Table 1
Evaluating the Allegation of Teratogenicity

Epidemiological Studies Controlled epidemiological studies consistently demonstrate an increased incidence of a particular spectrum of embryonic and/or fetal effects in exposed human populations.

Secular Trend Data Secular trends demonstrate a positive relationship between the changing exposures to a common environmental agent in human populations and the incidence of a particular embryonic and/or fetal effect

Animal Developmental Toxicity Studies An animal model can be developed which mimics the human developmental effect at clinically comparable exposures. Since mimicry may not occur in all animal species, animal models are more likely to be developed once there is good evidence for the embryotoxic effects reported in the human. Developmental toxicity studies in animals are indicative of a potential hazard in general rather than the potential for a specific adverse effect on the fetus when there are no human data on which to base the animal experiments.

Dose-Response Relationship Developmental toxicity in the human increases with dose (exposure) and the developmental toxicity in animal occurs at a dose that is pharmacokinetically (quantitatively) equivalent to the human exposure.

Biological Plausibility The mechanisms of developmental toxicity are understood and the effects are biologically plausible.

a. Mechanisms
b. Receptor agonistic or antagonistic studies
c. Enzyme suppression
c. Nature of the malformations
d. Teratology principles

* Modified from Brent.1986, 1995a,b

Table 2

Estimated pregnancy loss in 100 pregnancies versus time from conception

Time from Conception	Percent Survival To Term*	Percent Loss During Interval*
Preimplantation		
0-6 days	25	54.55
Postimplantation		
7-13 days	55	24.66
14-20 days	73	8.18
3-5 wks	79.5	7.56
6-9 wk	90	6.52
10-13 wk	92	4.42
14-17 wk	96.26	1.33
18-21 wk	97.56	0.85
22-25 wk	98.39	0.31
26-29 wk	98.69	0.30
30-33 wk	98.98	0.30
34-37 wk	99.26	0.34
38+ wk	99.32	0.68

* Data from Kline et al. 1980. An estimated 50 to 70 percent of all human conceptions are lost in the first 30 weeks of gestation and 78 percent are lost before term.

[+] Modified from Schardein 1993.

Table 3

Spontaneous Abortions can be classified into the following categories:

1) Abortions with Chromosomal abnormalities

The earlier the abortion, the higher the proportion of chromosomal abnormalities. (Boue 1975, Harlap 1980, Hertig 1967, Robert et al. 1975). Approximately 53% of spontaneous abortions in the first trimester are due to chromosomal abnormalities, 36% are due to chromosomal abnormalities in the second trimester and only 5% of stillbirths in the third trimester are due to chromosomal abnormalities. Over 95% of abortions with chromosomal abnormalities were due to autosomal trisomy, double trisomy, monosomy, triploidy or tetraploidy (Kajii et al. 1980, Simpson 1980). Most chromosomal abnormalities are not the cause of repetitive abortion, although in about 4% of couples with two or more spontaneous abortions, a normal-appearing parent could be a carrier for a balanced translocation or may be a mosaic with abnormal cells in the germ cell line. Environmental exposures during pregnancy cannot account for any of these abortions because most aneuploidies result from meiotic non-disjunction during gametogenesis or other events before conception.

2) Abortions with normal chromosomes (euploidy) (Hertig 1967, Kajii et al. 1980, Copp 1995, Garner 1995, Baldwin & Whitley 1989, Benirschke 1974, Brown & Scialli 1987, Foulon et al. 1990, Harter 1976, Holder 1972, Kriel et al. 1970, Lewis & Schulman 1973, Mead 1989, Ransome-Kuti 1972, Robert 1975, Shmoys & Kaplan 1990, Torpin 1941, Weber et. al.1988). Hertig (1967) and many other investigators reported the occurrence of malformed or blighted embryos as a cause of abortion. These embryonic losses may occur later in the first trimester and have been shown to have normal karyotypes in more recent studies (Kajii et al. 1980). The etiology of these abortions is manifold and is listed in Table 4.

