bw (guinea pigs and hamsters), 224 mg/kg bw (rabbits), and 240 mg/kg bw (dogs). For Theobromine, the reported LD_{50}s in rats and dogs (vehicle not specified) were 950 and 300 mg/kg bw, respectively. For Theophylline, the lowest LD_{50}s reported for mice, rats, guinea pigs, and rabbits are 235, 225, 183, and 350 mg/kg bw, respectively. The LC_{50} following aerosol exposure or Caffeine mixed with 2% of a

hydrophobic fumed silica was 4.94 mg/L. Following a dust aerosol exposure to Theophylline mixed with 1% and 2% silica, an LC_{50} of > 6.7 mg/L was established.

Caffeine (0.5%) was given to rats for 7 or 8 weeks. A statistically significant decrease in thymus weight and vacuolar degeneration was apparent. Similar results were seen when 0.8% Theobromine was given to rats for the same duration. In 90-day studies in which mice and rats were dosed with Caffeine in drinking water, the NOAEL were 167 and 179 mg/kg bw/day in male and female mice, respectively, and 151 and 174 mg/kg bw/day in male and female rats, respectively; the highest doses administered in these studies were 167 and 180 mg/kg bw/day to male and female mice, respectively, and ~272 and 287 mg/kg bw/day in male and female rats, respectively.

Immature and mature rabbits were fed a diet containing ≥ 1.5% Theobromine. In immature rabbits, mortality was present, and dose-dependent. Mature and immature rabbits displayed pulmonary congestion, ascites in the liver, kidney congestion, and redness of the gastro-intestinal mucosa.

In 16-day gavage studies involving Theophylline, the majority of rats died after being dosed with 200 mg/kg, and all rats died when dosed with 400 mg/kg. A 16-day feed study was also performed using rats given Theophylline at concentrations of up to 8000 ppm. All rats survived; the final mean body weight was statistically decreased in rats given 8000 ppm. Administration of Theophylline in corn oil to mice for 2 years via gavage resulted in decreases in the survival, and body weights of male mice dosed with 150 mg/kg were statistically significant. Mice given 4000 ppm Theophylline for 14 weeks displayed statistically significant decrease in mean body weights and increases in leukocyte, segmented neutrophil, and lymphocyte counts. Statistically significant decreases in the mean body weights and body weight gains of male mice was apparent after administration of 150 mg/kg bw via gavage for 14 weeks. In a 2 year study, mice were administered Theophylline at up to 150 mg/kg bw via gavage. Decreases in survival and body weights in treated mice were reported. In a different 2 year gavage study with Theophylline in corn oil using rats, no statistically significant differences in the survival between treated and control groups were seen.

Studies were performed evaluating the developmental toxicity of Caffeine in mice. Mice dosed with up to 350 mg/kg/d of Caffeine in drinking water did not display any consistent dose-related effects. In different studies, mice displayed reduced maternal body weight gain (100 - 400 mg/kg/d Caffeine; GD 6 - 15) a reduction of live male pups/litter, female body weight, and adjusted seminal vesicle weight (up to 88 mg/kg/d; 21 - 35 weeks). Mice were dosed with up to 1000 mg/kg/d for 100 or 140 days via drinking water. An increase of preimplantation loss and resorptions were seen. Dose-dependent maternal weight gain decreases were reported when rats were dosed with 10 - 40 , 40 - 80, 10 - 100, 100 and 70 – 2000 mg/kg. In a study where 61 Osborne-Mendel rats were treated with up to 125 mg/kg via gavage on GD 0 - 19, 6/61 females died at the highest dose level. At the 80 mg/kg dose level, 2 litters were resorbed, and at the 125 mg/kg dose level, 4 litters were resorbed. Resorptions was also noted at concentrations of 1500 and 2000 ppm in a different study involving 61 female rats given Caffeine in drinking water on days 0 -20 of gestation. A 23% average sperm radius decrease, as well as a decrease in sperm motility and velocity was observed in 8 rats given 25 mg/kg Caffeine via gavage. No teratogenic effects were observed when rabbits were given up to 125 mg/kg of Caffeine via gavage on gestation days 6 -16. Forty pregnant monkeys were given Caffeine in drinking water eight weeks before pregnancy to several months after pregnancy. A dose-related increase in stillbirths, decreased maternal weight, and miscarriages were present.

In a study where rats fed diets containing up to 99 mg/kg bw/d Theobromine, no maternal toxicity was reported, but a slight decrease in fetal body weight as well as an increase in skeletal variation frequency was apparent. Rats given Theobromine in the diet for 28 days displayed testicular atrophy, and rats fed 0.6% exhibited seminiferous tubular-cell degeneration. However, in a similar study where rats were fed 0.6% Theobromine, no testicular atrophy was noted. Rabbits given up to 63 mg/kg Theobromine bw via feed displayed little to no maternal toxicity. In a different study, rabbits were given up to 200 mg/kg bw Theobromine via gavage on GD 6 - 29. At the 200 mg/kg dose level, 40% of the dams died, but little to no maternal toxicity was reported in rabbits given 25 - 125 mg/kg. No testicular atrophy was noted when dogs were given up to 150 mg/kg/d Theobromine for one year.

A statistically significant decrease in testicular weight was observed at the 300 mg/kg bw dose level in mice dosed for 13 weeks via gavage or diet. Mice given 0.2% Theophylline via drinking water displayed an increased percentage of resorptions/litter and a reduced number of live fetuses.[4141,43] Dose-related decreases in the number of live pups/litter was also reported in a different study in which mice were given 0.3% Theophylline in feed. Decreases in litter size and fetal weight were noted in rats dosed with 0.4% Theophylline via drinking water on GD 6 – 15. Rats given 0.5% Theophylline for 19 or 14 - 75 weeks displayed testicular atrophy. Statistically significant decreases in testicular weight were reported after rats were administered 150 mg/kg bw/d via gavage.

Multiple tests were available regarding the genotoxicity of Caffeine. The majority of bacterial in vitro tests yielded positive results, however the majority of mammalian cell in vitro genotoxicity assays yielded negative results. Bacterial studies were mostly positive without metabolic activation, and mostly negative with metabolic activation. Results were negative in an

Ames test testing up to 5000 µg/plate Theobromine with and without metabolic activation. Negative results were also obtained in a chromosomal aberration assay performed with and without metabolic activation. CHO cells and cultured human lymphocytes were dosed with 1000 µg/mL. Positive results were obtained when metabolic activation was not present. Negative and positive results were also seen in genotoxicity studies involving Theophylline. However, in vivo genotoxicity assays using Caffeine produced predominately negative results.

IARC concluded there is inadequate evidence for the carcinogenicity of Caffeine in experimental animals and in humans; IARC had an overall evaluation that Caffeine is not classifiable as to its carcinogenicity to humans.

No statistically significant differences between the incidences of tumors in control and treated rats were present when Sprague-Dawley rats were given up to 2000 ppm Caffeine. Rats given 0.2% Caffeine in their drinking fluid for 12 months displayed pituitary adenomas and pituitary hyperplasia. Wistar rats were given either 0.1 or 0.2% Caffeine in drinking fluid. Tumor incidences was higher in untreated rats. Theophylline was not carcinogenic in rats and mice when administered up to 150 mg/kg bw/d in male B6C3F1 mice and up to 75 mg/kg in male and female Fischer 344 rats. Authors of an NTP study stated there was no evidence of carcinogenic activity based on 2 year gavage studies performed on F344/N rats and B6C3F1 mice.

Osborne-Mendel rats were given a diet consisting of 0.5% Caffeine, Theobromine, or Theophylline alone or with sodium nitrite. In the group fed both nitrite and the methylxanthines, the mortality rate was significantly increased. Food intake was decreased in Caffeine-treated rats, and a further reduction of food intake was noted in rats treated with both Caffeine and sodium nitrite. Food intake was not affected in rats treated with Theobromine alone, but rats treated with both Theobromine and sodium nitrite displayed significant decreases in intake. No neoplastic or pre-neoplastic lesions were observed. In a different study, rats were first given BBN followed by 110 mg/kg/d Caffeine or Caffeine and phenacetin for 21 months via drinking water. No carcinogenic potential was seen in rats given only Caffeine treated water, but an increase in tumor incidences was seen in rats given Caffeine and phenacetin combined. The increase in tumor incidences was greater in rats treated with both Caffeine and phenacetin versus rats treated with phenacetin alone. DEN injections were given to Fischer 344 rat followed by 90 mg/kg/d Caffeine via drinking water for 6 weeks. Carcinogenetic potential was evaluated by comparing the number and area of glutathione-*S*-transferase placental form positive (GST-P+) foci in the liver of treated rats with control rats. There was no increase in the number and area of GST-P+-foci in treated animals.

Mice were given an injection of DMBA once a week for 6 weeks. The test substance (250 and 500 mg Caffeine/L of drinking water) was given to groups of 54 - 55 BD2F1 or 37 - 43 C3H mice, one week after the end of DMBA injections. In BD2F1 mice, the low dose of the test substance revealed a 20% increase of mammary carcinoma multiplicity, while the high dose revealed a 40% increase. C3H mice had an increase of mammary carcinomas of 13% at 250 mg/L Caffeine, and an increase of 117% at 500 mg/L Caffeine.

The irritation index was 0 when White Vienna rabbits were subjected to 50% Caffeine under a semi-occlusive patch. Slight reddening was reported when White Vienna rabbits had semi-occlusive patches containing 0.5 g of 50% aqueous Theophylline applied to the skin. The reported irritation index was 0.0. In an LLNA using four female mice at concentrations of up to 5%, and an HRIPT (105 subjects; 6%), Caffeine was not considered a sensitizer.

Average irritation indices of 0.9 (corneal opacity), 0 (iritis), 1.6 (conjunctival erythema) and 0.6 (conjunctival edema) were recorded when undiluted Caffeine was instilled into the eyes of 3 rabbits. In a similar study, 51mg of undiluted Theophylline was instilled into the eyes of 3 White Vienna rabbits. Mean irritation indices for corneal opacity, iritis, conjunctival redness, and conjunctival swelling were 0.6, 0.0, 1.8, and 0.6 respectively.

Case-control studies regarding the carcinogenicity through the intake of coffee (≥ 7 cups/day) provided no evidence of a potential breast cancer risk. Multiple studies confirmed this result. Different studies showed no or irregular association between Caffeine intake and cancer in the reproductive organs or pancreas. Cohort studies showed no correlation between risks for bladder cancer and Caffeine consumption, while a number of case-control studies showed a weak positive association between bladder cancer and coffee intake.

