The Role of Environmental Toxicants and Estrogen Metabolism in the Development of Cancer and Chronic Disease:

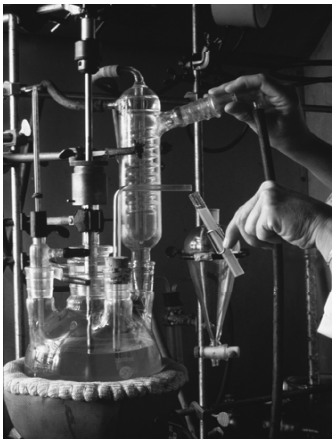
Novel Diagnostic and Therapeutic Approaches

David M. Brady, ND, DC, CCN, DACBN

Our polluted environment

We are continuously adding to the environmental burden by producing more new chemicals. Currently over 100,000 and increasing by about 3 new chemicals per day.

Surgeon General of the United States. Healthy people: the Surgeon General's report on health promotion and disease prevention. 1979, Washington:DHEW Pub. #(PHS)79-55071



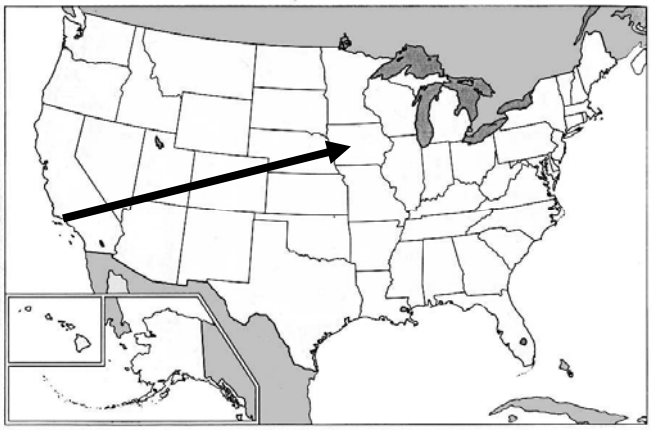
Our polluted environment

In 1989, 5,705,670,380 pounds of chemicals were released. Some went into our air, some our rivers and streams, and some into our ground water supply.

Toxics in the community: national and local perspectives. The 1989 toxics release inventory national report. 1992, United States Environmental Protection Agency, Office of Toxic Substances, Economics and Technology Division, Washington, DC.

Our polluted environment

This is enough to fill a line of semi-trucks stretching from Los Angeles to Des Moines, Iowa with cancer-causing toxins!



Our polluted environment

- **What is the Toxics Release Inventory and what do the data show for 2004?**
- **The Toxics Release Inventory (TRI) is a database containing detailed information on nearly 650 chemicals and chemical categories at over 23,000 industrial and federal facilities pertaining to disposal or other releases, and waste management for recycling, energy recovery, or treatment. This inventory was established under the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA) and expanded by the Pollution Prevention Act of 1990.**
- **For Reporting Year 2004, 23,675 facilities reported to EPA's TRI Program. These facilities reported 4.24 billion pounds of on-site and off-site disposal or other releases of the almost 650 toxic chemicals. Over 87 percent of the total was disposed of or otherwise released on-site; almost 13 percent was sent off-site for disposal or other releases.**

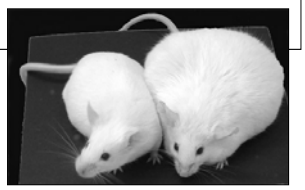
Our polluted environment

Sperm counts declined by an average of 1.5% per year in the U.S. and 3.1% per year in Europe between 1938 and 1990, according to a report by epidemiologists at the California Department of Health Services.

Chemical Week: December 10, 1997

Low doses, big effects: Scientists seek 'fundamental changes' in testing, regulation of hormone-like chemicals

Small doses can have big health effects. That is a main finding of a new report, three years in the making, published in March 2012 by a team of 12 scientists who study hormone-altering chemicals. Dozens of substances that can mimic or block hormones are found in the environment, the food supply and consumer products, including plastics, pesticides and cosmetics. One of the biggest controversies is whether the tiny doses that most people are exposed to are harmful. Researchers led by Tufts University's Laura Vandenberg concluded after examining hundreds of studies that health effects "are remarkably common" when people or animals are exposed to low doses. "Fundamental changes in chemical testing are needed to protect human health," they wrote.



Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses

Laura N. Vandenberg, Theo Colborn, Tyrone B. Hayes, Jerrold J. Heindel, David R. Jacobs, Jr., Duk-Hee Lee, Toshi Shioda, Ana M. Soto, Frederick S. vom Saal, Wade V. Welshons, R. Thomas Zoeller, and John Peterson Myers

Endocrine Reviews, June 2012, 33(3):0000–0000

Our polluted environment

Hum Reprod. 2002 Nov;17(11):2839-41.

Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure.

Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y.

Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.

BACKGROUND: There is broad human exposure to bisphenol A (BPA), an estrogenic endocrine-disrupting chemical widely used for the production of plastic products. BPA is reported to affect preimplantation of embryos or fetuses and alter their postnatal development at doses typically found in the environment. We measured contamination of BPA in various kinds of human biological fluids by a novel enzyme-linked immunosorbent assay.

CONCLUSION: These results suggest accumulation of BPA in early fetuses and significant exposure during the prenatal period, which must be considered in evaluating the potential for human exposure to endocrine-disrupting chemicals.

DANGER

PLASTIC BOTTLE SCARE

Retailers phase out containers made with the chemical BPA. Cause for concern?

Are plastic baby bottles a danger to the infants who drink from them? A preliminary report from the National Institutes of Health suggests that hard plastic made with an ingredient named bisphenol A (BPA), found in baby and reusable water bottles, may pose serious health risks. Retailers like Wal-mart and Toys-R-Us announced plans to pull the products from shelves. Here's what else you need to know:

WHAT IS BISPHENOL A?

Bisphenol A is a chemical used in a wide range of products like bicycle helmets, CDs and cell phones, as well as containers designed to hold baby formula and water. Many are marked on the bottom with the following recycling codes: the number 7 inside a triangle, or the letters PC. An epoxy containing BPA is also used in the lining of food cans.

IS BPA A HEALTH RISK?

A 2004 Centers for Disease Control study found traces of BPA—which can leach from containers into food and beverages—in the urine of 93 percent of 2,500 people tested. Whether it is harmful to humans has not been proven. But studies in animals suggest that BPA mimics the female hormone estrogen and can cause breast and prostate cancer, thyroid disease, early puberty in females and decreased sperm counts in males. "There is enough there that we can't dismiss the possibility there might be similar effects in humans," says Dr. Michael D. Shelby of the NIH's National Toxicology Program. The plastics industry, however, maintains the products are safe.

WHO IS AT RISK?

Because of their developing organs, fetuses, infants and children could be most affected by low-level exposure to BPA. The recent NIH study reports "negligible concern" for adults.

HOW CAN I AVOID EXPOSURE?

Use products stamped with recycling codes 1, 2 or 5, which denote BPA-free hard plastics. Glass bottles are safe, as are stainless steel or aluminum water bottles. Also, avoid heating BPA products in the microwave, says Michael Schade of the Center for Health, Environment and Justice: "BPA tends to leach faster with higher temperatures."

By Champ Clark

WHAT TO LOOK FOR

Check the bottom of a bottle. Many BPA products are stamped with a recycling code 7 inside a triangle, or the letters PC. Bottles with codes 1, 2 or 5 are

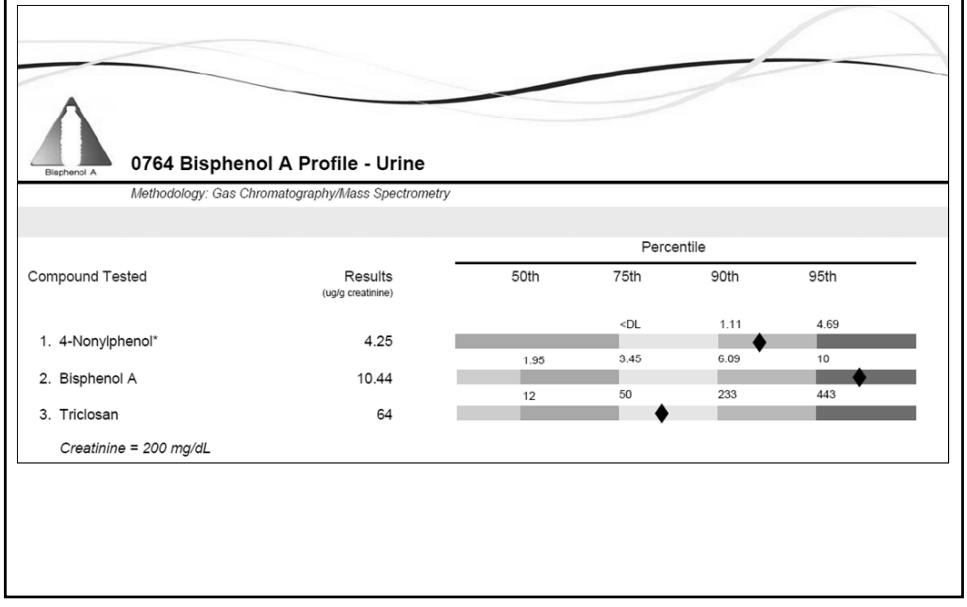
For more info on BPA, go to www.nieh.nih.gov or www.fda.gov

Bottle makers like Nalgene (right) plan to stop using BPA—but others, including Avent, insist the chemical is safe.

AVENT

nalgene

146 May 19, 2008 PEOPLE



Bisphenol A

0764 Bisphenol A Profile - Urine

Methodology: Gas Chromatography/Mass Spectrometry

Compound Tested	Results (ug/g creatinine)	Percentile 50th	75th	90th	95th
1. 4-Nonylphenol*	4.25		<DL	1.11	4.69
2. Bisphenol A	10.44		1.95	3.45	6.09	10
3. Triclosan	64		12	50	233	443

Creatinine = 200 mg/dL

Bisphenol A

- Weak estrogenic activity
  - **MCF-7 human breast cancer cells**
- Block epidermal cytosolic sulfotransferases and increase estrogen levels
- Methyl and propyl forms are potent mitochondrial toxins
  - **Possible role in male infertility**

Byford SJ. Et al J Steroid Biochem 2002;80:49-60
Prusakeiwicz JJ, et al. Toxicol 2007;232)258-56
Tavares RS, et al. Reprod Toxicol 2009;27:1-7

Our polluted environment

More than 80 percent of infants tested in a new study had been exposed to a potentially harmful group of chemicals known as **phthalates**. Some animal studies have found these substances to be harmful to development, and one study on human infants found an association between exposure to a particular phthalate and male reproductive problems. Baby lotion, baby shampoo and baby powder were all linked to phthalate exposure in the study.