Table 4

Etiology of Abortion

1. **Chromosomal abnormalities: pre-conceptional or periconceptional etiology.**
2. **Embryos and fetuses with severe congenital malformations or growth retardation.**
3. **Endometriosis.**
4. **Lupus anticoagulant (antiphospholipid antibodies) and other immunological problems related to reproduction.**
5. **Cervicitis; bacterial or viral infection.**
6. **Uterine abnormalities: subserosal myoma or hematoma, infantile uterus, bifid uterus, IUD, etc. (8-10% of recurrent aborters).**
7. **Some teratogens, especially those with cytotoxic properties and endocrine disrupters (RU 486).**
8. **Maternal Diabetes, alcoholism, hypothyroidism, illicit drug abuse, maternal phenylketonuria, hemorrhagic diatheses and many other chronic and acute maternal diseases.**
9. **Luteal phase hormonal deficiency.**
10. **Trauma, IUDs, lightening and other rare miscellaneous events**
12. **Hypersecretion of LH**
13. **Hyperandrogenemia**
14. **Hyperprolactinemia**
15. **Autoimmune thyroid disease**
16. **Thrombophilic abnormalities other than antiphospholipid antibody.**
17. **Vitamin B 12 deficiency**
18. **Elevated glutathione levels**
19. **Dietary factors; decreased with fruits and vegetable, increased with diet rich in fats.**
20. **Twenty seven percent of women with habitual abortion had a mutation G1691A in Factor V gene (Leiden mutation) of mutation C677T in the methylenetetrahydrofolate reductase gene. The Leiden mutation may play a considerable role for women having primary recurrent abortions.**
21. **Fourteen percent of women with unexplained recurrent abortion show highly skewed X-chromosome inactivation, which shows that they are carriers of X-linked lethal traits.**
22. **IgG auto anti-laminin antibodies and recurrent abortion.**
23. **HLA-G genotype and recurrent abortion**
24. **TH 1 type response associated with recurrent abortion (cytokines).**

Table 5

Etiology of Human Congenital Malformations Observed During the First Year of Life*

Suspected Cause		Percent of Total
Unknown		**65 to 75**
	Polygenic	
	Multi factorial (gene-environment interactions)	
	Spontaneous errors of development	
	Synergistic interactions of teratogens	
Genetic		**10 to 25**
	Autosomal and sex-linked genetic disease	
	New mutations	
	Cytogenetic (chromosomal abnormalities)	
Environmental		**10**
	Maternal conditions: Alcoholism; diabetes; endocrinopathies; phenylketonuria; smoking and nicotine; starvation; nutritional	4
	Infectious agents: Rubella, toxoplasmosis, syphilis, herpes, cytomegalic inclusion disease, varicella, Venezuelan equine encephalitis, parvo virus B19	3
	Mechanical problems (deformations): Amniotic band constrictions; umbilical cord constraint; disparity in uterine size and uterine contents	1 to 2
	Chemicals, prescription drugs, high dose ionizing radiation, hyperthermia	1-2

* Adapted from Brent (1976, 1985, 2004, 2008) and Brent and Holmes (1988).

Table 7

Summary of Supplemental Publications Reviewed by Species and Route of Administration