DISCUSSION

The 3 ingredients in this report are methylxanthines, each of which is commonly ingested in food products, can be naturally or synthetically derived. The Panel found that the data in this report were sufficient to support the safety of Caffeine, Theobromine, and Theophylline. In addition, the Panel noted that Caffeine, the methylxanthine with the highest frequency and concentration of use, is considered a GRAS foods substance in the US, with widespread frequent dietary exposure. Since the ingestion of this ingredient is safe, and exposure resulting from ingestion of food would be far greater than exposure due to cosmetic use, the concern for systemic toxicity was mitigated.

The Panel recognized the positive genotoxicity studies therein, but considered those to be potentially misleading. Indeed, positive results were only observed in in vitro studies without metabolic activation (those in vitro studies with metabolic activation were negative); the positive results of studies performed with mammalian cell cultures were also in sharp contrast to the in vivo mammalian studies which yielded negative results (suggesting that those positive results were not of concern).

Furthermore, the Panel noted the negative results of the carcinogenicity studies performed by the NTP, further mitigating any concern of the positive genotoxicity studies. Positive results for development and reproductive studies were also noted, but were considered negligible considering these effects were only seen at concentrations much higher than what would be used in cosmetics.

The Panel discussed the issue of incidental inhalation exposure from formulations that may be aerosolized (e.g., face/neck products and face powders at up to 6%). The acute inhalation data suggest little potential for respiratory effects at relevant doses. Also, the Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. The Panel considered other data available to characterize the potential for Caffeine, Theobromine, and Theophylline to cause systemic toxicity, irritation, sensitization, reproductive and developmental toxicity, and genotoxicity. They noted the lack of systemic toxicity at high doses in acute and chronic oral exposure studies, minimal or no irritation or sensitization in tests of dermal exposure at relevant concentrations, and the absence of relevant genotoxicity in multiple assays. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The CIR Expert Panel concluded that Caffeine, Theobromine, and Theophylline are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment[1,CIR staff]

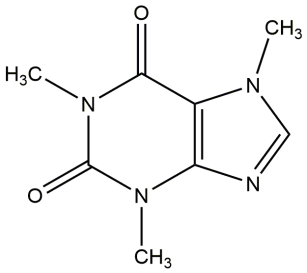
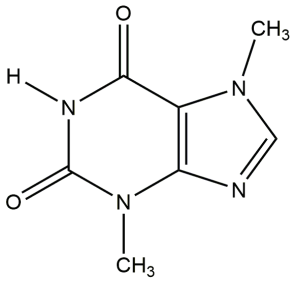
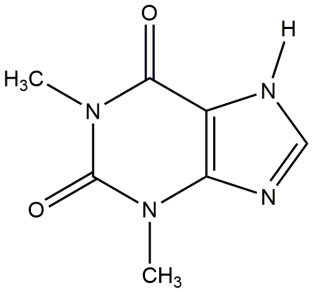
Ingredient CAS No.	Definition & Structure	Function(s)
Caffeine 58-08-2	Caffeine is the heterocyclic organic compound that conforms to the formula: 	Fragrance Ingredients; Skin-Conditioning Agents-Miscellaneous
Theobromine 83-67-0	Theobromine is the heterocyclic compound that compound that conforms to the formula: 	Fragrance Ingredients; Skin-Conditioning Agents-Miscellaneous
Theophylline 58-55-9	Theophylline is the xanthine derivative that conforms to the formula: 	Skin-Conditioning Agents-Miscellaneous

Table 2. Chemical Properties of Caffeine, Theobromine, and Theophylline

Property	Value	Reference
Caffeine		
Physical Form	Powder Prismatic crystals Hexagonal prisms	11 11 11
Color	White	11
Odor	Odorless	11
Molecular Weight (g/mol)	194.194	11
Density/Specific Gravity (@ 18 ºC)	1.23	11
Vapor pressure (mmHg @ 25 ºC)	9.0×10^{-7}	11
Melting Point (ºC)	238	11
Boiling Point (ºC)	178	11
Water Solubility (g/L @ 20 ºC)	22	5
Ethanol Solubility (g/L 20 ºC)	8	5
log K_{ow}	-0.07	11
Disassociation constant - pKa (@ 25 ºC)	14	11
Theobromine		
Physical Form	Crystalline powder Monoclinic needles	12 12
Color	White	12
Molecular Weight (g/mol)	180.167	12
Density/Specific Gravity (@ 20 ºC)	1.52	12
Vapor Pressure (mmHg@ 25 ºC)	1.13×10^{-11}	12

Table 2. Chemical Properties of Caffeine, Theobromine, and Theophylline

Property	Value	Reference
Melting Point (ºC)	357	12
Boiling Point (ºC)	290 - 295	12
Water Solubility (g/L @ 25 ºC)	0.33	54
log K_{ow}	-0.78	12
Disassociation constants - pKa (@ 25 ºC)	9.9	12
Theophylline		
Physical Form	Crystalline powder Needles or plates	13 13
Color	White	13
Odor	Odorless	13
Molecular weight (g/mol)	180.167	13
Vapor Pressure (mmHg @ 25 ºC)	$5x10^{-9}$	13
Melting Point (ºC)	273	13
Water Solubility (g/L @ 20 ºC)	8.3	6
log K_{ow}	-0.02	13
Disassociation constants - pKa (@ 25 ºC)	8.81	13

Table 3. Frequency and Concentration of Use

	# of Uses[17]	Max Conc of Use (%)[18]	# of Uses[17]	Max Conc of Use (%)[18]	# of Uses[17]	Max Conc of Use (%)[18]
	CAFFEINE		THEOBROMINE		THEOPHYLLINE	
Totals*	**1033**	**0.00005-6**	**5**	**0.00002-0.0025**	**5**	**NR**
Duration of Use						
Leave-On	*882*	*0.00005-6*	*2*	*0.0025*	*5*	*NR*
Rinse-Off	*151*	*0.0004-0.37*	*3*	*0.00002*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*0.05*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	206	0.01-1.5	NR	0.0025	NR	NR
Incidental Ingestion	4	0.0004-0.2	NR	NR	NR	NR
Incidental Inhalation-Spray	2; 237[a]; 293[b]	0.2; 0.001-1[b]	2[a]	NR	2[a];1[b]	NR
Incidental Inhalation-Powder	3; 237[a]; 1[c]	2; 0.0001-6[c]	2[a]	NR	NR	NR
Dermal Contact	945	0.00005-6	5	0.00002-0.0025	5	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	76	0.001-1	NR	NR	NR	NR
Hair-Coloring	4	NR	NR	NR	NR	NR
Nail	NR	0.0001-0.2	NR	NR	NR	NR
Mucous Membrane	24	0.0004-0.2	3	NR	NR	NR
Baby Products	1	NR	NR	NR	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[a] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[b] It is possible these products are sprays, but it is not specified whether the reported uses are sprays..
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders
NR – no reported use

Table 4. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/Results	Reference
				DERMAL		
Caffeine	Rat	10	olive oil	semi-occlusive patch applied for 24 hrs	> 2000 mg/kg bw	5
Theophylline	Rat	10	olive oil	semi-occlusive patch applied for 24 hrs	> 2000 mg/kg bw	6
				ORAL		
Caffeine	Mouse	NR	NR	NR	127 mg/kg bw	5
Caffeine	Mouse	12	NR*	NR	185 mg/kg bw	5
Caffeine	Mouse	10	water	NR	200 mg/kg bw	5
Caffeine	Rat	NR	water	0, 160, 180, 200, 220 mg/kg	192 mg/kg bw	5
Caffeine	Rat	12	trioctanoin	50, 100, 200, 400, 800 mg/kg	200-400 mg/kg bw	5
Caffeine	Rat	NR	NR	NR	233 mg/kg bw	5
Caffeine	Rat	NR	NR	NR	247 mg/kg bw	5
Caffeine	Rat	10	carboxymethyl cellulose	178, 261, 383 mg/kg bw; gavage, observed for 14 days	261 - 383 mg/kg bw, three males and all three males died within 24 hours when treated with 383 mg/kg bw	5
Caffeine	Rat	NR	NR	NR	344 mg/kg bw	5
Caffeine	Rat	NR	NR	NR	355 mg/kg bw	5
Caffeine	Rat	NR	NR	NR	421 mg/kg bw	5
Caffeine	Rat	NR	corn oil	90, 130, 200, 200, 300, 450, 670, 1000, 1500 mg/kg	450 mg/kg bw	5

Table 4. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/Results	Reference
Caffeine	Rat	5	corn oil	90, 130, 200, 300, 450, 670, 1000, 1500 mg/kg	483 mg/kg bw	5
Caffeine	Rat	10	gum Arabic	NR	700 mg/kg bw; muscular rigidity and tremor noted	5
Caffeine	Hamster	NR	NR	NR	230 mg/kg bw	5
Caffeine	Guinea Pig	NR	NR	NR	230 mg/kg bw	5
Caffeine	Rabbit	NR	NR	NR	224 mg/kg bw	5
Caffeine	Rabbit	NR	NR	NR	246 mg/kg bw	5
Caffeine	Dog	NR	NR	NR	140 mg/kg bw	5
Theobromine	Rat	NR	NR	NR	950 mg/kg bw	10
Theobromine	Dog	NR	NR	NR	300 mg/kg bw	10
Theophylline	Mouse	NR	NR	NR	235 mg/kg bw	6
Theophylline	Mouse	NR	NR	NR	332 mg/kg bw	6
Theophylline	Mouse	NR	NR	NR	600 mg/kg bw	6
Theophylline	Rat	NR	NR	NR	225 mg/kg bw	6
Theophylline	Rat	20	0.5% Tragacanth in distilled water	0, 100, 215, 261, 316, 464, 1000 mg/kg	272 mg/kg bw	6
Theophylline	Guinea Pig	NR	NR	NR	183 mg/kg bw	6
Theophylline	Rabbit	NR	NR	NR	350 mg/kg bw	6
INHALATION						
Caffeine; test substance was mixed with 2% of a hydrophobic fumed silica	Rat	10	aerosol	2.48, 4.94 mg/L; rats were exposed to an aerosol for 4 hours	4.94 mg/L; no deaths at the low concentration level; In the high dose group, 6/10 rats died. Congestion, bloody ulcers in the glandular stomachs and hyperemia was discovered in the rats that died.	5
Theophylline; test substance mixed with 1% and 2% of a hydrophobic fumed silica	Rat	10	aerosol	2.39, 6.7 mg/L; rats were exposed to a dust aerosol of the test substance using a head-nose inhalation system for 4 hours	> 6.7 mg/L; no deaths occurred	6