Phthalates are a group of widely used chemicals that make plastic softer and help stabilize fragrance in personal care products. These chemicals are found in children's toys, infant care products, cosmetics, food packaging, vinyl flooring, blood storage containers and more, according to the U.S. Centers for Disease Control and Prevention (CDC). Phthalates are banned from use in personal care products and in some toys in Europe.

"*We believe that there is potential value in the study of metabolized phthalates. But we take great exception to any effort to draw unfounded conclusions that suggest human health risks are associated with the mere presence of very low levels of metabolized phthalates in urine*," Marian Stanley, manager of the Phthalates Esters Panel of the American Chemistry Council, a plastics industry trade group, said in a statement. ***Pediatrics. 2008 Feb;121(2):e260-8.***

0740 Phthalates and Parabens - Urine

Methodology: Gas Chromatography/Mass Spectrometry

Compound Tested	Results ug/g creatinine	Percentile 50th	75th	90th	95th
Phthalates - Metabolites of DEHP (Di-2-ethylhexyl phthalate)					
1. MEHHP	95	19	41	99	179
2. MEHP	21	2.4	5.2	11.8	22.1
3. MEOHP	42	11	22	53	107
4. MEtP	178	115	312	835	1500
Parabens					
5. Butylparaben	23.6	<DL	1.2	9.8	23.1
6. Ethylparaben	78.1	<DL	5.5	32.7	78.8
7. Methylparaben	1000	61	228	535	817
8. Propylparaben	234	8	50	138	235

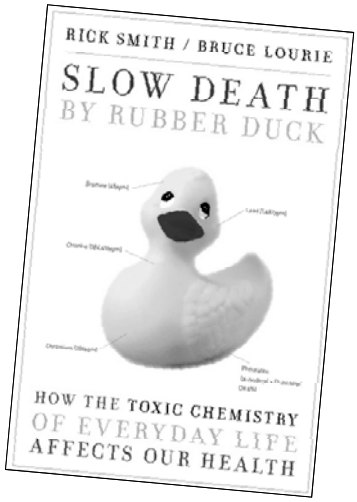
Creatinine = 205 mg/dL

Breast Cancer

- Highest levels of MEP (phthalates) had an odds ratio (OR) of 2.2 for developing breast cancer
- For premenopausal women the OR was 4.13

Lopez-Carrillo L, et al. Environ Health Perspect 2010;118:539-44

Slow Death by Rubber Duck



- 2 day avoidance, followed by 2 day exposure
  (normal modern living)
- MEP went from 64 to 1,410 ng/ml after 2 days of normal modern living
- Mostly personal care products

Chlorinated Pesticides

0760 Chlorinated Pesticides - Serum

Methodology: Gas Chromatography/Mass Spectrometry

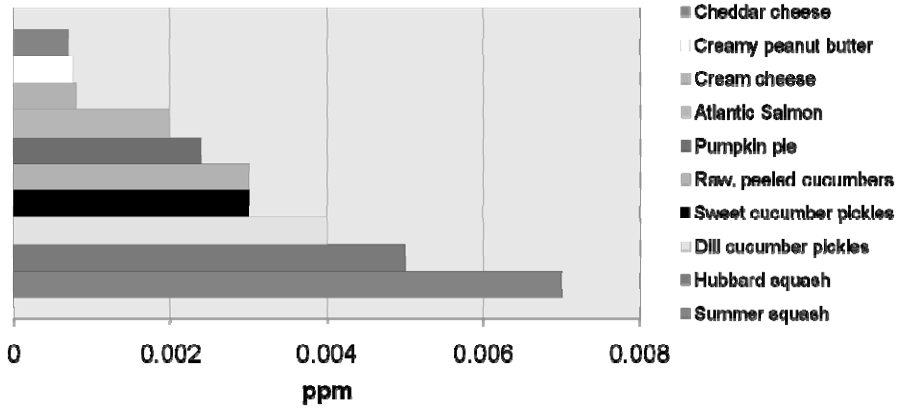
Compound Tested	Results ppb	95th Percentile** ppb	Lipid Adjusted Results (ng/g lipid)	95th Percentile** (ng/g lipid)
1. DDE	Detected	12.1	Detected	1860
	0.08 - 0.26*		14.4 - 46.8*	
2. DDT	Not Detected	0.13	N/A	19.5
3. Dieldrin	Detected	0.14	Detected	19.0
	1.22		218	
4. Heptachlor Epoxide	Not Detected	0.13	N/A	18.9
5. Hexachlorobenzene (HCB)	Not Detected	0.19	N/A	28.9
6. Mirex	Not Detected	0.09	N/A	13.2
7. Oxychlordane	Not Detected	0.27	N/A	37.7
8. trans-Nonachlor	Not Detected	0.47	N/A	68.3

Cholesterol	180	<= 200	mg/dL
Triglycerides	155	35 - 160	mg/dL
Total Lipids (calc.)†	6		g/L

Dieldrin Health Effects

- Disruption of dopamine transport in the brain
  - **Significantly associated with Parkinson's Dz.**
- Endocrine:
  - **Increases rates of hypothyroidism**
  - **Reduces production of testosterone (Leydig Cells) - infertility**
- Cancers – lung, breast, pancreatic
- Increases superoxide production and neutrophil inflammatory responses

Food Exposure to Dieldrin Source: FDA - TDS



HCE Exposure Sources

- Household dust
- Outdoor dirt and dust brought indoors
- Diet:
  - **Butter**
  - **Cream cheese**
  - **Atlantic Salmon**
  - **Ground beef**
  - **Swiss and cheddar cheeses**
  - **Hubbard squash**
  - **Breast milk**

HCE Adverse Health Effects

- Initiator, promoter and progressor of <u>breast cancer</u>
- Increased risk of non-Hodgkins Lymphoma (OR 1.82 to 3.41)
- Neurotoxic to the dopaminergic system and may lead to increased risk for Parkinsonism
- Increased rates of atherosclerosis

Quintana PJ, et al. Environ Health Perspect 2004;112:854-61
Richardson JR, et al. Neurotoxicity 2008;29:855-863
Pines A, et al. Sci Total Environ 1986;54:135-55
Pierik FH, et al. Environ Res 2007:105:364-9
Cassidy RA, et al. Breast Cancer Res Treat 2005;90:55-64
Khanjani N, et al. Arch Environ Contam Toxicol 2006;50:452-61

PCBs

0761 Polychlorinated Biphenyls (PCBs)

Methodology: Gas Chromatography/Mass Spectrometry

Compound Tested	Results (ppb)	95th Percentile** (ppb)	Lipid Adjusted Results (ng/g lipid)	95th Percentile** (ng/g lipid)
Dioxin-like Polychlorinated Biphenyls				
1. PCB 118	Not Detected	0.22	N/A	31.3
2. PCB 126	Not Detected	0.00048	N/A	0.069
3. PCB 156	Not Detected	0.10	N/A	15.3
4. PCB 169	Not Detected	0.00027	N/A	0.041
Non-Dioxin-like Polychlorinated Biphenyls				
5. PCB 74	Not Detected	0.15	N/A	22.3
6. PCB 138	Detected	0.48	Detected	75.3
	0.29		52.03	
7. PCB 153	Detected	0.62	Detected	97.1
	0.63		113	
8. PCB 180	Detected	0.53	Detected	81.5
	0.08 - 0.26*		14.4 - 46.6*	

Cholesterol	185	<= 200	mg/dL
Triglycerides	152	35 - 160	mg/dL
Total Lipids (calc.)†	6		g/L

PCB Exposure Sources

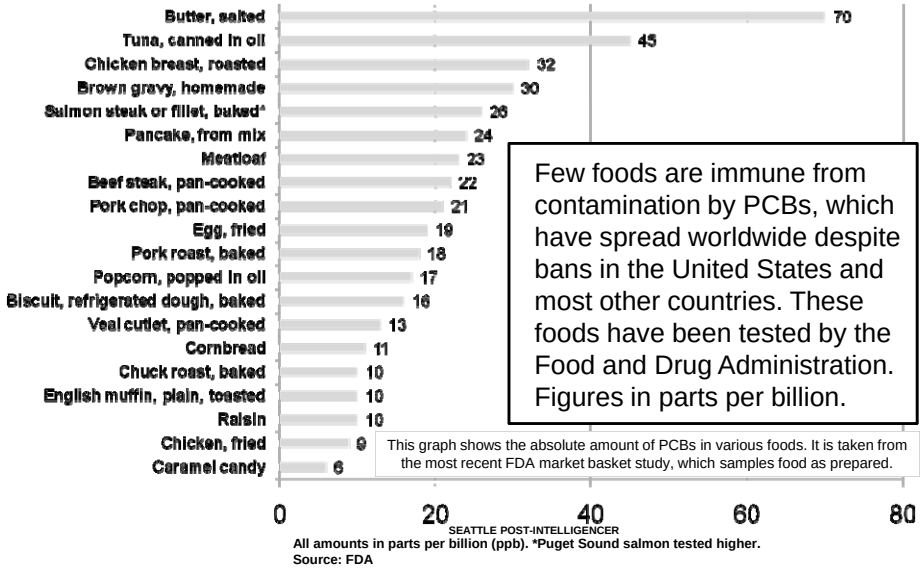
- The greatest exposure source for PCBs currently is Atlantic (farmed) salmon, followed by butter
- Persons who fish in the Great Lakes and consume their catch can also have very elevated levels of PCBs in their blood

Anderson HA, et al. *Environ Health Perspect* 1998;106:279-289

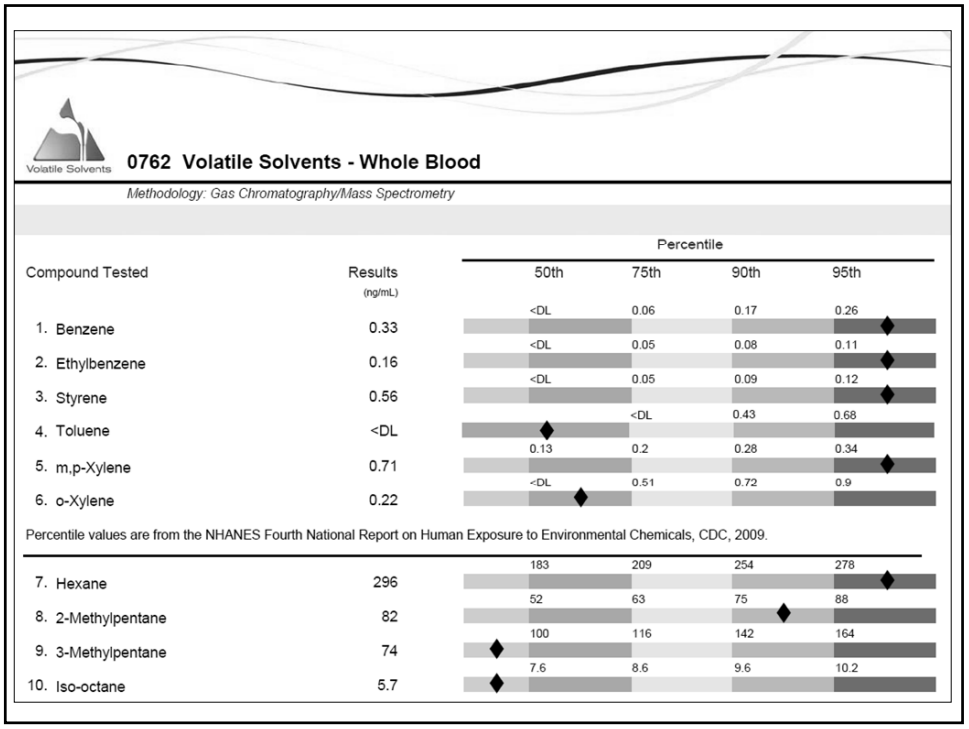
More on Exposure Sources

- PCBs also concentrate in breast milk giving infants who are breast fed daily exposures
  - **Numerous studies from around the globe have consistently documented PCBs in breast milk samples**
- PCB exposure has also been attributed to inhalation of indoor air in buildings that have old electrical fixtures present that still contain PCBs

Avoid Foods Containing PCBs



Few foods are immune from contamination by PCBs, which have spread worldwide despite bans in the United States and most other countries. These foods have been tested by the Food and Drug Administration. Figures in parts per billion.