Publication	Species	Route	Dose	Investigated Effect
Fenster et al., 1998	Humans	PO daily consumption	> or < 150 mg/day	Rate of caffeine metabolism and risk of spontaneous abortion.
Klebanoff et al., 2002	Humans	PO daily consumption	-	Effect of 3d trimester maternal serum concentration of paraxanthine on birth weight.
du Preez et al., 1999	Humans	IV	4.0-7.7 mg/kg	The clearance rate and volume of distribution of theophylline in apneic premature neonates.
Mazkereth et al.,1997	Humans	IV	6 mg/kg	Effect of aminophylline dosage on urinary output in premature infants.
Maza et al.,2001	Rats	IV	6 mg/kg	Effect of hepatic regeneration after partial hepatectomy on theophylline pharmacokinetics.
Jorritsma et al.,2000	Rats	IP	10 mg/kg	Induction of P4501A with caffeine in therapeutic model of hyperbilirubinemia in Gunn rats.
Pelissier-Alicot et al., 2002	Rats	SC	25 mg/kg	Effect of administration (a.m. vs. p.m.) of caffeine on daily rhythms of heart rate, body temperature and locomotor activity.
Schrader et al., 1999	Rats	Analytical method	-	Development of reverse-phase HPLC method for analyzing caffeine + all 8 metabolites simultaneously from rat urine.
Buters et al., 1996	Mice	IP	2 mg/kg	Confirmed involvement of CYP1A2 in PK and metabolism of caffeine in CYP1A2-/- and CYP1A2+/+ mice.
Derkenne et al., 2005	Mice	IP	8 mg/kg	Replaced mouse Cyp1a2 (-/-) with human CYP1A2 gene to restore metabolism of caffeine and change it to human profile.
Kolarovic et al., 1999	Mice	IP	20 mg/kg	Use of caffeine as a biomarker for estimation of xenobiotic biotransformation and possible hepatotoxicity.
Labedzki et al., 2002	Mice/human microsomes	*In vitro* comparison	-	*In vitro* comparison of murine and human CYP1A2-mediated metabolism of caffeine and quinolones.
Janus and Antoszek, 2000	Cattle	IV	5 mg/kg	Effect of gender and age on the pharmacokinetics of caffeine in Holstein cattle.
Janus et al., 2001	Cattle (calves)	IV	5 mg/kg	Effect of 4-day starvation or water deprivation on the pharmacokinetics of caffeine in calves.
Peck et al., 1997	Horses	IV	2.5 mg/kg	Compared the pharmacokinetic disposition of caffeine and its metabolites in horses and donkeys.

PO = Oral; IV = Intravenous; IP = Intraperitoneal; SC = Subcutaneous

Table 7 cont'd

Summary of Supplemental Publications Reviewed by Species and Route of Administration

Publication	Species	Route	Dose	Investigated Effect
Todi et al., 1999	Horses	IV	2 g or less	Detection of caffeine in serum and urine after doses of caffeine or theophylline in race horses.
Wasfi et al., 2000	Camels	IV	2.35 mg/kg	The pharmacokinetics, metabolism and urinary detection time of caffeine was characterized in camels.
Fort et al., 1998	Frog (*Xenopus*)	*In vitro*	-	The developmental toxicities of caffeine and 13 metabolites were investigated in the FETAX Teratogenesis Assay.

IV = Intravenous

Table 8

Species Considerations Between Rats and Humans in the Metabolism of Caffeine					
	Route	Dosage	Peak Exposure	$T_{1/2}$	Metabolites
Rats					
8 wk old males	Gavage	4 mg/kg/day		2.12 hours	1,3,7-diaminouracil, or 6-amino-5-N-formylmethylaminol-1,3-dimethyluracil Dimethylxanthenes: (theophylline, theobromine and paraxanthine)
Pregnant females	Gavage or drinking water (GDs 0-11)		0.10-5.74 µg/mL		
Pregnant females	Oral (GDs 12-15)	80 mg/kg	60-63 µg/mL	1.7-2.6 hours	
Pregnant females	2 bolus gavage	5, 25 mg/kg	20 umol/L	8.9 hours	
Non pregnant	2 bolus gavage	5, 25 mg/kg	2 umol/L	3.8 hours	
Humans					
Newborns				4 days	Paraxanthine or 1,7-dimethylxanthine Dimethylxanthenes: (theophylline, theobromine and paraxanthine)
6-13 yr olds				2.3 hours	
Adults **a**				2-6 hours	
Pregnant women				10-20 hours	
Humans **b**	Drinking liquids	1-2 mg/kg 3-5 mg/kg	1-2 µg/mL 5 µg/mL		
FDA (1980)	Pregnant women limit caffeine to less that 400 mg/day	6.7 mg/kg for a 60 kg human			