NR = Not Reported; * = the dosing solution included an unspecified concentration of sodium benzoate

Table 5. Repeated Dose Oral Toxicity Studies

Ingredient	Animals/Group	Study Duration	Vehicle	Dose/Concentration	Results	Reference
Caffeine	B6C3F1 mice (12/sex)	90 days	drinking water	21.4, 43.6, 85.4, 130.5, 167.4 mg/kg bw/d (male); 24.6, 46.6, 87.9, 134.4, 179.4 mg/kg bw/d (female)	Body weight was statistically significantly decreased, but was not dose-dependent. Significant decreases in the levels of serum amylase (1500 ppm, male and female), serum aspartate aminotransferase (375 ppm, female), and alanine aminotransferase (1500 ppm, females) were present. Water consumption was decreased in the higher dosed animals. No gross morphology or irregular microscopic findings were observed. NOAEL male: 167.4 mg/kg bw/d NOAEL female: 179.4 mg/kg bw/d	5
Caffeine	male Sprague-Dawley rats (# of rats not stated)	7 or 8 weeks	feed	0.5%	Statistically significant decreased in body weight and food consumption was noted. Decreases in thymus weight were apparent. Treated rats displayed vacuolar degeneration of spermatogenic cells.	39
Caffeine	Fischer 344 rats (12/sex)	90 days	drinking water	19.7, 41.8, 85.4, 151, 271.9 mg/kg/d (male); 23.1, 51, 104.2, 174.2, 287 mg/kg/d (female)	Body weight gain in all groups was decreased in all dose levels, however, the effect was only statistically significant in the highest dose only. In animals given the highest dose, a body weight reduction of 26% and 20% was observed in males and females, respectively. Water consumption was decreased in the high dosed groups. Microscopic evaluation of sex organs revealed no difference between treated and control rats. NOAEL male: 151 mg/kg bw/d NOAEL female: 174.2 mg/kg bw/d	5
Theobromine	male Sprague-Dawley rats (# of rats not stated)	7 or 8 weeks	feed	0.8%	Statistically significant decreases in body weight and food consumption were noted. Decreases in thymus weight were apparent. Treated rats displayed severe testicular atrophy and spermatogenic cell degeneration/necrosis.	39
Theobromine	8 rabbits/group	20 days (immature rabbits) or 120 days (mature rabbits)	feed	0, 0.5, 1, 1.5%	Mature rabbits dosed for 120 days displayed a dose-dependent increase in the severity of lesions in the thymus, heart and testes. For rabbits given 1 or 1.5% Theobromine in the diet, 1/8 survived the duration of 120 days. 4/8 mature rabbits exposed to 0.5% Theobromine in the diet survived. Statistically significant decreases in weight gain were present in a dose-dependent manner. In rabbits that died, severe pulmonary congestion, slight to moderate hydropericardium and scattered foci of myocardial necrosis were present. Congestion of the capillaries and intraalveolar edema was apparent. Degeneration of the heart was seen, and in severe cases, fragmentation of the fibers associated with macrophage infiltration was apparent. Damage to the seminiferous tubules was also noted. In immature rabbits dosed, mortality was apparent in a dose-dependent manner. Gross lesions were similar to those that appeared in mature rabbits. The thymus of treated animals showed edema and widespread hemorrhages.	40
Theophylline	B6C3F1 mice (5 male/5 female per group)	16 day (gavage)	corn oil	0, 12.5 (twice daily), 25 (daily), 50 (daily), 50 (twice daily), 100 (daily), 200 (daily), 200 (twice daily), 400 (daily) mg/kg bw	Three out of five males and 5/5 females dosed with 400 mg/kg once daily died on day 1. No statistically significant decreases in body weight were found. No other findings were reported.	41

Table 5. Repeated Dose Oral Toxicity Studies

Ingredient	Animals/Group	Study Duration	Vehicle	Dose/Concentration	Results	Reference
Theophylline	B6C3F1 mice (10 male/10 female per group)	14 week (gavage)	corn oil	0, 75, 150, 300 mg/kg bw	Three males and 10 females given 300 mg/kg, one 75 mg/kg male, and one control female died before study completion. The decrease in mean body weights and body weight gains of male mice given 150 and 300 were statistically significant. The increase in mean cell volume and mean cell hemoglobin of male mice dosed with 300 mg/kg was also statistically significant. Slight dose-depended increases in the incidences of mesenteric periarteritis in both sexes were apparent. No other findings were reported.	41
Theophylline	B6C3F1mice (10 male/10 female per group)	14 week (feed)	feed	0, 1000, 2000, 4000 ppm	Decreases in mean body weight and body weight gains were statistically significant in all mice. One animal per sex died before completion of the study. The increase in leukocyte, segmented neutrophil, and lymphocyte counts of male mice dosed with a concentration of 4000 ppm was statistically, significantly increased compared to controls. Increases in the leukocyte and segmented neutrophil counts of female mice dosed with concentrations of 2000 and 4000 ppm was statistically greater than controls. Slight dose-depended increases in the incidences of mesenteric periarteritis in both sexes were apparent. No other findings were reported.	41
Theophylline	B6C3F1 mice (50 male/50 female per group)	2 year (gavage)	corn oil	0, 15, 50, 150 mg/kg/d (male); 0, 7.5, 25, 75 mg/kg/d (female)	The decrease in the survival and body weights of 150 mg/kg males were statistically significant. Decreases in the final body weights of 150 mg/kg males, and 25 and 75 mg/kg females were also statistically significant. No treatment-related increases in the incidence of nonneoplastic lesions or neoplasms were reported. There were decreased incidences of hepatocellular adenomas compared to control mice. Male mice showed a pattern of nonneoplastic liver lesions along with silver-staining helical organisms in the liver (*Helicobacter hepaticus*). These lesions were significantly more prominent in control males compared to males treated with 150 mg/kg/d/	41
Theophylline	F344 N rats (5 male/5 female per group)	16 day (gavage)	corn oil	0, 12.5, (twice daily) 25 (daily), 50 (daily), 50 (twice daily), 100 (daily), 200 (once daily), 200 (twice daily), 400 (daily) mg/kg bw	All rats that received 400 mg/kg once daily died, and all but one female rat receiving 200 mg/kg twice daily died. Final mean body weights and body weight gains of groups receiving Theophylline twice per day were similar to those receiving the same doses once per day. Body weight gains of males receiving 100 or 200 mg/kg and of females receiving 50-200 mg/kg were less than the weight gain of the control animals. Uterine weights of females that were dosed with 100 or 200 mg/kg once per day were significantly less than females receiving 50 mg/kg daily. Uterine atrophy was apparent in 3 females receiving 200 mg/kg twice per day. Periarteritis was observed in 2 male and 2 female animals dosed with 400 mg/kg twice daily. No other findings were reported.	41
Theophylline	F344 N rats (5 male/5 female per group)	16 days (feed)	feed	0, 500, 1000, 2000, 4000, 8000 ppm	All rats survived. The decrease of final mean body weight was statistically significant in rats given 8000 ppm. Testis weights were statistically significantly decreased in male rats given 4000 ppm. All rats survived until completion of the study. The incidence of uterine hypoplasia was observed in treated females.	41
Theophylline	F344 N rats (10 male/10 female per group)rats	14 week (feed)	feed	0, 1000, 2000, 4000 ppm	The increase in the final mean body weights in rats given 1000 ppm compared to the control group was statistically significant. Segmented neutrophil counts in all dosing groups were significantly greater than the control group. Kidney weight was increased in rats given 4000 ppm. A dose –related, statistically significant increase in the severity of nephropathy in males and incidences of mesenteric and/or periarteritis in males and females was apparent.	41

Table 5. Repeated Dose Oral Toxicity Studies

Ingredient	Animals/Group	Study Duration	Vehicle	Dose/Concentration	Results	Reference
Theophylline	F344 N rats (50 male/50 female per group)	2 year (gavage)	corn oil	7.5, 25, 75 mg/kg bw	No statistically significant differences in survival between treated and control groups. Dosed rats had final mean body weights that were statistically, significantly lower than the control group. No statistically significant increases in the frequency of neoplasms were found. Chronic inflammation of the mesenteric arteries was increased in males given 75 mg/kg bw. Dose-related negative trends in the incidence of mammary gland fibroadenomas and fibrodenomas or carcinomas combined in females.	[41]

NOAEL= no-observed-adverse-effect-level

Table 6. Developmental and Reproductive Toxicity Studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
			ORAL			
Caffeine	CD-1 mice (20 pairs of animals/generation; both sexes treated)	Water	0, 4 - 5, 12 - 18, 25 - 39 mg/kg/d	A population of mice was bred for four generations, and administered Caffeine via drinking water, continuously. Twenty pairs of mice were bred in each generation.	No dose-related effects on fertility, sexual maturity, litter size, offspring weight, sex ratio, or abnormalities were observed.	[42]
Caffeine	CD-1 mice; 40 animals/sex for the control and 20 animals/sex for the treated groups	Water	0, 22, 44, 88 mg/kg/d	Animals were given drinking water containing Caffeine during a 7 day premating period and during the 100 day cohabitation period. A crossover mating trial was then conducted.	F0: no effect on body weight, alopecia in 55% on mice at 44 mg/kg/d and 50% of mice at 88 mg/kg/d; F1: the number of live pups/litter decreased as Caffeine dosage increased; male body weight was reduced by 8%, no change in female body weight No effect on the average number of litters per pair or mean number of pups/litter was observed. There was a 20% reduction of live male pups/litter. The amount of pups born alive decreased as Caffeine dosage increased. No differences between control and tested groups in mating and fertility indices. Female body weight decreased by 5%. Testis weight dropped by 7% and adjusted seminal vesicle weight dropped by 12%.	[5]
Caffeine	CD-1 Mice (# of animals not stated; female mice)	Water	350 mg/kg	Mice were dosed once daily on days 8 - 18 of gestation.	No difference was noted between the control group and treated mice regarding embryonic resorption, growth, skeletal development, or terata. Supernumary ribs were the only observed fetal affect with a linear inverse relationship between maternal body weight gain during gestation.	[5]
Caffeine	CD-1 Mice (# of animals/sex not stated)	NR	50, 100, 250, 400 mg/kg/d	Mice were given daily doses via gavage on days 6-15 of gestation.	Reduced maternal body weight gain was reported at the 100 mg/kg/d level. Developmental effects on fetal weight and ossification were observed at the 250 mg/kg/d level and higher.	[55]
Caffeine	mice (# animals not stated; only males treated)	Water	50, 100, 200, 400, 600, 800, 1000 mg/kg/d	Males were exposed for 100 or 140 days, mated to untreated females for 3 weeks or more	Increase in preimplantation loss and resorptions, however results were not dose-dependent	[5]