Volatile Solvents

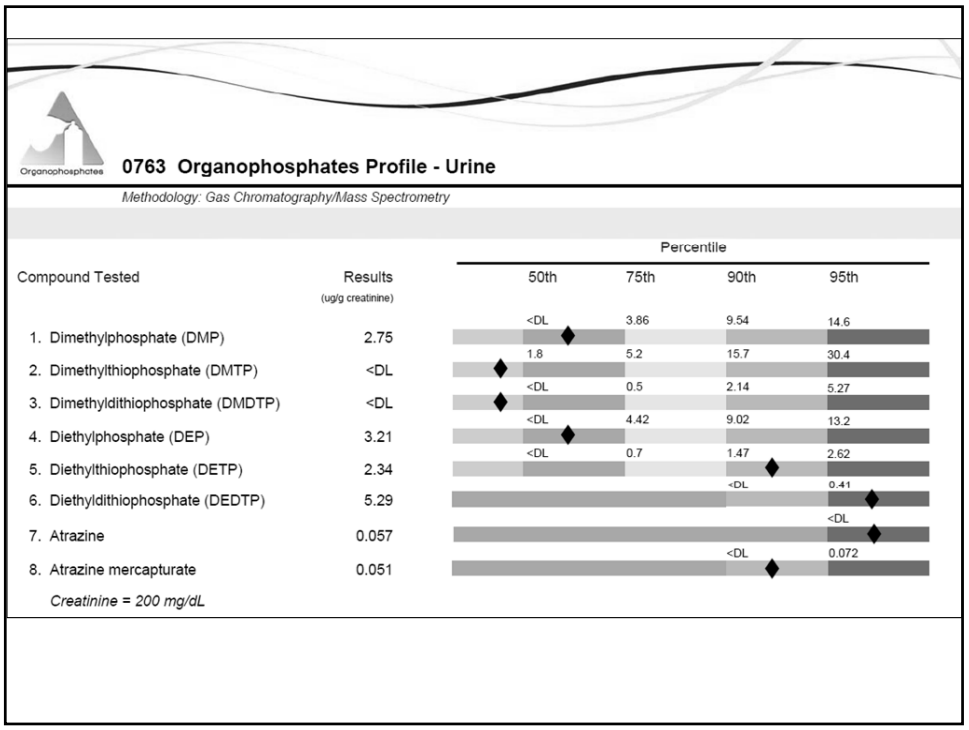
0762 Volatile Solvents - Whole Blood

Methodology: Gas Chromatography/Mass Spectrometry

Compound Tested	Results (ng/mL)	Percentile 50th	75th	90th	95th
1. Benzene	0.33	<DL	0.06	0.17	0.26
2. Ethylbenzene	0.16	<DL	0.05	0.08	0.11
3. Styrene	0.56	<DL	0.05	0.09	0.12
4. Toluene	<DL	<DL		0.43	0.68
5. m,p-Xylene	0.71	0.13	0.2	0.28	0.34
6. o-Xylene	0.22	<DL	0.51	0.72	0.9

Percentile values are from the NHANES Fourth National Report on Human Exposure to Environmental Chemicals, CDC, 2009.

7. Hexane	296	183	209	254	278
8. 2-Methylpentane	82	52	63	75	88
9. 3-Methylpentane	74	100	116	142	164
10. Iso-octane	5.7	7.6	8.6	9.6	10.2



Organophosphates

0763 Organophosphates Profile - Urine

Methodology: Gas Chromatography/Mass Spectrometry

Compound Tested	Results (ug/g creatinine)	Percentile 50th	75th	90th	95th
1. Dimethylphosphate (DMP)	2.75	<DL	3.86	9.54	14.6
2. Dimethylthiophosphate (DMTP)	<DL	1.8	5.2	15.7	30.4
3. Dimethyldithiophosphate (DMDTP)	<DL	<DL	0.5	2.14	5.27
4. Diethylphosphate (DEP)	3.21	<DL	4.42	9.02	13.2
5. Diethylthiophosphate (DETP)	2.34	<DL	0.7	1.47	2.62
6. Diethyldithiophosphate (DEDTP)	5.29			<DL	0.41
7. Atrazine	0.057				<DL
8. Atrazine mercapturate	0.051			<DL	0.072

Creatinine = 200 mg/dL

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Common Herbicide Disrupts Human Hormone Activity In Cell Studies

ScienceDaily (May 8, 2008) — A common weedkiller in the U.S., already suspected of causing sexual abnormalities in frogs and fish, has now been found to alter hormonal signaling in human cells, scientists from the University of California San Francisco (UCSF) report.

enlarge

The herbicide atrazine is the second most widely used weed killer in the U.S., applied to corn and sorghum fields throughout the Midwest and also spread on suburban lawns and gardens. It was banned in Europe after studies linked the chemical to endocrine disruptions in fish and amphibians.

See also:

Health & M
- Human
- Hormon
- Pregnar
  Childbirt

Earth & Cli
- Environ
  Issues
- Sustaina
- Geoche

Reference
- Herbicide
- Oily fish
- Dead zone (ecology)
- Antifreeze protein

In studies with human placental cells in culture, the UCSF scientists found that atrazine increased the activity of a gene associated with abnormal human birth weight when

ed weedkiller
um fields
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EDSP testing
Endocrine Disruptor Screening tests ecotoxicology

Our polluted environment

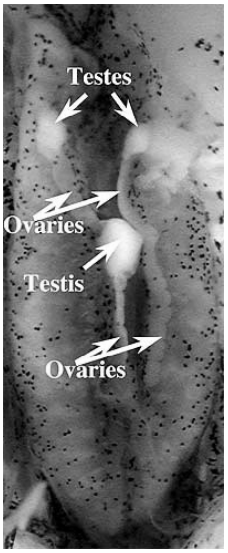
In an article in the April 16, 2002 issue of *Proceedings of the National Academy of Sciences,* University of California, Berkeley, developmental endocrinologist Tyrone B. Hayes, associate professor of integrative biology, and his colleagues report that atrazine at levels often found in the environment demasculinizes tadpoles and turns them into hermaphrodites - creatures with both male and female sexual characteristics. The herbicide also lowers levels of the male hormone testosterone in sexually mature male frogs by a factor of 10, to levels lower than those in normal female frogs.

Our polluted environment

Popular Pesticide Faulted for Frogs' Sexual Abnormalities

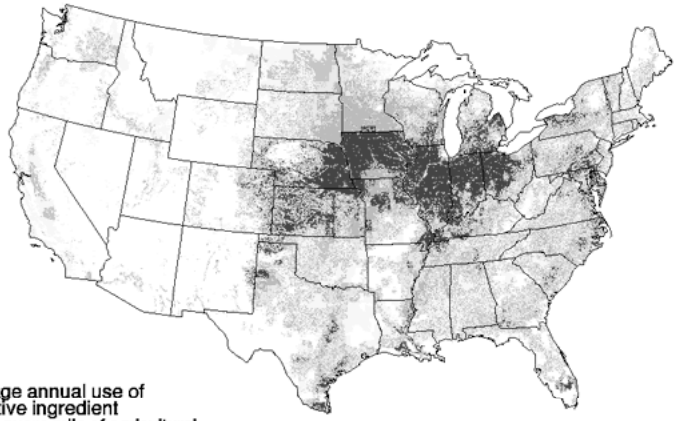
Scientists from the *Environmental Protection Agency* say there is "sufficient evidence" to conclude that the country's most widely used pesticide, atrazine, causes sexual abnormality in frogs.

JENNIFER LEE
(NY Times June 19, 2003)



ATRAZINE - herbicide

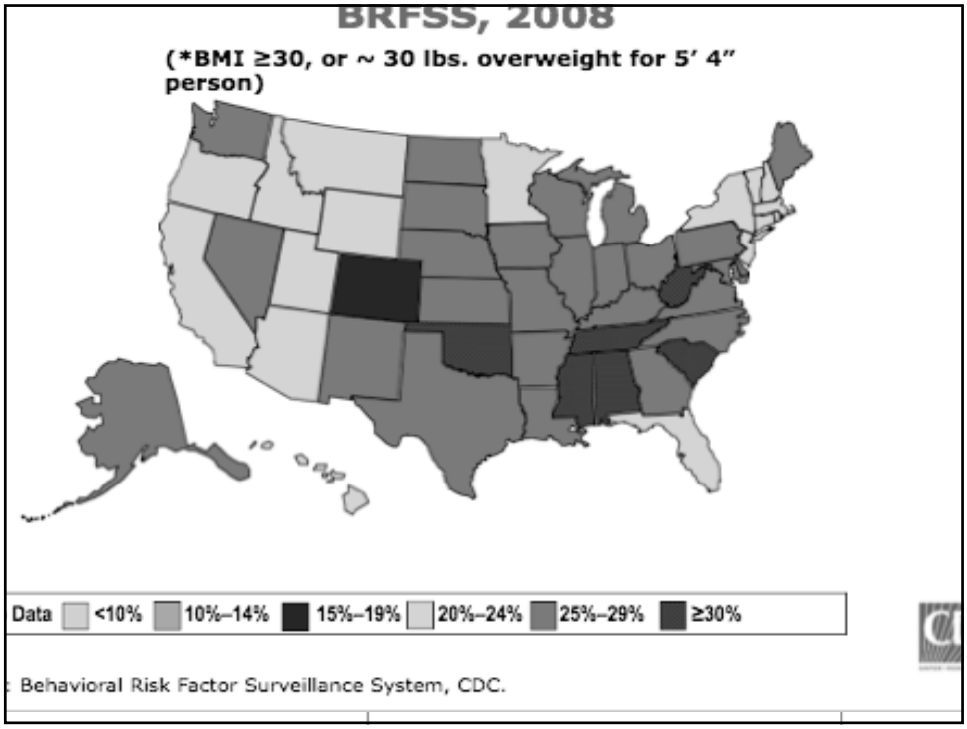
1997 estimated annual agricultural use



Average annual use of active ingredient (pounds per square mile of agricultural land in county)