Table developed from information provided in Christian and Brent, 2001.

a. Healthy non-smoking adults
b. General population; no age or sex distinction

Table 9
Comparing the Pharmacokinetics and Toxicokinetics of Caffeine in Humans and Animals

Method of Administration	Exposure	Plasma caffeine level	Teratogenic Effect
1 to 2 cups of coffee/day in humans; 1 to 2 mg/kg	100 to 200 mg of caffeine	1 to 3 ug/ml peak level	Not teratogenic
3-5 cups of coffee/day in humans; 3 to 5 mg.kg	500 to 600 mg of caffeine	5-6 ug/ml peak level	Not teratogenic
Caffeine in the drinking water in the rat	80 mg/kg/day	5.7 +/- 2.3 mg/kg/day	Not teratogenic
Caffeine in the drinking water in the rat	205 mg/kg/day	Peak ?	Not teratogenic
Caffeine by once a day gavage in the rat	80 mg/kg/day	Peak >60 ug/ml	Teratogenic
Caffeine in the drinking water in the rat	330 mg/kg/day	Peak >60 ug/ml	Teratogenic
Caffeine in drinking water in the rat	80 mg/kg/day	0.10 to 5.74 µg/mL	Not teratogenic
Caffeine bolus of 25mg, 24 hours later in non pregnant rat	25 mg/kg	2 umol/L 0.4 ug/ml	A pharmacokinetic study
Caffeine bolus of 25mg, 24 hours later in 20 day pregnant rat	25mg/kg	20 umol/L 4 ug/ml	A pharmacokinetic study
Human exposure during pregnancy of a mother who drank 9 to 24 cups of coffee/day (Bodineau et al. 2003, Khanna and Somani 1984)	900 to 2400 mg/day 9mg/kg to 24mg.kg/day	80ug/ml at birth, estimated 40.3ug/ml.at the 12^{th} postpartum day. Maternal serum level on the 10^{th} postpartum day, 18.4ug/ml	No teratogennesis, growth retardation. Liveborn who is doing well and was weight-appropriate for the gestational age.

Table 10
Mechanisms of Action of Environmental Teratogens

1. **Cytotoxicity or mitotic delay beyond the recuperative capacity of the embryo or fetus (ionizing radiation, chemotherapeutic agents, alcohol).**

2. **Inhibition of cell migration, differentiation and cell communication.**

3. **Interference with histogenesis by processes such as cell depletion, necrosis, calcification or scarring.**

4. **Biologic and pharmacological receptor-mediated developmental effects (i.e., etretinate, isotretinoin, retinol, sex steroids, streptomycin, thalidomide).**

5. **Metabolic inhibition (i.e., warfarin, anticonvulsants, nutritional deficiencies).**

6. **Physical constraint, vascular disruption, inflammatory lesions, amniotic band syndrome.**

7. **Interference with nutritional support of the embryo by decreasing maternal food intake or affecting yolk sac or chorioplacental function or transport.**

Table 11

Magnitude of congenital malformation, spontaneous abortion and growth retardation risks

from exposure to caffeine during pregnancy

Effect	Risk	Quality of the Data
Congenital Malformations	**Unlikely**	**Good**
Spontaneous abortion	**Minimal**	**Fair to good**
Fetal growth retardation	**Unlikely**	**Fair to good**

Conclusion: Consumption of caffeinated beverages during pregnancy is unlikely to increase the risk of congenital malformations and growth retardation; and poses a minimal risk for miscarriage, possibly at very, very high caffeine exposures. These risk estimates include the evaluation of inconsistent epidemiology studies and the utilization of animal (mammalian) reproductive studies.