Table 6. Developmental and Reproductive Toxicity Studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Caffeine	Osborne-Mendel Rat (61 rats; both sexes)	Water	6, 12, 40, 80, 125 mg/kg	Rats were treated by gavage each day on days 0 - 19 of gestation.	Six females died at the 125 mg/kg dose level. A statistically significant decrease in weight gain as well as food consumption was observed in treated animals compared to controls. Two litters were resorbed at the 80 mg/kg dose level and 4 litters were resorbed at the 125 mg.kg dose level. Statistically significant decreased in fetal weight and crown-rump length was reported at 40 mg/kg. Ectrodactyly was seen at the dose levels of 80 and 125 mg/kg. At this dose level, skeletal ossification problems such as misshapen centra, missing centra, reduced dorsal arch, reduced pubis, missing hind phalanges, reduced metacarpals, and reduced metatarsals were also seen.	[5]
Caffeine	CD rats (12 rats/group; females)	Water	10, 20, 40 mg/kg/d	Rats were administered the test substance via gavage on days 1 - 20 of gestation, and allowed to litter.	No consistent effects were reported, however, Caffeine caused a reduced maternal weight gain at all three dose levels.	[5]
Caffeine	Sprague-Dawley Rat (20 pairs/group; both sexes)	Water	0, 12.5, 25, 50 mg/kg	Rats of both sexes were given Caffeine in deionized water via gavage; 1 week pre-cohabitation exposure; 16-week cohabitation exposure	Pup weight decreased by 7, 7, and 8% in the 12.5, 25, and 50 mg/kg dose groups, respectively. The average sperm radius was decreased by 23 and 26% in the 25 and 50 mg/kg groups, respectively. Sperm motility was reduced by 4%, and sperm velocity was reduced by 9%. Organ weight was decreased in all groups.	[5]
Caffeine	CD rats (20 rats/group; females)	Water	0, 40, 80 mg/kg bw	Rats were given doses via gavage on days 1 - 19 of gestation.	At both dose levels, a statistically significant reduction of maternal weight gain was apparent. However, this did not have an effect on the rate of prenatal death or malformation. Fetal weight was significantly reduced in the high dosed-group.	[5]
Caffeine	Rat (# of animals not stated; females)	Water	10, 100 mg/kg	Pregnant rats were given either a single bolus a day, or 4 separate boluses (in 3 hour intervals, per day), containing Caffeine for 15 days via gavage on days 6 - 20 of gestation.	Caffeine treated animals displayed a dose-dependent decrease in maternal body-weight gain during the first week of treatment. During the second week of treatment, all groups showed similar weight gain patterns, however, rats treated with the high dose had lower final body weights than control rats and low-dose treated rats. Increases in late resorptions, retarded ossification of the fetal skeleton, and incidences of malformed fetuses were present in the group given 100 mg/kg Caffeine once a day. Significant decreases in fetal weight and length was observed in groups treated with 100 mg/kg Caffeine once daily or 25 mg/kg Caffeine 4x daily.	[5]

Table 6. Developmental and Reproductive Toxicity Studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Caffeine	Osborne-Mendel Rats (Sixty females were mated in the control and lowest dose groups and 30 females were mated for the mid and high dose group)	Water	180, 360, 700 ppm	Rats were given Caffeine in drinking water continuously on days 0 to 20 of gestation. Animals were killed on either gestation day 20, postnatal day 0 or postnatal day 6.	Dams dosed with a concentration of 700 ppm had decreased body weight gain. Animals killed on gestation day 20 at the 700 ppm level had a decreased number of viable fetuses and an increased occurrence of sternebral variations. Pups of animals killed on postnatal day 0 displayed affected sternebral development at the 700 ppm level. Pups of animals killed on postnatal day 6 had impaired weight gain and reduced sternebral ossifications at the 700 ppm dose level.	[5]
Caffeine	Osborne-Mendel Rats (61 females/group)	Water	70, 180, 360, 700, 1000, 1500, 2000 ppm	Rats were given Caffeine continuously in the drinking water on days 0 - 20 of gestation. Dams were killed on day 20 of gestation.	The decrease of maternal food and water consumption was statistically significant at concentrations of 1000 ppm and higher. Maternal body weight gain was statistically significantly decreased at concentration levels of 1000 ppm and higher. At doses of 1500 and 2000 ppm, decreased implantation efficiency, increased resorptions, and decreased mean number of viable fetuses was observed.	[5]
Caffeine	Rabbits (# of animals not stated; females)	Water	14, 40, 125 mg/kg	Rabbits were given test substance via gavage or drinking water; no other details regarding dosing were provided	No teratogenic effects were observed.	[5]
Caffeine	Monkey (*Macaca fascicularis*; 40 female monkeys)	Water	10 - 15, 25 - 30 mg/kg/d	Forty pregnant monkeys were continuously given Caffeine in drinking water eight weeks before pregnancy to several months after pregnancy.	Dose-related increases in stillbirths, miscarriages and decreased maternal weight were present. Infant body weights were reduced over the first 30 days in males, but the deficits were reversible and not evident after one year of age. These effects were seen at all dose levels.	[5]
Theobromine	Sprague-Dawley Rats (# of animals not stated; females)	NR	53 or 99 mg/kg bw/d	Rats were fed diets containing Theobromine on gestation days 6-19	No maternal toxicity reported; slight decrease in fetal body weight and increase in skeletal variation frequency at high dose. No other developmental/reproductive toxicity information was noted.	[10]
Theobromine	Male Rats (# of animals not stated)	Feed	0.2 - 1.0%	Rats were given Theobromine in the diet for a period of 28 days.	At the 0.8% level, rats displayed severe testicular atrophy. At the 0.6% level, rats exhibited seminiferous tubular-cell degeneration. Testicular changes occurred only at lethal concentrations.	[10]
Theobromine	Male Rats (# of animals not stated)	Feed	0.6%	Rats were fed 0.6% Theobromine in the diet for 28 days.	No testicular atrophy was observed.	[10]

Table 6. Developmental and Reproductive Toxicity Studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Theobromine	New Zealand white rabbits (# of animals not stated; sex not stated)	Feed	21, 41,63 mg/kg bw	Rabbits were diets containing Theobromine. No other details regarding this study were provided.	Little or no maternal toxicity was observed. A fetal body weight and increase in the frequency of skeletal variations was observed at the 41 and 63 mg/kg bw levels. No other developmental/reproductive toxicity information was noted.	10
Theobromine	New Zealand white rabbits (# of animals not stated; sex not stated)	NR	up to 200 mg/kg bw	Rabbits were given test substance via gavage on gestation days 6 -29.	40% of rabbits receiving 200 mg/kg died; little or no maternal toxicity was observed at the lower doses; decreases in fetal body weight and an increase in malformations was seen at doses of 125 or 200 mg/kg.	10
Theobromine	male Dogs (# of animals not stated)	NR	25, 50, 100, 150 mg/kg/d	Dogs were given Theobromine over the course of 1 year. Route of administration was not stated.	No testicular atrophy was observed at any dose level.	10
Theophylline	male B6C3F1 mice (# of animals not stated)	Water or Feed	75-300 mg/kg bw/d	Mice were dosed for 13 weeks via gavage or diet. No other study details were provided.	Mice given a gavage dose of 300 mg/kg bw/d displayed a slight but significant decrease in testicular weight. Doses of 150 mg/kg bw/d or less had no effect. No effect on sperm motility, sperm density, or the number of abnormal sperm was observed.	10
Theophylline	CD-1 Mice (# of animals not stated; females)	Water	0.2%	Mice were given 0.2% Theophylline in drinking water on gestation days 6 through 15.	An increased percentage of resorptions per litter and reduced number of live fetuses/fetal weight as well as decreases in gravid uterine weight were noted. No other developmental/reproductive toxicity information was noted.	41
Theophylline	Swiss CD-1 Mice (# of animals not stated; sex not stated)	Feed	0.075, 0.15, 0.30%	Mice were given Theophylline in the diet for one week before mating and during 13 weeks of cohabitation. Litters were removed one day after birth, except for one litter which was raised for 21 days.	A dose-related decrease in the number of live pups/litter was reported in the high-dose groups. A statistically significant decrease in live pup weight as well as number of litters/breeding pairs was also reported in the high-dose groups. No other developmental/reproductive toxicity information was noted.	10
Theophylline	CD Rats (# of animals not stated; females)	Water	0.4%	Rats were dosed with drinking water containing 0.4% Theophylline on gestation days 6 to 15	A reduction of litter size and fetal weight was noted, but no increases in malformations. No other developmental/reproductive toxicity information was noted.	41
Theophylline	Holtzman Rats (# of animals not stated; sex not stated)	Feed	0.5%	Rats were fed 0.5% Theophylline for 19 weeks. No other study details were provided.	86% of treated animals displayed testicular atrophy	10
Theophylline	Rats (# of animals not stated; sex not stated)	Feed	0.5%	Rats were feed 0.5% Theophylline for 14 - 75 weeks. No other study details were provided.	Bilateral testicular atrophy with variable atrophic changes in the epididymis, prostate gland, and seminal vesicles was observed.	41

Table 6. Developmental and Reproductive Toxicity Studies

Test Article	Animals/Group	Vehicle	Dose/Concentration	Procedure	Results	Reference
Theophylline	Fischer 344 rats (# of animals not stated; sex not stated)	Water or Feed	75-300 mg/kg bw/d	Rats were given up to 300 mg/kg bw/d via gavage or feed for 13 weeks. No other study details were provided.	A significant decrease in testicular weight was apparent in rats with 150 mg/kg bw/d by gavage, but not in rats given 75 mg/kg bw or less. No effect on sperm motility, sperm density, or the number of abnormal sperm was observed.	[10]