- no estimated use
- 0.001 to 0.358
- 0.359 to 2.151
- 2.152 to 9.855
- 9.856 to 32.77
- >= 32.771

Crops	Total pounds applied	Percent national use
corn	62, 381, 038	84. 00
sorghum	6, 750, 038	9. 09
summer fallow	2, 539, 169	3. 42
sugarcane	2, 203, 421	2. 97
sweet corn	340, 452	0. 46
sod harvested	30, 214	0. 04
other hay	13, 224	0. 02
seed crops	5, 833	0. 01



Chronic Exposure to the Herbicide, Atrazine, Causes Mitochondrial Dysfunction and Insulin Resistance

Soo Lim[1], Sun Young Ahn[4], In Chan Song[2], Myung Hee Chung[3], Hak Chul Jang[1], Kyong Soo Park[1], Ki-Up Lee[5], Youngmi Kim Pak[4]*, Hong Kyu Lee[1]*

1 Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea, 2 Department of Radiology, Seoul National University College of Medicine, Seoul, Korea, 3 Department of Pharmacology, Seoul National University College of Medicine, Seoul, Korea, 4 Age-Related and Brain Diseases Research Center, Department of Nanopharmaceutical and Life Sciences, Department of Physiology, Kyung Hee University College of Medicine, Seoul, Korea, 5 Department of Internal Medicine, University of Ulsan College of Medicine, Seoul, Korea

Abstract

There is an apparent overlap between areas in the USA where the herbicide, atrazine (ATZ), is heavily used and obesity-prevalence maps of people with a BMI over 30. Given that herbicides act on photosystem II of the thylakoid membrane of chloroplasts, which have a functional structure similar to mitochondria, we investigated whether chronic exposure to low concentrations of ATZ might cause obesity or insulin resistance by damaging mitochondrial function. Sprague-Dawley rats (n = 48) were treated for 5 months with low concentrations (30 or 300 μg kg^{-1} day^{-1}) of ATZ provided in drinking water. One group of animals was fed a regular diet for the entire period, and another group of animals was fed a high-fat diet (40% fat) for 2 months after 3 months of regular diet. Various parameters of insulin resistance were measured. Morphology and functional activities of mitochondria were evaluated in tissues of ATZ-exposed animals and in isolated mitochondria. Chronic administration of ATZ decreased basal metabolic rate, and increased body weight, intra-abdominal fat and insulin resistance without changing food intake or physical activity level. A high-fat diet further exacerbated insulin resistance and obesity. Mitochondria in skeletal muscle and liver of ATZ-treated rats were swollen with disrupted cristae. ATZ blocked the activities of oxidative phosphorylation complexes I and III, resulting in decreased oxygen consumption. It also suppressed the insulin-mediated phosphorylation of Akt. These results suggest that long-term exposure to the herbicide ATZ might contribute to the development of insulin resistance and obesity, particularly where a high-fat diet is prevalent.

Citation: Lim S, Ahn SY, Song IC, Chung MH, Jang HC, et al. (2009) Chronic Exposure to the Herbicide, Atrazine, Causes Mitochondrial Dysfunction and Insulin Resistance. PLoS ONE 4(4): e5186. doi:10.1371/journal.pone.0005186

Editor: German Malaga, Universidad Peruana Cayetano Heredia, Peru

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Funding: This work ... based on nanosenso... a grant from the 21C... had no role in study...

Competing Interes...

* E-mail: ykpak@khu...

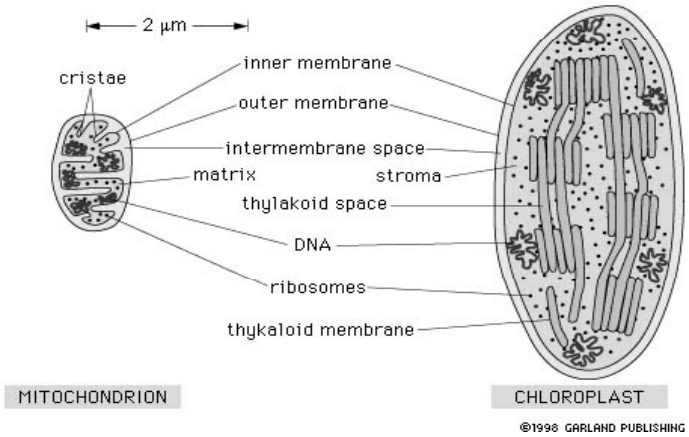
Mitochondria in skeletal muscle and liver of ATZ-treated rats were swollen with disrupted cristae. ATZ blocked the activities of oxidative phosphorylation complexes I and III, resulting in decreased oxygen consumption. These results suggest that long-term exposure to the herbicide ATZ might contribute to the development of insulin resistance and obesity, particularly where a high-fat diet is prevalent.

Introduction

A close associa... insulin resistance i... found that artific... induced insulin re... studies showing tha... toxin causes insulin...

Persistent organ... and water may acc... up the food chain, ... recently been associ... concentration-depe... atrazine (ATZ, ... azine), has been extensively used in the USA since the early 1960s, a time frame that corresponds to the beginning of the present obesity epidemic [7,8]. Because it is moderately persistent under normal soil condition and has low to moderate water-solubility, ATZ is routinely found as a contaminant in many surface and ground waters [9,10]. Maps of ATZ usage show that the Corn Belt region of the Midwest USA has the heaviest ... similar Q_i binding sites, we hypothesized that ATZ might bind to these mitochondrial sites, resulting in the suppression of mitochondrial oxidative phosphorylation. Previous studies have shown that exposure to ATZ reduces metabolic activity in the gills of fish [13] and induces cellular DNA damage [14–18], tumorigenesis [19–22], and hermaphroditism of exposed male frogs [23]. In the present study, we found that chronic exposure to low concentra...

Designed to Kill Plants- Kills Mitochondria



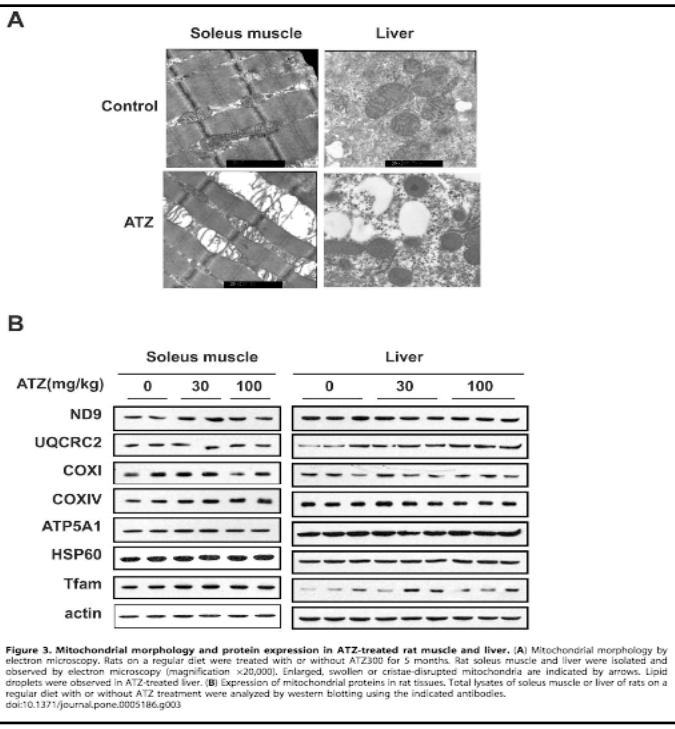


Figure 3. Mitochondrial morphology and protein expression in ATZ-treated rat muscle and liver. (**A**) Mitochondrial morphology by electron microscopy. Rats on a regular diet were treated with or without ATZ300 for 5 months. Rat soleus muscle and liver were isolated and observed by electron microscopy (magnification ×20,000). Enlarged, swollen or cristae-disrupted mitochondria are indicated by arrows. Lipid droplets were observed in ATZ-treated liver. (**B**) Expression of mitochondrial proteins in rat tissues. Total lysates of soleus muscle or liver of rats on a regular diet with or without ATZ treatment were analyzed by western blotting using the indicated antibodies.
doi:10.1371/journal.pone.0005186.g003

Our polluted environment

THE GLOBE AND MAIL

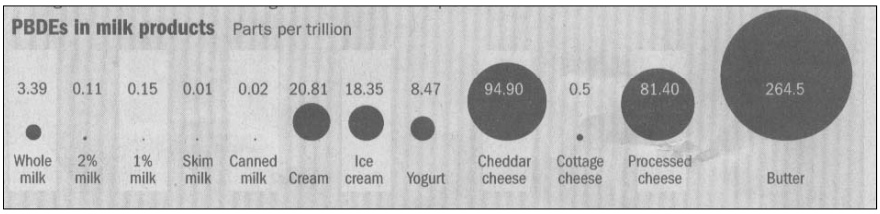
TUESDAY, MAY 30, 2006

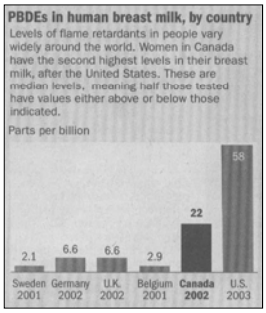
Ottawa plans to snuff out flame retardants

Researchers link carpets to stereos, televisions and computers. could offer a clue for the sudden rise of these childhood disorders in

Flame retardants contain polybrominated diphenyl ethers (PBDEs). Regulators are considering drastic action, including placing these chemicals on a banned list in Canada, because of studies linking PBDEs to behavior changes that bear an uncanny resemblance to attention-deficit disorder common in children. These flame-retardant chemicals are not staying put in consumer products, but have been migrating from mattresses and computers in ways that are not completely understood, into the environment and into people.

Our polluted environment

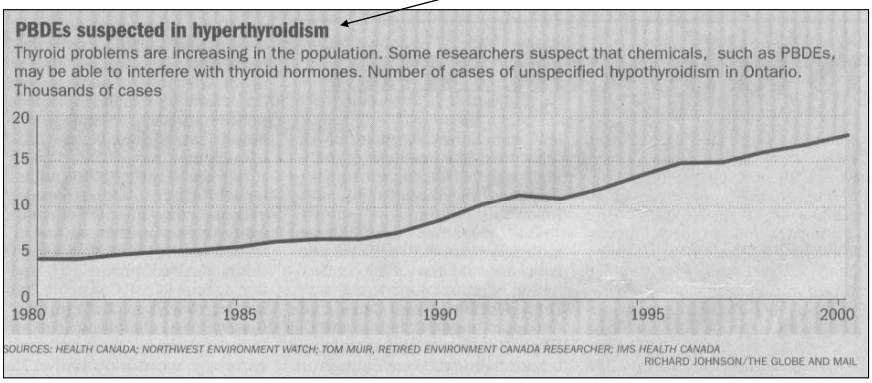




Our polluted environment

PBDEs and Hypothyroidism

*Typo (should be hypothyroidism)



Our polluted environment

Toxicity and Diabetes

A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002.

Lee DH, **Lee IK**, **Song K**, **Steffes M**, **Toscano W**, **Baker BA**, **Jacobs DR Jr**.

OBJECTIVE: Low-level exposure to some persistent organic pollutants (POPs) has recently become a focus because of their possible link with the risk of diabetes.

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Low-level exposure to some persistent organic pollutants (POPs) has recently become a focus because of their possible link with the risk of diabetes.

CONCLUSIONS: There were striking dose-response relations between serum concentrations of six selected POPs and the prevalence of diabetes.

CONCLUSIONS: There were striking dose-response relations between serum concentrations of six selected POPs and the prevalence of diabetes. The strong graded association could offer a compelling challenge to future epidemiologic and toxicological research.

Diabetes Care. 2006 Jul;29(7):1638-44.

Published on Tuesday, November 9, 2010 by Environment News Service

Chemicals in Fast Food Wrappers Show Up in Human Blood

TORONTO, Ontario, Canada - Chemicals used to keep grease from leaking through fast food wrappers and microwave popcorn bags are migrating into food, being ingested by people and showing up as contaminants in blood, according to new research at the University of Toronto.

The contaminants are perfluoroalkyls, stable, synthetic chemicals that repel oil, grease, and water. They are used in surface protection products such as carpet and clothing treatments and coating for paper and cardboard packaging.

Chemicals used to keep grease from leaking through fast food wrappers and microwave popcorn bags are migrating into food, being ingested by people and showing up as contaminants in blood, according to new research at the University of Toronto. (photo by Flickr user permanently scatterbrained / eric molina)

Earlier research by University of Toronto environmental chemists Scott Mabury and Jessica D'eon, established in 2007 that the wrappers are a source of these chemicals in human blood. Their new study shows that perfluorinated chemicals can migrate from wrappers into food.

PHYSICAL
- **Injury**
- **Inflammation**
- **Excessive Exercise**

NUTRITIONAL
- **Excess Food**
- **Additives**
- **Alcohol**
- **Trans-fatty acids**

TOTAL TOXIC EXPOSURE

INFECTION
Endotoxins & Exotoxins
- **Bacterial**
- **Fungal**
- **Parasitic**

CHEMICAL
- **Xenobiotics**
- **Organics**

Vegetable Toxins

- The average person consumes 5,000 to 10,000 plant toxins per year
- 20% of cancer deaths are due to natural compounds contained in the diet
- Development of “bitterness” as a taste promoted avoidance of plant based toxins, which are mostly bitter

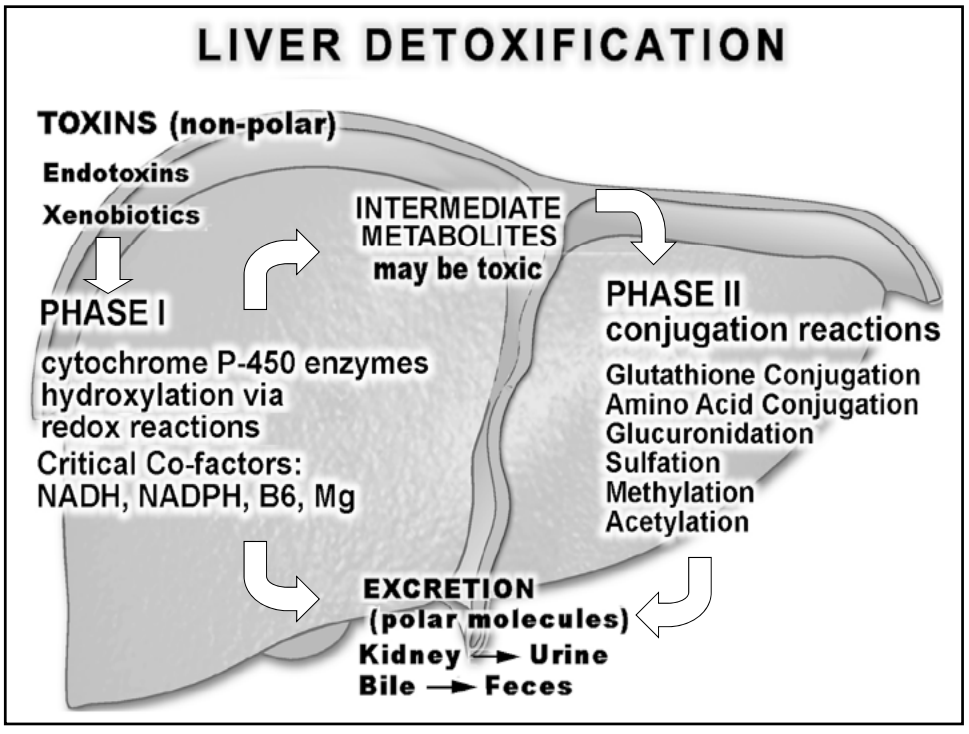
1. AMA Handbook of Poisonous and Injurious Plants (Chicago Review Press, 1985).
2. Toxicants Occurring Naturally in Food (National Academy of Sciences, 1973).
3. Soranzo N, et al. Positive selection on a high-sensitivity allele of human bitter-taste receptor TAS2R16. Curr Biol 15(14):1257-1265, 2005.

Vegetable Toxins: Worse in Organic?

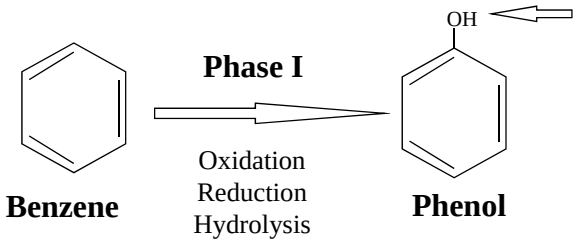
A Few Examples:

- Nightshades (tomato, potato): solanine
- Celery: psoralen
- Cassava (tapioca): konzo (cyanide analog)
- Jimsonweed: scopalamine
- Fava Beans: vicine and convicine
- Cayenne: capsaicin

1. Muller JL. Love potions and the ointment of witches: historical aspects of the nighshade alkaloids. J Toxicol Clin Toxicol 36(6):617-627, 1998.
2. Ljunggren B. Severe photoxic burn following celery ingestion. Arch Dermatol 126(10): 1334-1336, 1990.
3. Ernesto M, et al. Persistent konzo and cyanogenic toxicity from cassava in northern Mozambique. Acta Trop 82(3):357-362, 2002.
4. Berkov S, et al. Alkaloid patterns in some varieties of Datura stramonium (Jimsonweed). Fitoterapia 77(3):179-182, 2006.
5. Golenser J, et al. Inhibitory effect of a fava bean component on the in vitro development of Plasmodium falciparum (Malaria) in normal and G6PD defeicient erythrocytes. Blood 61(3):507-510, 1993.
6. Gazerani P, et al. The impact of ethnic differences in response to capsaicin-induced trigeminal sensitization. Pain 117(1-2): 223-229, 2005.