NOAEL= no-observed-adverse-effect-level; NR = Not Reported

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VITRO		
Caffeine	5000 µg/mL	*Escherichia coli* K12	Ames test without metabolic activation	positive, frameshift mutation observed	[5]
Caffeine	1000 µg/mL	*E. coli* K12	Ames test without metabolic activation	positive	[5]
Caffeine	up to 1940 µg/plate	*E. coli* K12	Bacterial gene mutation assay with and without metabolic activation, activation with S-9 mix made from mouse liver homogenate	negative	[5]
Caffeine	6000 µg/well	*E. coli* PolA+, PolA-	Ames test	positive, inhibition zone was 5 and 11 mm for tester strain PolA+ and PolA-, respectively	[5]
Caffeine	NR	*S. typhimurium*	5 strains of Salmonella were subjected to a microsome assay with and without metabolic activation	negative	[56,57]
Caffeine	up to 1940 µg/plate	*S. typhimurium* TA 98, TA100, TA535, TA1537	Ames test with and without metabolic activation, activation with S-9 mix made from rat liver homogenate	negative	[5]
Caffeine	4, 20, 100, 500, 2500 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1538	Ames test with and without metabolic activation; activation with S-9 mix made from rat liver homogenate	negative	[5]
Caffeine	100, 333.3, 1000, 3333.3, 10000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537	Ames test with and without metabolic activation	negative	[5]
Caffeine	3.3, 10, 33.3, 100, 333.3, 1000, 3333.3 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537	Ames test with and without metabolic activation	negative	[5]
Caffeine	6000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537	Ames test with and without metabolic activation	negative	[5]
Caffeine	58, 97, 291, 583, 971, 2524, 2913, 10098, 20100 µg/plate	*S. typhimurium* BA13	Ames test	negative	[5]
Caffeine	250, 500, 1000 µg/mL	(CHL)	Chromosomal aberration study without metabolic activation, cells were exposed for 24 or 48 hrs, 100 metaphases/dose evaluated	positive; dose related increased in chromosomal aberration frequency at mid and high dose levels	[5]
Caffeine	194-777 µg/mL	Chinese hamster cells	Chromosomal aberration study without metabolic activation	positive, increase of chromosomal aberration frequency occurred in a dose-dependent manner	[5]
Caffeine	100 µg/mL	Indian muntjac skin fibroblasts	Sister chromatid exchange assay without metabolic activation	positive, SCE frequency greatly increased in presence of test substance	[5]

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
Caffeine	19, 97, 194 µg/mL	Human xeroderma pigmentosum cell lines (XP19, XP20)	Sister chromatid exchange assay without metabolic activation	positive, increase in SCE frequency at concentrations at all dose levels	5
Caffeine	194-777 µg/mL	CHL	Sister chromatid exchange assay without metabolic activation	positive, dose-dependent SCE frequency increase	5
Caffeine	1550 µg/mL	*E. coli* WP2, WP-B	DNA damage and repair assay without metabolic activation	positive	5
Caffeine	583, 1942, 5826 µg/mL	Chinese hamster V79 cells (V79)	DNA damage and repair assay with and without metabolic activation	negative	5
Caffeine	971, 1942, 3884 µg/mL	Rat kidney cell line NRK-49F	Micronucleus test without metabolic activation; cells incubated for 1 hr, ≤ 4000 interphase cells scored	positive, 4.5% increase of micronucleated cells at high does level, control had a 0.3% increase; cell viability was 92.3% at the 1942 µg/mL dose level, and 63.6% in control after 0 - 3 days of incubation	5
Caffeine	24, 49, 97, 194, 388, 777, 971, 1359 µg/mL	Preimplantation mouse embryo	Micronucleus test without metabolic activation	positive, linear increase in micronuclei at concentrations of 194 µg/mL and above	5
Caffeine	5, 50, 500 µg/mL	Human hepatoma (Hep-G2) cells	Micronucleus test without metabolic activation, incubation for 24 hours, 1000 cells/dose level scored	positive, dose-dependent increase in micronuclei frequency	5
Caffeine	0.05, 0.1, 0.25, 0.5, 1.0 µg/mL	Human peripheral blood leukocytes	Cytogenetic assay without metabolic activation	positive, chromosome damage seen at the DNA-synthesis phase, S-phase most sensitive, G1 and G2 not affected	5
Caffeine	0.05, 0.1, 0.25, 0.5, 1.0 µg/mL	Human embryonic fibroblasts	Cytogenetic assay without metabolic activation	positive , chromosome damage seen at DNA-synthesis phase, S-phase most sensitive, G1 and G2 not affected; gaps and breaks, no exchanges observed	5
Caffeine	5, 10, 25, 50, 75, 100 µg/mL	Human lymphocytes	Cytogenetic assay without metabolic activation, activation with S-9 mix made from animal liver homogenate	negative	5
Caffeine	250, 500, 750 µg/mL	Human lymphocytes	Cytogenetic assay without metabolic activation	positive	5
Caffeine	485, 971 µg/mL	Human lymphocytes	Cytogenetic assay without metabolic activation	positive, increased frequency of chromatid breaking and decreased G2 duration in X-ray irradiated and nonirradiated cells	5
Caffeine	40, 60, 80, 120, 140, 160 µg/mL	Rat MCTI cells	Cytogenetic assay without metabolic activation	negative, only minimal effects after 1 - 4 weeks of treatment	5
Caffeine	252, 505, 757 µg/mL	Human diploid fibroblasts	Cytogenetic assay without metabolic activation, incubation with test substance for 24 hours, colcemid added 24 hours later, 200 metaphases/dose scored	positive, mitotic index reduction of 58% at the 252 µg/mL dose level with significant clastogenicity, no exchanges observed	5
Caffeine	1.0, 1.5, 2.0 mg/mL	Human diploid fibroblasts	Cytogenetic assay with and without metabolic activation, activation with S-9 mix made from rat liver homogenate, incubated with test substance, colmecid added 24 hours later, 100 metaphases/dose scored	positive, increase in chromosome breaks at high concentration in presence of S-9, no exchanges observed	5
Caffeine	5, 10 or 20 µg/mL	HeLa cells	Cytogenetic assay, cultures exposed for 6 and 9 weeks, cells examined twice a week	negative, no breaks or growth effects reported	44

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
Caffeine	40, 60, 80, 120, 140, 160 µg/mL	HeLa cells	Cytogenetic assay without metabolic activation	positive, significant and dose-dependent increase in terminal break frequency at concentrations of 40 µg/mL and higher	5
Caffeine	583, 1942, 5826 µg/mL	HeLa cells	Cytogenetic assay, suspended in culture for 7 - 11 days	negative; no chromatid break increase was observed when cells were exposed to 5 and 20 µg/mL of Caffeine.	44
Caffeine	4850 µg/mL	CHL	Cytogenetic assay	positive result observed at 4850 µg/mL dependent on temperature, but not ATP content	5
Caffeine	194 µg/mL	V79	Mammalian cell gene mutation assay without metabolic activation	negative, no induction of ouobain-resistant mutants was observed	5
Caffeine	97, 146, 194, 388, 583 µg/mL	Human lymphocytes	Unscheduled DNA synthesis without metabolic activation, lymphocytes obtained from healthy donors and patients with systemic lupus erythematosus (SLE)	negative, no inhibition of DNA repair in normal lymphocytes, no further reduction of DNA repair in SLE cells	5
Caffeine	0, 5, 10, 15, 20 mM	Human lymphoblast lines MIT-2 and HH-4	Human lymphoblast mutation assay; cell lines were maintained in RPMI 1640 supplemented with either 10% of 15% fetal calf serum; after treatment, cells centrifuged; 4000 cells were plated and incubated for 2 weeks	At significantly toxic concentrations, Caffeine was not mutagenic.	45
Caffeine	0, 5, 10, 15, 20 mM	*S. typhimurium*	Cells were exposed with and without a drug-metabolizing system; cells were resuspended in phosphate-buffered solution and plated after exposure	No mutations were observed by Caffeine alone or with the drug-metabolizing system.	45
Theobromine	0.5-5000 µg/plate	*S. typhimurium*	Ames test with and without metabolic activation	negative	46
Theobromine	0-1000 µg/mL	CHO	Chromosomal aberration tests with and without metabolic activation	negative	46
Theobromine	0-1000 µg/mL	CHO and cultured human lymphocytes	Sister chromatid exchange assay with and without metabolic activation	Results in CHO cells were positive, in a dose-dependent manner, without metabolic activation; with the S-9 system, results were equivocal and not dose-related	46
Theophylline	1-10,000 µg/plate	*S. typhimurium* TA97$_a$, TA100, TA102, TA104	Ames test with and without metabolic activation, activation with S-9 mix made from rat liver homogenate	negative, very weak mutagenic activity (factor up to 1.5) observed in TA104 and TA102 in presence of S-9	6
Theophylline	100, 333, 1000, 3333, 10000 µg/plate	*S. typhimurium* TA97, TA98, TA100, TA1535	Ames test with and without metabolic activation, activation with S-9 mix made from liver homogenate of rats and hamsters	negative	6
Theophylline	10 mg/mL	*S. typhimurium* TA100, TA98	Ames test with and without metabolic activation, test substance consisted of Theophylline dissolved in distilled water, concentrations of 0.01-1 mg/petri dish tested	positive; the higher concentrations had negative effects on bacteria; a weak mutagenic effect was evident in TA 100 without the S9 fraction, however mutagenicity was not present when the same bacteria was tested with Theophylline and the S9 fraction; bacterial strain TA 98 showed no mutagenic effects.	47