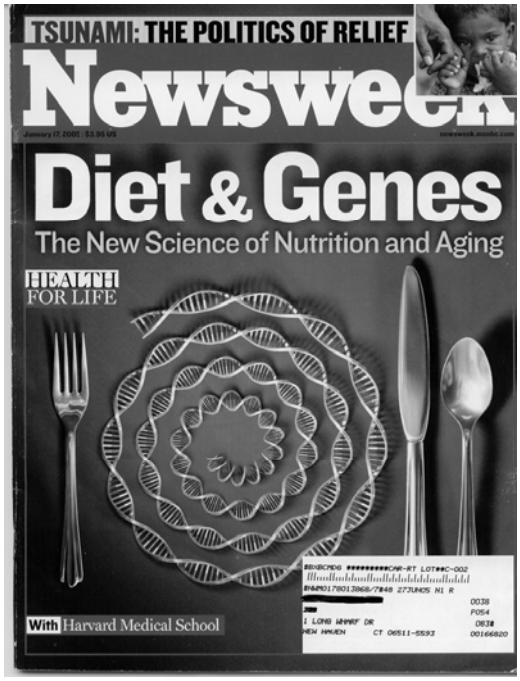


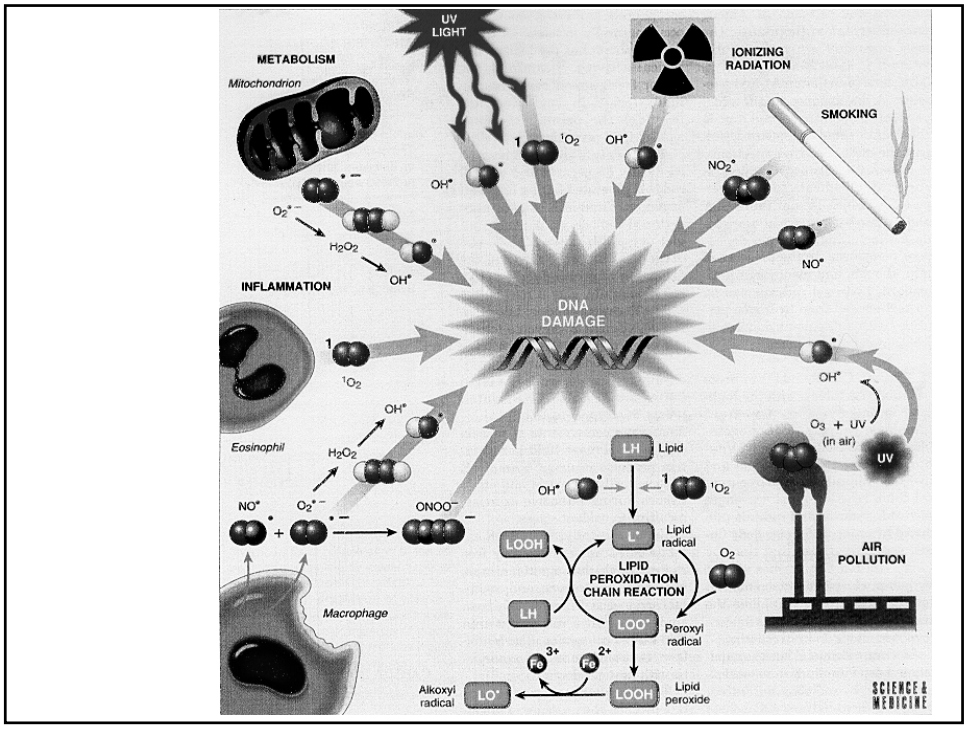
Detoxification pathways in the liver

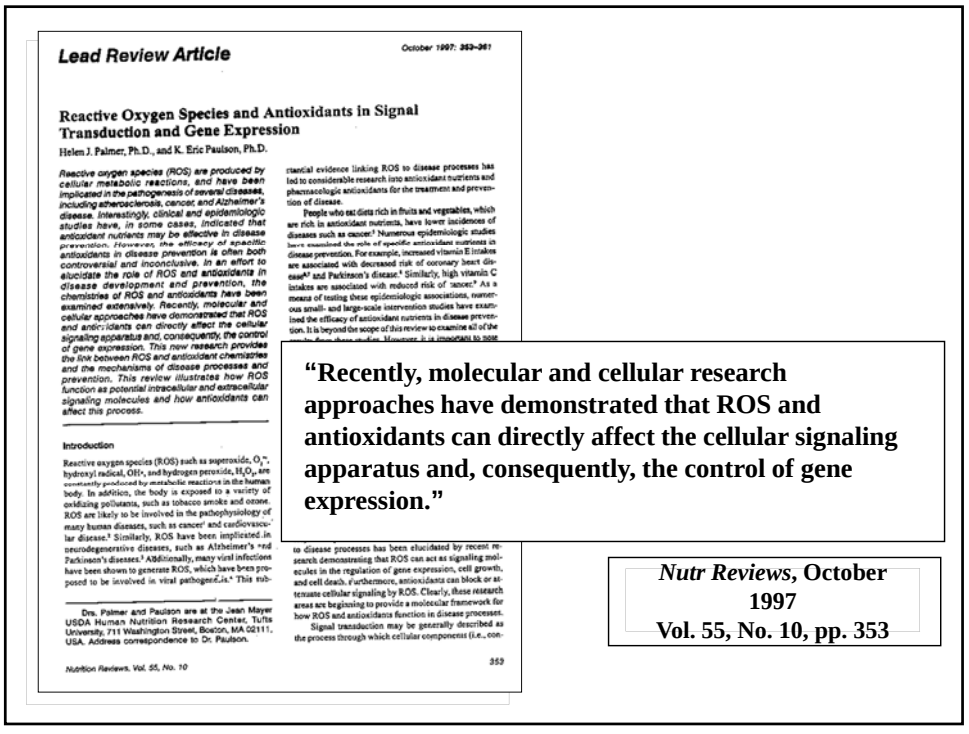


This modification sets the stage for Phase II, ultimately allowing the elimination of the toxin.

Timbrell JA. Principles of biochemical toxicology. London: Taylor & Francis, 1991:75-124







Lead Review Article

October 1997: 353–361

Reactive Oxygen Species and Antioxidants in Signal Transduction and Gene Expression

Helen J. Palmer, Ph.D., and K. Eric Paulson, Ph.D.

Reactive oxygen species (ROS) are produced by cellular metabolic reactions, and have been implicated in the pathogenesis of several diseases, including atherosclerosis, cancer, and Alzheimer's disease. Interestingly, clinical and epidemiologic studies have, in some cases, indicated that antioxidant nutrients may be effective in disease prevention. However, the efficacy of specific antioxidants in disease prevention is often both controversial and inconclusive. In an effort to elucidate the role of ROS and antioxidants in disease development and prevention, the chemistries of ROS and antioxidants have been examined extensively. Recently, molecular and cellular approaches have demonstrated that ROS and antioxidants can directly affect the cellular signaling apparatus and, consequently, the control of gene expression. This new research provides the link between ROS and antioxidant chemistries and the mechanisms of disease processes and prevention. This review illustrates how ROS function as potential intracellular and extracellular signaling molecules and how antioxidants can affect this process.

Introduction

Reactive oxygen species (ROS) such as superoxide, $O_2^{\bullet-}$, hydroxyl radical, $OH^{\bullet}$, and hydrogen peroxide, H_2O_2, are constantly produced by metabolic reactions in the human body. In addition, the body is exposed to a variety of oxidizing pollutants, such as tobacco smoke and ozone. ROS are likely to be involved in the pathophysiology of many human diseases, such as cancer[1] and cardiovascular disease.[2] Similarly, ROS have been implicated in neurodegenerative diseases, such as Alzheimer's[3] and Parkinson's diseases.[4] Additionally, many viral infections have been shown to generate ROS, which have been proposed to be involved in viral pathogenesis.[5] This substantial evidence linking ROS to disease processes has led to considerable research into antioxidant nutrients and pharmacologic antioxidants for the treatment and prevention of disease.

People who eat diets rich in fruits and vegetables, which are rich in antioxidant nutrients, have lower incidences of diseases such as cancer.[6] Numerous epidemiologic studies have examined the role of specific antioxidant nutrients in disease prevention. For example, increased vitamin E intakes are associated with decreased risk of coronary heart disease[7] and Parkinson's disease.[4] Similarly, high vitamin C intakes are associated with reduced risk of cancer.[6] As a means of testing these epidemiologic associations, numerous small- and large-scale intervention studies have examined the efficacy of antioxidant nutrients in disease prevention. It is beyond the scope of this review to examine all of the

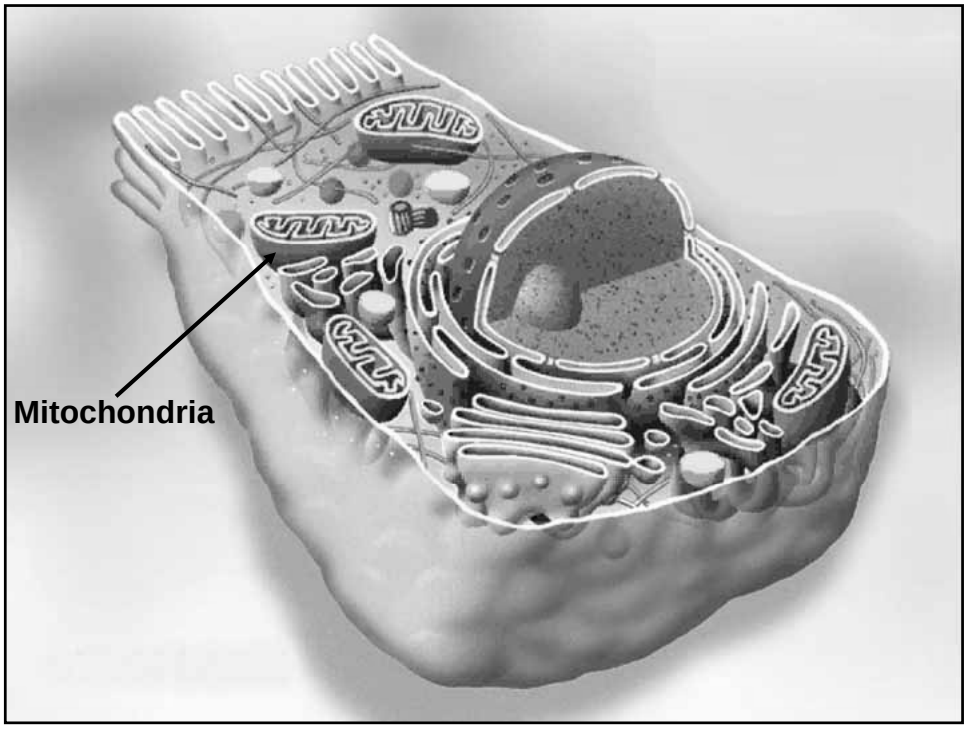
to disease processes has been elucidated by recent research demonstrating that ROS can act as signaling molecules in the regulation of gene expression, cell growth, and cell death. Furthermore, antioxidants can block or attenuate cellular signaling by ROS. Clearly, these research areas are beginning to provide a molecular framework for how ROS and antioxidants function in disease processes.

Signal transduction may be generally described as the process through which cellular components (i.e., con-

Drs. Palmer and Paulson are at the Jean Mayer USDA Human Nutrition Research Center, Tufts University, 711 Washington Street, Boston, MA 02111, USA. Address correspondence to Dr. Paulson.

"Recently, molecular and cellular research approaches have demonstrated that ROS and antioxidants can directly affect the cellular signaling apparatus and, consequently, the control of gene expression."

***Nutr Reviews*, October 1997**
Vol. 55, No. 10, pp. 353



Mitochondrial Uncoupling in the Toxic Patient

Implications for Detoxification

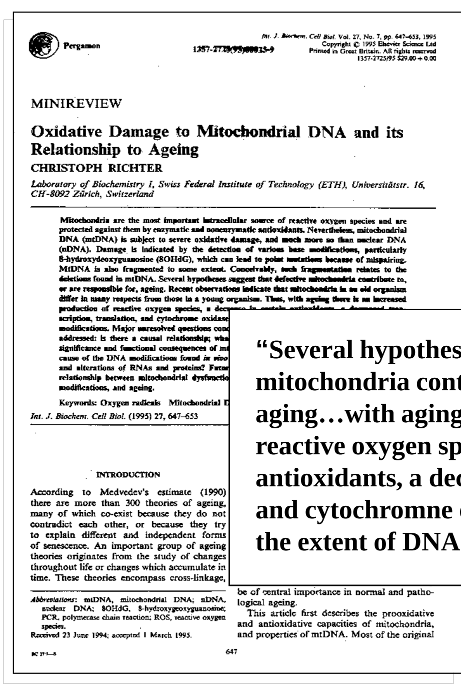
Mitochondria

Mitochondrial DNA is 20X more susceptible to oxidative damage than nuclear DNA

Without adequate energy production, all functional systems are impaired

Important nutrients:

Coenzyme Q_{10}, carnitine, lipoic acid, vitamins C & E, and more



Pergamon

1357-2725(95)00015-9

Int. J. Biochem. Cell Biol. Vol. 27, No. 7, pp. 647–653, 1995

1357-2725/95 $29.00 + 0.00

MINIREVIEW

Oxidative Damage to Mitochondrial DNA and its Relationship to Ageing

CHRISTOPH RICHTER

Laboratory of Biochemistry I, Swiss Federal Institute of Technology (ETH), Universitätstr. 16, CH-8092 Zürich, Switzerland

**Mitochondria are the most important intracellular source of reactive oxygen species and are protected against them by enzymatic and nonenzymatic antioxidants. Nevertheless, mitochondrial DNA (mtDNA) is subject to severe oxidative damage, and much more so than nuclear DNA (nDNA). Damage is indicated by the detection of various base modifications, particularly 8-hydroxydeoxyguanosine (8OHdG), which can lead to point mutations because of mispairing. MtDNA is also fragmented to some extent. Conceivably, such fragmentation relates to the deletions found in mtDNA. Several hypotheses suggest that defective mitochondria contribute to, or are responsible for, ageing. Recent observations indicate that mitochondria in an old organism differ in many respects from those in a young organism. Thus, with ageing there is an increased production of reactive oxygen species, a dec…
scription, translation, and cytochrome oxidase…
modifications. Major unresolved questions conc…
addressed: is there a causal relationship; wha…
significance and functional consequences of m…
cause of the DNA modifications found *in vivo*…
and alterations of RNAs and proteins? Futu…
relationship between mitochondrial dysfunctio…
modifications, and ageing.**

Keywords: Oxygen radicals Mitochondrial D…

Int. J. Biochem. Cell Biol. (1995) 27, 647–653

INTRODUCTION

According to Medvedev's estimate (1990) there are more than 300 theories of ageing, many of which co-exist because they do not contradict each other, or because they try to explain different and independent forms of senescence. An important group of ageing theories originates from the study of changes throughout life or changes which accumulate in time. These theories encompass cross-linkage,

…be of central importance in normal and pathological ageing.