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
Theophylline	20 mg/mL	Hamster cells	Ames test with and without metabolic activation, test substance consisted of Theophylline dissolved in distilled water; test substance was tested with and without S9 fraction; results were compared with negative and positive control	positive; weak mutagenic effect was seen in in the absence of S9 cells in hamster cells; in the presence of S9, a decreased level of spontaneous mutation was evident	47
Theophylline	20 mg/mL	Human cells	Ames test with and without metabolic activation, test substance consisted of Theophylline dissolved in distilled water	negative, no DNA damage observed	47
Theophylline	NR	Chinese hamster Don-6 cells and human diploid fibroblasts	Sister chromatid exchange assay without metabolic activation	positive	6
Theophylline	1, 10, 100 µg/mL	Human lymphocytes	Sister chromatid exchange assay without metabolic activation	negative	6
Theophylline	18, 90, 360 µg/mL	CHO	Sister chromatid exchange assay without metabolic activation, incubated with test substance for 26 hours or 46 hours	positive, number of SCEs slightly increase (factor up to 2.8), potentiated toxic effects of methylnitrosurea and reduced cloning efficiency of cellular growth rate	6
Theophylline	13 mg/mL	HeLa cells	Cytogenetic assay without metabolic activation, incubated with 1.3% solution of test substance for 1 hour and fixed after 30 hours	positive, chromatid breaks: 68/2776 in treated cells (2.4%) and 3/3208 in control cultures (0.1%)	6
Theophylline	18, 180, 1800 µg/mL	Human lymphocytes	Cytogenetic assay without metabolic activation, incubated with test substance for 72 hrs, 1000 cells/culture examined	negative, mitotic rate was 16% of control at low concentration, no mitosis seen at mid and high concentration; no chromosome damage observed	6
Theophylline	1, 10, 100 µg/mL	Human lymphocytes	Cytogenetic assay without metabolic activation	positive	6
Theophylline	250, 500, 750 µg/mL	Human lymphocytes	Cytogenetic assay without metabolic activation	positive, increase in breaks at dose of 500 µg/mL and above, mitotic indices reduced at 500 µg/mL and above	6
Theophylline	577, 1135 µg/mL	FM3A cells	Cytogenetic assay without metabolic activation, cells incubated with test substance for 24 and 48 hours	positive; the low dose produced 20 aberrant metaphases after 24 hours and 68% aberrant metaphases after 48 hours; high dose produced 46 and 56% aberrant metaphases after 24 and 48 hours, respectively	6
Theophylline	0, 100, 150, 200 µg/mL	Human lymphocytes	Cytogenetic assay	positive	6
Theophylline	510, 555, 600 µg/mL	CHO	Cytogenetic assay with and without metabolic activation, activation with S-9 mix made from liver homogenate of rats,	negative	6
Theophylline	NR	*E. coli* 15+m-	Bacterial gene mutation assay without metabolic activation	positive	6
Theophylline	150 µg/mL	*E. coli*	Bacterial gene mutation assay	positive	6
Theophylline	5, 7, 9 µg/mL	V79	HGPRT assay with and without metabolic activation	negative	6
Theophylline	up to 5 mg/mL	L5178Y tk +/- cells	Mouse lymphoma assay	negative, treatments greater than 24 hours produced weakly positive results	6

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VIVO		
Caffeine	46 mg/kg/d	Rat (oral feed); 30 rats	Cytogenetic assay, 117 weeks of treatment	negative, no significant difference between treated and control rats	[5]
Caffeine	800mg/daily	Human (tablet); 9 volunteers	Chromosomal damage assay; volunteers treated for 4 weeks, 200 mg 4x a day	negative, no significant increase in chromosome damage, single exposure of cells at the 48 h time produced gaps and breaks in the 250-750 µg/mL range	[5]
Caffeine	4, 13, mg/kg	Mouse (drinking water); 5 male mice/group	Dominant lethal assay, 16 week exposure, each male mated to five females a week for 7 weeks	negative, litter size and fertility unaffected	[5]
Caffeine	3.6, 13.4, 49, 122 mg/kg/d	Mouse (drinking water)	Dominant lethal assay, 8 weeks of exposure	negative	[5]
Caffeine	90 mg/kg, 112 mg/kg	Mouse (oral)	Dominant lethal assay, 3 (112 mg/kg) or 6 weeks (90 mg/kg)	negative	[5]
Caffeine	90 mg/kg, 112 mg/kg	Mouse (drinking water, gavage); 50 mice/group	Dominant lethal assay, 8 weeks (112 mg/kg) drinking water or 5 days (90 mg/kg) gavage; administered drinking water, 10 male mice administer i.p. injection	negative, no mutagenic induction of dominant lethals, preimplantation losses, or depression of female fertility observed	[5]
Caffeine	200 mg/kg d (drinking water), 168-240 mg/kg (i.p.)	Mouse (drinking water, i.p.)	Dominant lethal assay	negative	[5]
Caffeine	NR	Mouse (drinking water); 6 mice/group	Dominant lethal assay, 254 - 550 days of exposure, each male mated with five females each week for 8 weeks	negative, no significant increase in embryonic deaths, males given highest doses produced less pregnancies	[5]
Caffeine	1000 mg/kg/d	Mouse (drinking water); 3 mice/group	Sister chromatid exchange assay, exposure period of 5, 10, or 15 days, given test substance; colchicine and BrdU injections given at 19 and 2 hours before mice were killed; 25 metaphase/animal scored	positive, SCE/cell frequency increase in exposure time-related manner	[5]
Caffeine	0, 20, 100, 200, 400 mg/kg	Chinese hamster (gavage)	Sister chromatid exchange assay, given 2 doses within 24 hours in an aqueous solution	positive	[5]
Caffeine	0, 45, 75, 100, 300 mg/kg/d	Chinese hamster (gavage); 8 hamsters/group	Micronucleus assay, given 1 or 2 gavage doses of test substance in water, bone marrow removed from femur and studied	positive, induction seen at highest dose level	[5]
Caffeine	0, 45, 75, 150, 300 mg/kg	Chinese hamster (gavage); 8 hamsters/group	Sister chromatid exchange assay, given single dose , BrdU tablets implanted at 2 h prior to dosing; animals injected with 0.02 mg vincristine and killed 3.5 hours later	positive, slight increase in SCE at 150 mg/kg and higher	[5]
Theophylline	75, 150 300 or 75, 150 mg/kg bw/d	Mouse (gavage); 7-10 mice/sex/group	Micronucleus assay, 14-week exposure	negative, no increase in micronucleated cells	[6]
Theophylline	175, 400, 800 or 225, 425, 850 mg/kg bw/d	Mouse (oral feed); 10 mice/sex/group	Micronucleus assay, 14-week exposure	negative, no increase in micronucleated cells observed	[6]
Theophylline	230 mg/kg bw/d	Rat (oral feed); 6 treated and 5 untreated rats	Cytogenetic assay, 75 week exposure	negative	[6]
Theophylline	0, 30, 75, 150, 225, 300, 450, 600 mg/kg	Hamster (gavage)	Sister chromatid exchange assay, implantation of BrdU tablets	positive	[58]

BrdU = Bromodeoxyuridine; CHL = Chinese Hamster Cell Line; CHO = Chinese Hamster Ovary Cells; *E. coli* = *Escherichia coli*; HeLa = Henrietta Lacks (uterine cell variety; deceased patient); HGPRT = Hypoxanthine-Guanine Phosphoribosyl Transferase; MCT1 = Monocarboxylate Transporter 1 Cells; NR = Not Reported; SCE = Sister Chromatid Exchange; SHE = Syrian Embryo Cells; *S. typhimurium* = *Salmonella typhimurium*

Table 8. Carcinogenicity studies

Ingredient	Animal (#/group)	Dose/Vehicle	Procedure	Results	Reference
Caffeine	Sprague-Dawley rats (50/sex)	21, 26, 49, 102 mg/kg/d (male); 15, 37, 80, 170 mg/kg/d (female)	Rats were given the test substance in drinking water continuously for 104 weeks.	No statistically significant difference between the incidences of tumors in control and treated rats were apparent except for mammary fibroadenomas. The incidence of mammary fibroadenomas showed a significant inverse dose-response relationship. Fifty percent of the control animals displayed mammary fibroadenomas, while 26% of the highest dosed female rats showed mammary fibroadenomas.	5
Caffeine	Wistar rats (40 females)	0.2% (2000 mg/L)	Caffeine solution was placed in the drinking water for 12 months. The average consumption was 13.5 g per rat. Thirty rats were given untreated water.	Twenty-two out of the 40 treated rats had pituitary adenomas, while 9 out of the 30 untreated rats had pituitary adenomas. Pituitary hyperplasia was seen in 5/40 treated rats, and in 1/30 untreated rats	48
Caffeine	Wistar rats (50/sex/group)	0, 0.1%, 0.2%	Three groups each of 50 male and 50 female Wistar rats were maintained on a basal diet and given either tap-water (controls), a 0.1% solution of synthetic Caffeine (purity 100%), or a 0.2% Caffeine solution as the drinking fluid for 78 weeks.	A total of 65/96 untreated rats had developed tumors. In the 0.1% solution group, 75/88 rats were tumor-bearing, and in the 0.2% group, 55/94 rats were tumor-bearing.	10
Theophylline	B6C3F1 Mice (50/sex/group)	15, 50, 150 mg/kg bw/d (male); 7.5, 25, 75 mg/kg bw/d (female)	Mice were given of test substance in corn oil via gavage, 5 days a week, for 2 years.	Males given high doses experienced a high mortality rate. Mean body weights at the end of the two year period were greatly increased in high dose males and females, and mid dose females. An increase in neoplasms and neoplastic lesions was not observed. Frequencies of hepatocellular carcinomas and adenomas were decreased in treated mice. Low and mid dose treated male mice had a combination of lesions (non-neoplastic) and silver staining helical organisms in the liver. High dose treated male mice had a greatly reduced number of liver lesions. Authors attributed the increase in hepatocellular neoplasms in male mice to *Helicobacter hepaticus* infection when mice were also affected by hepatitis. The authors found it difficult to interpret the decreased incidence of liver neoplasms in high dose treated male mice.	6
Theophylline	Fischer 344 Rats (50/sex)	0, 7.5, 25, 75 mg/kg bw/d	For 2 years, rats were given the test substance in corn oil via gavage	No significant increases in the frequency of neoplasms were found. Chronic inflammation of the mesenteric arteries was increased in males given 75 mg/kg bw.	6,41

Table 9. Epidemiological Studies

Ingredient	Test Article/Exposure	Procedure	Results	Reference
		Bladder/Renal		
Caffeine	30+ cups coffee/wk, 3+ cups tea/wk	332 white male bladder cancer patients between the age of 21 and 84 with 686 population-based controls	A 2.5-fold increase in the risk of developing bladder cancer was observed in men who drank 30 or more cups of coffee/week	53
Caffeine	Caffeinated coffee and tea (Control patients reported no coffee or tea consumption, other dose levels included 1 - 20, 21 - 40, or 40+ cups/week)	424 bladder cancer patients identified through the Utah Cancer Registry, and 889 controls obtained through random digit dialing and the Health Care Financing Administration	Patients who drank more than 40 cups of coffee or tea a week had an increased risk of bladder cancer; for non-smokers, the odds ratio was doubled with consumption of one or more cups of caffeinated tea, smokers had a tripled odds ratio	53
Caffeine	Coffee (caffeinated and decaffeinated), tea, cocoa, soda, artificial sweetener	826 bladder cancer patients; 792 randomly selected population controls matched by age, sex, and area of residence	No association was reported between Caffeine consumption and bladder cancer	53

Table 9. Epidemiological Studies

Ingredient	Test Article/Exposure	Procedure	Results	Reference
Caffeine	Coffee consumption (regular ground, instant, decaffeinated)	195 male and 66 women with lower urinary tract bladder cancer, identified in Hawaii between 1977 and 1986. Each case was matched for sex, age, and ethnic group (Caucasian or Japanese) to 2 population-based controls.	No association between duration and amount of coffee consumed and development of lower urinary tract cancer; inverse relationship between lower urinary tract cancer and the consumption of regular ground coffee (p = 0.02), but not with any other types of coffee	53
Caffeine	Coffee consumption (caffeinated and decaffeinated), other methylxanthine-containing beverages	303 male and 61 female Italian bladder cancer patients	The relative risk was 1.2 for those who drank 1 cup of coffee or less/day; the relative risk for those who drank 3 or more cups/day was 1.5, those who drank 4 or more cups/day had a relative risk of 1.4. The results indicated a higher prevalence of coffee consumption in bladder cancer cases; however, the prevalence was not clearly dose-dependent.	53
		Breast		
Caffeine	up to 7 cups coffee/day	2651 newly-diagnosed breast cancer patients in the eastern US; 1,501 controls with nonmalignant conditions	No association between coffee consumption and breast cancer risk observed.	53
Caffeine	Consumption of coffee, tea, soda, chocolate, chocolate drinks (amount not reported)	1617 breast cancer patients, ages 20 to 79 in eastern New York; 1617 randomly selected controls matched with age, sex, and area of residence	No association between Caffeine consumption and breast cancer risk observed.	53
Caffeine	Median Caffeine intake of 212 mg/d in women who developed breast cancer and 201 mg/d in women who remained free of the disease	34,800 postmenopausal women from Iowa were monitored from 1986-1990. Caffeine intake was assessed by food frequency questionnaire. 580 with breast cancer	No association between breast cancer occurrence and Caffeine intake.	53
Caffeine	> 100 mg Caffeine/d; 0.5 mmol/d	755 breast cancer patients under the age of 36 in the United Kingdom; 755 age-matched general population controls	No association between Caffeine consumption and breast cancer risk.	53
		Pancreas		
Caffeine	5 or more cups coffee/day	99 pancreatic cancer patients, aged 40 - 79 years in Sweden; 138 population controls and 163 hospital controls	No relation between coffee consumption and pancreatic cancer incidence reported	53
Caffeine	Coffee (5+ cups/d), decaffeinated coffee (2+ cups/d), tea (1+ cups/day)	150 Italian women with pancreatic cancer, 605 controls with acute, non-neoplastic diseases unrelated to coffee consumption	Increased incidence of pancreatic cancer in patients who drank 2+ cups coffee/day (RR=1.72, 95% CI 0.95-3.11); RR of 1.44 (95% CI 0.74-2.80) for those who drank 3-4 cups/day and 1.06 (95% CI 0.41-2.70) who drank 5+ cups/day	53
		Reproductive Organs		
Caffeine	Coffee (5+ cups/d), decaffeinated coffee (5+ cups/d), tea (5+ cups/day)	290 patients with ovarian cancer in the United States; 580 controls with non-malignant conditions of acute onset and 476 controls with cancer of other sites	Increased risk of ovarian cancer associated with drinking 4-5 cups coffee/d; RR=1.1 (95% CI 0.6-2.0) using the controls with non-malignant conditions and RR=1.0 (95% CI 0.5-1.8) using the controls with cancer. No association between ovarian cancer and decaffeinated beverage consumption	53
Caffeine	Caffeinated coffee, decaffeinated coffee, and tea consumption (amount not reported)	201 patients with vulvar cancer; 342 community controls	Elevated risk in patients who drank 1, 2, or 4 cups of coffee, but not 3 cups/day. No association between vulvar cancer and decaffeinated coffee seen.	53
Caffeine	Coffee, tea, and Caffeine consumption (amount not reported)	362 white Utah men with prostate cancer, 685 age-matched controls	No association between prostate cancer risk and coffee/tea/Caffeine consumption	53

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FDA Frequency of Use Data

Caffeine

01B - Baby Lotions, Oils, Powders, and Creams	58082	1
03B - Eyeliner	58082	1
03C - Eye Shadow	58082	10
03D - Eye Lotion	58082	119
03E - Eye Makeup Remover	58082	1
03F - Mascara	58082	4
03G - Other Eye Makeup Preparations	58082	71
04A - Cologne and Toilet waters	58082	1
04E - Other Fragrance Preparation	58082	4
05A - Hair Conditioner	58082	11
05B - Hair Spray (aerosol fixatives)	58082	2
05E - Rinses (non-coloring)	58082	2
05F - Shampoos (non-coloring)	58082	24
05G - Tonics, Dressings, and Other Hair Grooming Aids	58082	34
05I - Other Hair Preparations	58082	3
06H - Other Hair Coloring Preparation	58082	4
07B - Face Powders	58082	3
07C - Foundations	58082	17
07E - Lipstick	58082	4
07F - Makeup Bases	58082	1
07H - Makeup Fixatives	58082	1
07I - Other Makeup Preparations	58082	17
10A - Bath Soaps and Detergents	58082	10
10E - Other Personal Cleanliness Products	58082	10
11A - Aftershave Lotion	58082	11

11E - Shaving Cream	58082	4
11G - Other Shaving Preparation Products	58082	2
12A - Cleansing	58082	54
12C - Face and Neck (exc shave)	58082	109
12D - Body and Hand (exc shave)	58082	124
12E - Foot Powders and Sprays	58082	4
12F - Moisturizing	58082	201
12G - Night	58082	36
12H - Paste Masks (mud packs)	58082	29
12I - Skin Fresheners	58082	14
12J - Other Skin Care Preps	58082	82
13B - Indoor Tanning Preparations	58082	8

Theobromine

10A - Bath Soaps and Detergents	83670	3
12C - Face and Neck (exc shave)	83670	1
12D - Body and Hand (exc shave)	83670	1
	58559	2

Theophylline

12D - Body and Hand (exc shave)		
12J - Other Skin Care Preps	58559	2
13B - Indoor Tanning Preparations	58559	1

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Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: September 18, 2018

SUBJECT: Draft Report: Safety Assessment of Xanthine Alkaloids as Used in Cosmetics (draft prepared for the September 24-25, 2018 CIR Expert Panel meeting)

The Council respectfully submits the following comments on the draft report, Safety Assessment of Xanthine Alkaloids as Used in Cosmetics.

The following comments are in addition to the comments previously provided on the SLR on August 30, 2018 (many of which still need to be addressed).

Key Issues

Memorandum - Although the memo indicates that the LLNA on Caffeine provided by the Council on August 8, 2018, has been added to the report, it is not found in the report. The data profile also needs to be updated to indicate that there is a sensitization study (the LLNA) on Caffeine.

Genotoxicity, Table 7 - It is not clear why genotoxicity studies of Theobromine are not included in the CIR report as published studies[1] (abstracts attached) are available.

Other Relevant Studies, Tumorigenicity - The studies presented in this section should be moved to the Carcinogenicity section.

Additional Considerations

Search strategy table - Although the page is titled Xanthine Alkaloids, the table says "Hydrogen Peroxide" and the CAS number given is for Hydrogen Peroxide. As there are three ingredients in this report, it would be helpful to use the search strategy table to show the

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[1]Renner HW, Münzner R. 1982. Genotoxicity of cocoa examined by microbial and mammalian systems. *Mutat Res* 103(3-6): 275-281.

Brusick D, Myhr B, Galloway S, et al. 1986. Genotoxicity of theobromine in a series of short-term assays. *Mutat Res* 169(3): 105-114.

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search results for each ingredient included in the CIR report.

Natural Occurrence - Please use either "cacao" or"cocoa" for *Theobroma cacao* throughout the report.

Non-cosmetic Use - Please state that Theobromine and Theophylline are used orally as bronchodilators.

ADME, Human, Oral - What dose of Caffeine was used in the "similar study using 4 subjects"? What dose of Caffeine was used in the study in 9 pregnant and 4 post-partum women?

Dermal Penetration, In Vitro - As the composition of the receptor fluid influences dermal penetration, the identity of the receptor fluid should be stated. In the description of reference 28, what is the meaning of "permeation"? Is this the amount recovered in the receptor fluid, or the amount recovered in the receptor fluid plus the amount recovered in the skin?

Short-Term, Sub-chronic and Chronic - Please correct: "and 75 mg/kg females were significantly less than the control group" e.g., what endpoint in the 75 mg/kg females was less than the control group?

DART - Reference 38, titled "Results of genotoxicity testing of theophylline on bacteria and two lines of mammalian cells" appears to be an incorrect reference for the mouse developmental study of Caffeine (treated in drinking water at doses up to 30 mg/kg/day).

The units ppm represent a concentration rather than a dose.

Please correct: "lumber" (should be "number")

Genotoxicity, In Vitro - What cell types were used in the cytogenetic tests of Caffeine?

Co-Carcinogenicity - The study in which BBN was given to rats followed by Caffeine in the drinking water, and the study in which rats were treated with DEN followed by Caffeine in the drinking water should be moved to the Tumor Promotion subsection.

Tumorigenicity - Was the 13.5 g/rat dose a daily dose?

Ocular Irritation - As Table 2 indicates Caffeine and Theophylline are solids, please check the units (ml) used in the ocular irritation studies of undiluted Caffeine and undiluted Theophylline.

Summary - Please include the compound(s) tested in the multiple reproductive toxicity studies performed in rats.

What compound was tested in rabbits given up to 63 mg/kg bw via feed?