This article first describes the prooxidative and antioxidative capacities of mitochondria, and properties of mtDNA. Most of the original

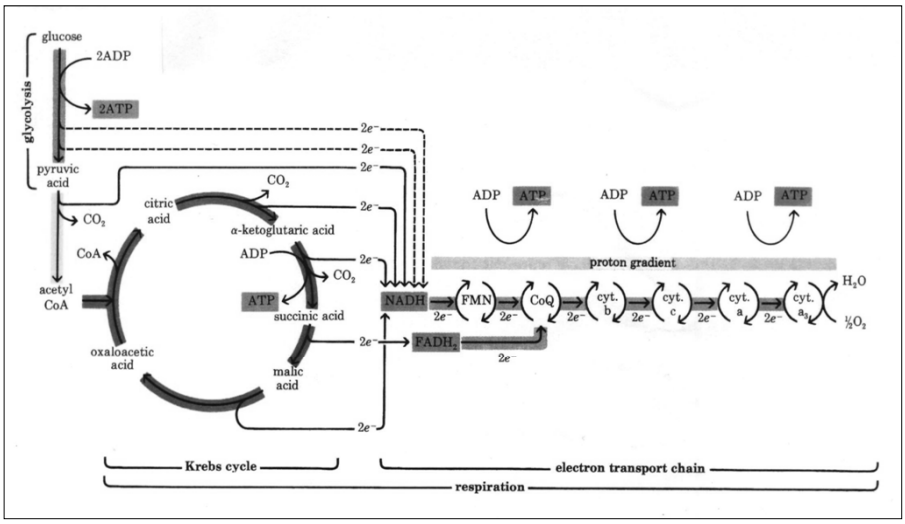
Abbreviations: mtDNA, mitochondrial DNA; nDNA, nuclear DNA; 8OHdG, 8-hydroxydeoxyguanosine; PCR, polymerase chain reaction; ROS, reactive oxygen species.

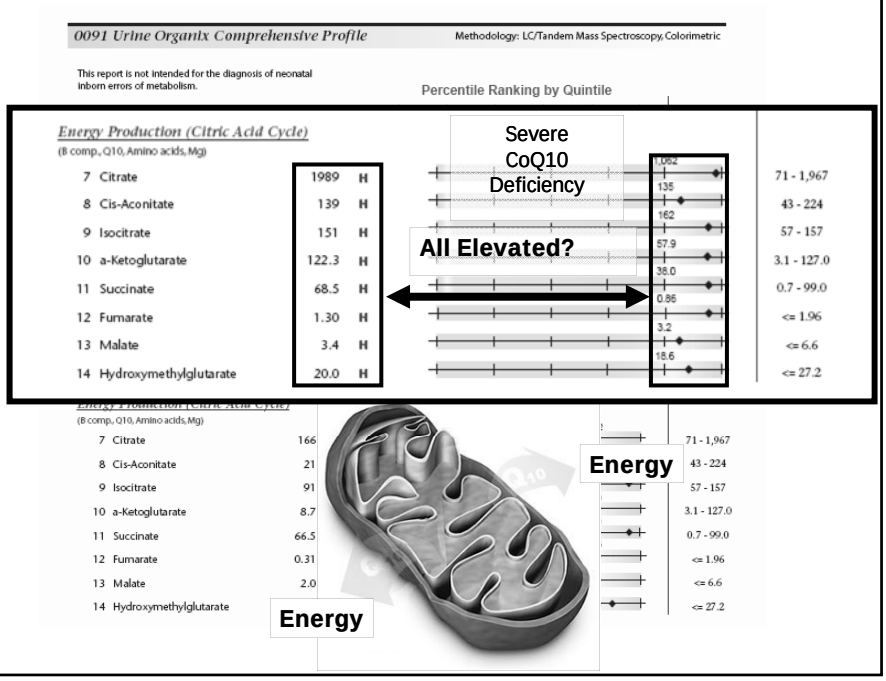
Received 23 June 1994; accepted 1 March 1995.

"Several hypotheses suggest that defective mitochondria contribute to, or are responsible for aging…with aging there is an increased production of reactive oxygen species, a decrease in certain antioxidants, a decreased transcription, translation, and cytochromne oxidase content, and an increase in the extent of DNA modifications."

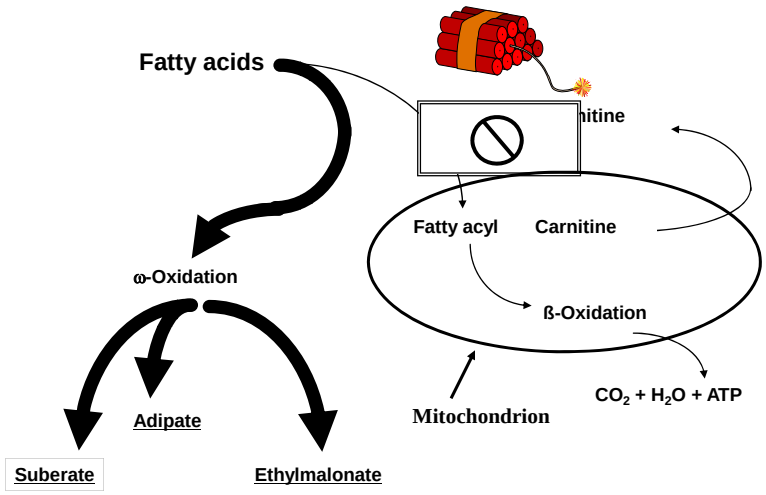
Int J Biochem Cell Biol,
Vol. 27, No. 7, pp. 647-653, 1995

Energy Production

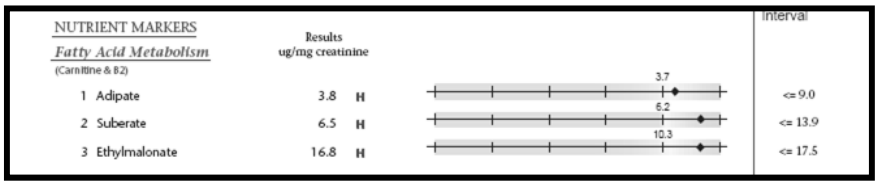




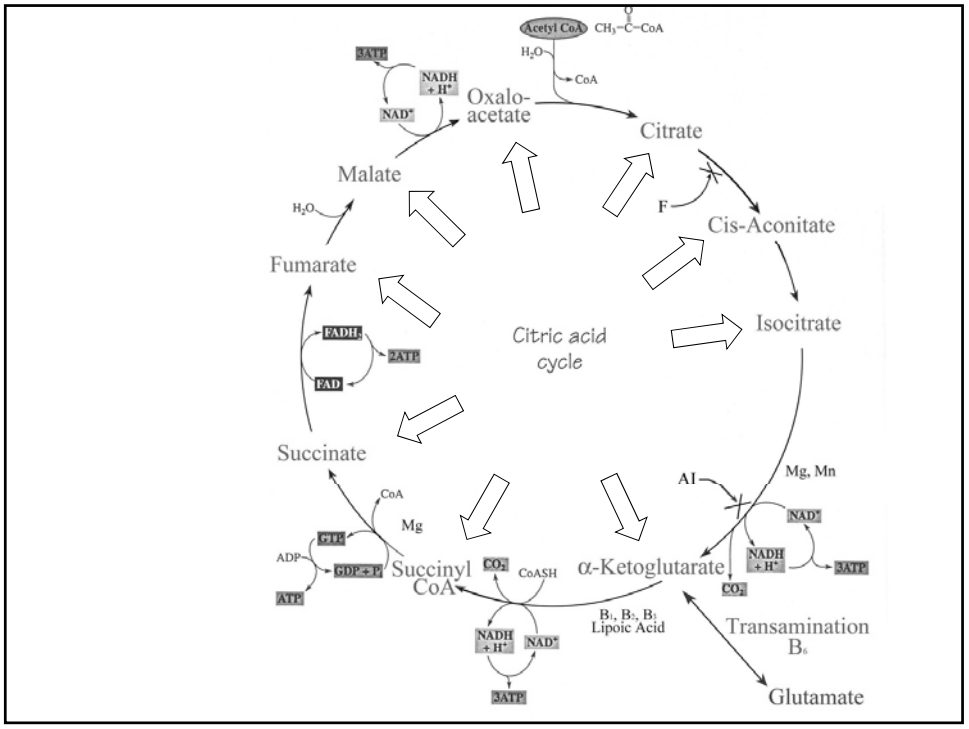
Organic Acids from Carnitine Deficiency



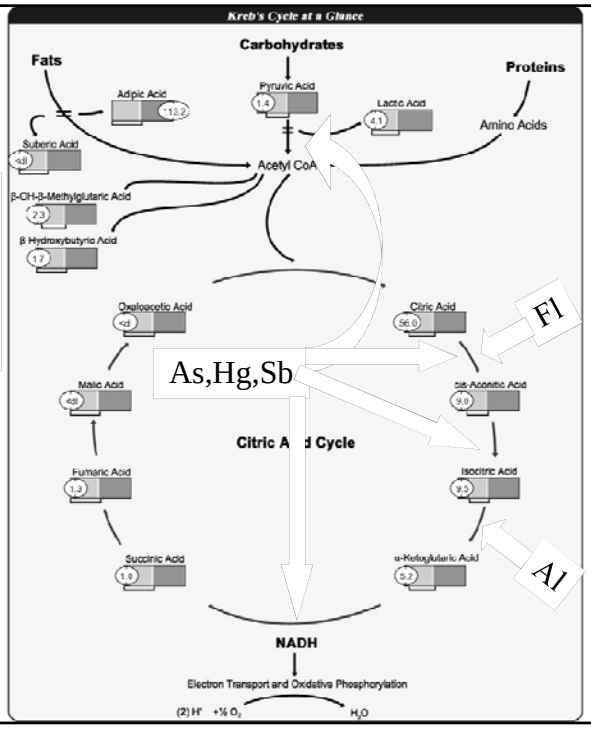
L-Carnitine Markers



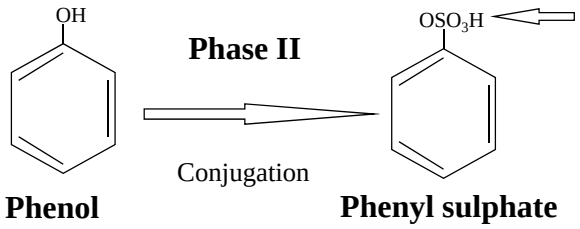
NUTRIENT MARKERS *Fatty Acid Metabolism* (Carnitine & B2)	Results ug/mg creatinine		Interval
1 Adipate	3.8	H	<= 9.0
2 Suberate	6.5	H	<= 13.9
3 Ethylmalonate	16.8	H	<= 17.5



Toxic Elements that may interfere



Phase II



During Phase II, the toxin is further modified by the addition of a molecule making the substance polar and therefore water soluble.