What type of results were observed in Ames tests at doses up to 6000 μg/well? What compound was tested?

What compound was tested in multiple dominant lethal assays?

PubMed

Format: Abstract

Full text links

ELSEVIER FULL-TEXT ARTICLE

Mutat Res. 1982 Mar;103(3-6):275-81.

Genotoxicity of cocoa examined by microbial and mammalian systems.

Renner HW, Münzner R.

Abstract

Unroasted or roasted cocoa powder dispersed in water and applied to Chinese hamsters by stomach tube caused elevated numbers of SCEs in the sister-chromatid exchange test (bone-marrow cells). Roasted cocoa freed from fat produced distinctly higher SCE values with a linear dose-response relationship, whereas cocoa butter had no influence on SCE levels. Positive results in the SCE test (1.5-fold values of the controls) were obtained after application of about 5 g cocoa/kg b.w. Presumably, because of the smaller quantities that could be administered in this way, positive test results were not found when cocoa was given in the diet instead of being administered by stomach tube. Cocoa from which **theobromine** was extracted by chloroform did not affect SCE levels. Pure **theobromine** increased SCE levels in a dose-dependent way. **Theobromine** was also positive in the micronucleus test at 2 X 40 mg/animal and negative in the chromosome aberration test at 1 X 40 mg/animal. Cocoa and the **theobromine** were negative in the Salmonella/mammalian microsome mutagenicity test both with and without metabolic activation.

PMID: 7045646

[Indexed for MEDLINE]

MeSH terms, Substances

LinkOut - more resources

PubMed

Format: Abstract

Full text links

ELSEVIER FULL TEXT ARTICLE

Mutat Res. 1986 Mar;169(3):105-14.

Genotoxicity of theobromine in a series of short-term assays.

Brusick D, Myhr B, Galloway S, Rundell J, Jagannath DR, Tarka S.

Abstract

Theobromine (3,7-dimethylxanthine) was evaluated for genotoxic activity in a series of in vitro assays. **Theobromine** was not mutagenic in the Ames assay up to a maximum concentration of 5000 micrograms/plate either with or without S9 activation. The compound also failed to induce significant levels of chromosome aberrations in CHO cells (with and without S9 activation) or transformation in Balb/c-3T3 cells. At the maximum tolerated concentration **theobromine** increased the frequency of TK-/- mutants in mouse lymphoma L5178Y cells. Increased frequencies were observed both with and without S9 activation and they were reproducible in 2 independent experiments. Statistically significant increases in SCEs were obtained in human lymphocytes and in CHO cells under nonactivation test conditions. The spectrum of results in this battery of tests indicate that **theobromine** treatment results in the expression of genotoxic potential in some assays and the observed activity appears qualitatively and quantitatively similar to that of caffeine, a closely related methylxanthine.

PMID: 3512993

[Indexed for MEDLINE]

Publication types, MeSH terms, Substances

LinkOut - more resources

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: October 23, 2018

SUBJECT: Tentative Report: Safety Assessment of Methylxanthines as Used in Cosmetics (posted October 5, 2018)

The Council respectfully submits the following comments on the tentative report, Safety Assessment of Methylxanthines as Used in Cosmetics.

Key Issues

Dermal Penetration; Summary - Unfortunately, *in vitro* dermal penetration studies do not use terms such as permeation or absorption consistently. When describing this type of study, please state the percentage of compound that was recovered in the receptor fluid and the percentage of compound that was recovered in the various parts of the skin, e.g., tape strips (epidermis), dermis (this information is often in a table rather than found in the text); rather than non-precise terms such as "absorption" or "permeation". If the non-precise terms are used, please explain how the authors defined them, e.g., does absorption mean what was recovered in the receptor fluid, or the amount recovered in the receptor fluid plus the amount in the dermis?

DART, Table 6 - Descriptions of all DART studies should state when in relation to mating the animals were treated. The study descriptions should also clearly state if just females, just males or if both sexes were treated.

In many places in this report, descriptions of effects on body weight are not precise. Animal studies are generally completed in young animals that are growing. Therefore, most studies look at changes in body weight and compare body weights of treated animals over time compared with the body weights of control animals. A statement such as the following included in the Summary is misleading as it suggests the animals lost weight, where it is more likely that body weight gain was lower than controls resulting in final body weights lower than controls. "Statistically significant decreases in body weight of male mice was apparent after administration of 150 mg/kg bw via gavage for 14 weeks."

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Summary - The NTP completes cancer bioassays in 50 animals/sex/dose group, does careful statistical analysis of each endpoint, and the studies undergo rigorous peer review. Therefore, regarding an NTP study, it is not appropriate to state that the NTP "claimed" that there was no evidence of carcinogenic activity. Please state the conclusion as stated by NTP.

Additional Considerations

Impurities, Caffeine - Although the *Food Chemical Codex* (reference 15) is published by USP, specifications from the Codex should not be called "USP-grade". The *Food Chemical Codex* states that Caffeine should be not less than 98.5% and not more than 101% Caffeine. It should be made clear that "1,3,7-trimethyl-3,7-dihydro 1*H*-purine-2,6-dione" is caffeine.

Impurities, Theobromine - It should be made clear that "3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione" is Theobromine.

Impurities, Theophylline - USP specifications should not be cited to IARC (reference 10).

Non-Cosmetic Use - It should be made clear that the Caffeine intake study (reference 30) was completed in the United States.

Dermal Penetration; Summary - The description of reference 31 suggests that there was a difference in Caffeine "absorption" depending on follicular density. Although the means may have been different, when the standard deviations are considered the values overlap and do not appear to significantly different. Did the study authors complete any statistical analyses? The values of 36.1 ± 9.85%, 43.4 ± 9.73% and 47.1 ± 9.10% should not be considered different without some type of statistical analysis.

ADME, Theophylline - Please correct: "I-methlxanthine..."

ADME, Human, Oral, Caffeine - The oral section starts with the following sentence: "A <u>dermal</u> absorption study was performed using four male subjects given 0, 1, 5 and 10 mg/kg per <u>oral</u>." If this was a dermal study, it should be moved to the dermal subsection. If it is an oral study "dermal" needs to be deleted.

Short-Term, Subchronic, and Chronic Toxicity Studies; Summary - If rabbits were fed diets containing Theobromine at concentrations of 0, 0.5, 1 or 1.5%, they were fed diets containing less than or equal to (≤) 1.5% not greater than or equal to (≥) 1.5% as stated in the Short-Term, Subchronic, and Chronic Toxicity Studies section and the Summary.

Short-Term, Subchronic, and Chronic Toxicity Studies - In the mouse study of Theophylline, it states that the mice were treated with 4000 ppm. Was this in drinking water or the diet?

In the last paragraph, please revise: "in corn oil of up to 150 mg Theophylline/kg bw". Were rats really treated with Theophylline in "corn"? Please provide references for the studies described in the last paragraph of this section.

DART - Compound subheadings would be useful for this section as the second paragraph does not clearly state which compound was tested until the next to last sentence.

DART - If no details of the study described in reference 44 were provided, how is it known that the next study (cited to reference 5) was "similar"?

Genotoxicity, In Vivo, Caffeine - What type of cells were assessed in the human study? Did the micronucleus assay in hamsters include any doses that were negative?

Co-Carcinogenicity - What was "The test substance"?

Dermal Irritation, Theophylline - It is unlikely that the rats were treated with doses ">2 g/kg". It is more likely that they were treated with a dose of 2 g/kg and that the LD_{50} was reported as being >2 g/kg.

Is the following correct: "50% aqueous solution and Theophylline"? What did the 50% aqueous solution contain? Should this be "50% aqueous solution of Theophylline"?

Sensitization, In Vitro - Local lymph node assays are generally completed in mice. This study should not be presented under an "In Vitro" subheading.

Sensitization, In Vivo - It should be made clear that the"test substance" was a body care product.

Summary - The paragraph summarizing the acute studies begins with the following sentence: "Studies involving acute dermal and inhalation toxicity of Caffeine and Theophylline reported low toxicity levels." With the exception of the second sentence and the last sentence, most of the studies summarized in this paragraph are oral studies and they include studies of Theobromine. What is meant by "low toxicity levels"?

In the Summary, it should be made clear that the bacterial tests were positive without metabolic activation and mostly negative with metabolic activation.

The IARC conclusion should be included in the Summary.

Table 1 - Although "idealized structure" is necessary for many cosmetic ingredients that are often mixtures of compounds with various structures, is the word "idealized" necessary for the compounds in this report?

Table 4 - Although reference 5 does say "Vehicle: Other: sodium benzoate", it is unlikely that sodium benzoate was the vehicle because it is a solid. It is more likely that it was included as a preservative. Perhaps the vehicle column should be changed for this study to indicate the vehicle was not reported with a footnote to indicate that the dosing solution included an unspecified concentration of sodium benzoate.

Tables 5 and 6 - As all of the studies in these tables are by the oral route of exposure, oral should be added to the title of these tables rather than included as a subsection of the table.

Table 5 - How many male rats were used in the dietary studies of Caffeine and Theobromine (reference 41)?

Please correct "oedoema" (to either edema (the usual US spelling) or oedema (acceptable spelling variation)

Table 6 - Please revise "Testis weight dropped by 7% and adjusted seminal vesicle weight dropped by 12%." As organ weights can only be measured once, it is not known if the weight "dropped". It is more likely that testis weight were 7% below controls and adjusted seminal vesicle weight was 12% below controls (most likely that the animals did not grow as well as controls). At which dose was this observed?

For the mouse study (50, 100, 200, 400, 600, 800 or 1000 mg/kg/day) and rat study (0, 12.5, 25, 50) (both studies of Caffeine) please state which sex (or both) was treated. In the rat study, were the rats only treated before mating, or did dosing continue during gestation?

Table 8 - The Animal column (first row) says "rats"; the Procedure column (first row) says "Mice" - which is correct?

Please correct: "affected my hepatitis"

Table 9 - As these are retrospective studies that really on memory for reported caffeinated beverage consumption, please changed "Test Article/Dose" to "Test Article/Exposure". The "Test Article/Exposure" column of the second study should be revised from "Control patients received 0 cups..." to "Control patients reported no coffee or tea consumption, other exposure levels reported included...."

Table 9, Pancreas - Please correct "cancer is patients"

References 16, 17, 18 - These three references are the same (*British Pharmacopoeia*).