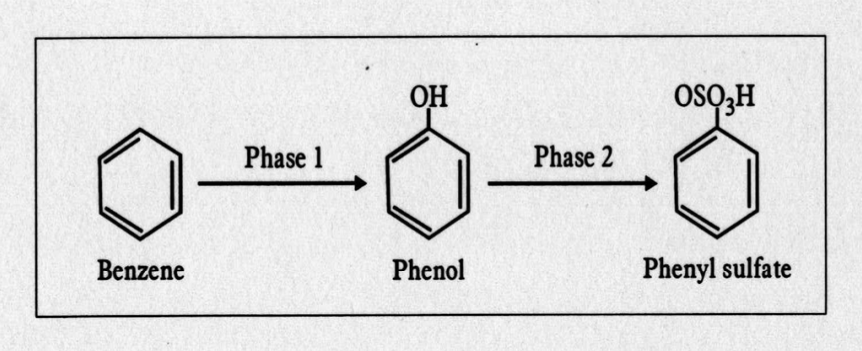
Timbrell JA. Principles of biochemical toxicology. London: Taylor & Francis, 1991:75-124

Detoxification pathways

- Phase II
  - **Conjugation**
    - Sulfate
    - Glycine
    - Glutathione
    - Glucuronide

Timbrell JA. Principles of biochemical toxicology. London: Taylor & Francis, 1991:75-124

Detoxification Chemistry



Symptom Picture of General Toxicity

- General Malaise
  - fatigue, headache, joint & muscle pain
- Chronic Mucous Production
- Poor Exercise Tolerance
- Skin Rashes, Peri-orbital edema
- Immune Weakness
- Environmental and Chemical Sensitivity
- Mental Status Changes
  - lack of concentration, depression, mood changes, confusion, memory loss, sleep disturbance, anxiety, PMS, etc.

Neurological disorders

Illnesses such as Alzheimer's and Parkinson's have been linked to "xenobiotics" (toxic chemicals).

- Steventon GB, et al. Xenobiotic metabolism in Alzheimer's disease. Neurology 1990;40: 1095-98.
- Steventon GB, et al. Xenobiotic metabolism in Parkinson's disease. Neurology 1989;39: 883-87.

Pesticides and Parkinson's Disease

In a study comparing 496 people with newly diagnosed PD to 541 matched controls, "People who recalled using in-home pesticides on at least 160 days of their lives were 70% more likely to develop PD than those who never used pesticides… Using garden pesticides for same number of days conferred a 50% increase in risk." (No relationship was seen with fungicides)

Lorene Nelson, PhD, Stanford University

American Academy of Neurology annual meeting, 2000

Clinical Detoxification

Treatment goals:

- reduce toxin exposure
- increase antioxidant support
- support mitochondrial function
- supplement phase II conjugating substances
  (e.g. glutathione, sulfates, glycine, taurine)

The role of nutritional support

Significant evidence exists to support the contention that diet and nutritional supplementation plays an important role in helping maintain these detoxification pathways.

- Guengerich FP. Effects of nutritive factors on metabolic processes involving bioactivation and detoxication of chemicals. *Annu. Rev. Nutr.* 1984; 4:207-31
- Anderson KE, Kappas A. Dietary regulation of cytochrome P450. *Annu. Rev. Nutr.* 1991; 11:141-167
- Parke DV and Ioannides C. The role of nutrition in toxicology. Annu. Rev. Nutr. 1981; 1:207-34

Macronutrients and detoxification

Total protein and energy requirements

- **In animals, depression of detoxification pathways occurs in as little as 36 hours of fasting**

- Guengerich FP. Effects of nutritive factors on metabolic processes involving bioactiva-tion and detoxication of chemicals. *Annu. Rev. Nutr.* 1984; 4:207-31

Macronutrients and detoxification

"Fasting may…inhibit the metabolism of some xenobiotics. Fasting by depleting glycogen can prevent glucuronidation, leading to a secondary enhancement of P450 activity."

Zimmerman HJ. *Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver*, *Second Edition,* Lippincott Williams & Wilkins, Philadelphia, 1999, p. 28.

Micronutrients and detoxification

B vitamins, vitamin C and minerals

- **Insufficient amounts in any of these nutrients can slow detoxification pathways.**

- Guengerich FP. Effects of nutritive factors on metabolic processes involving bioactivation and detoxication of chemicals. *Annu. Rev. Nutr.* 1984; 4:207-31
- Anderson KE, Kappas A. Dietary regulation of cytochrome P450. *Annu. Rev. Nutr.* 1991; 11:141-167
- Parke DV and Ioannides C. The role of nutrition in toxicology. Annu. Rev. Nutr. 1981; 1:207-34

Micronutrients and detoxification

Sulfhydryl amino acids

- **Sulfation is an important component of Phase II detoxification pathways.**

- Guengerich FP. Effects of nutritive factors on metabolic processes involving bioactivation and detoxication of chemicals. *Annu. Rev. Nutr.* 1984; 4:207-31
- Parke DV and Ioannides C. The role of nutrition in toxicology. Annu. Rev. Nutr. 1981; 1:207-34

Micronutrients and detoxification

Glutathione

- **One of the first known functions of glutathione is as an important detoxification factor.**

- Beutler E. Nutritional and metabolic aspects of glutathione. *Annu. Rev. Nutr.* 1989; 9:287-302.

Micronutrients and detoxification

NAC (N-acetylcysteine)

- **Exogenous NAC increases cellular glutathione production, as well as provides sulfhydryl groups to support Phase II.**

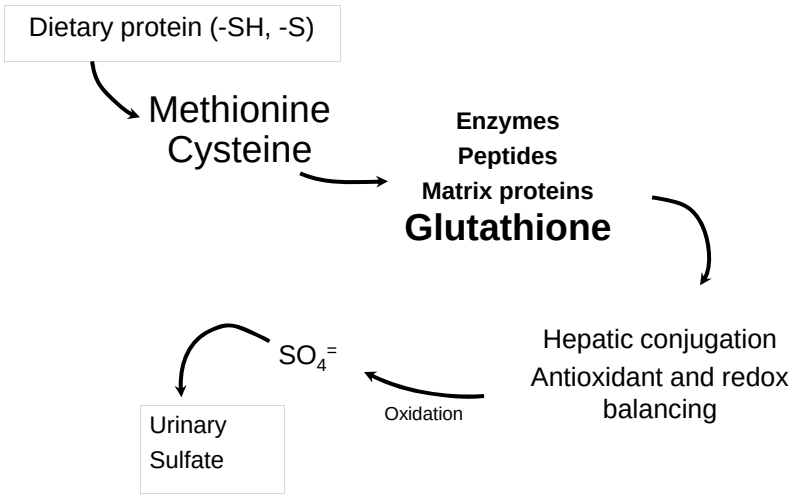
- Lauterburg BH. Mechanism of action of N-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in vivo. *J Clin. Invest.* 1983; 71:980-91.

Patient assessment & support strategies

Multiple Test Profile Use

- Find total toxic burden
  - **Organotoxins, toxic metals, endotoxins**
- Pinpoint metabolic effects
  - **Detoxification pathways**
    - Glutathione status markers
  - **Oxidative stress**
    - Polyunsaturated fatty acids as pro-oxidants
    - Antioxidant protection
  - **Markers of mitochondrial function**

Detoxification Markers: Inorganic Sulfate from Organic Sulfur



Other Detox Markers

- **Citrate**
- **cis-Aconitate**
- **Isocitrate**
- **Orotate**

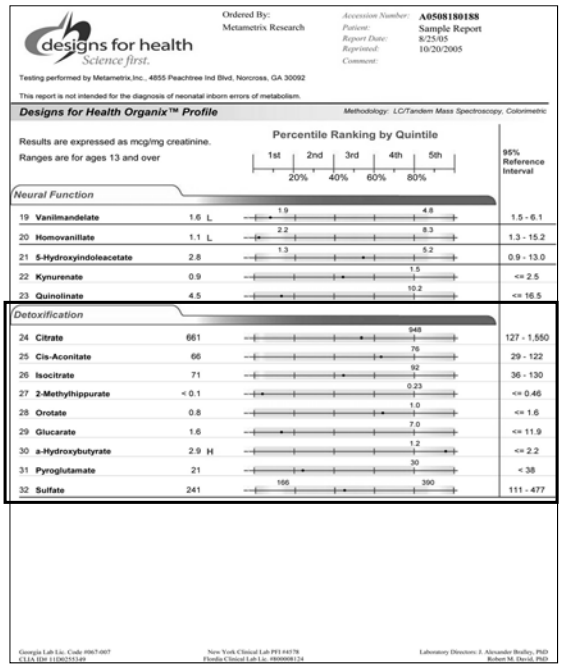
→ **Ammonia toxicity markers**
Need for arginine

- **2-Methylhippurate** → **Detox by-product of xylene**
Need for glycine and B5

- **Glucarate** → **Glucaronidation marker**
Need for glycine, glutathione, NAC

- **Alpha-Hydroxybutyrate** → **Indicates high cellular glutathione demand**
Need for glutathione, NAC

- **Pyroglutamate** → **Glutathione, glycine, methionine depletion**



designs for health
Science first.

Ordered By:
Metametrix Research

Accession Number: A0508180188
Patient: Sample Report
Report Date: 8/25/05
Reprinted: 10/20/2005
Comment:

Testing performed by Metametrix,Inc., 4855 Peachtree Ind Blvd, Norcross, GA 30092

This report is not intended for the diagnosis of neonatal inborn errors of metabolism.

Designs for Health Organix™ Profile

Methodology: LC/Tandem Mass Spectroscopy, Colorimetric

Results are expressed as mcg/mg creatinine.
Ranges are for ages 13 and over

Percentile Ranking by Quintile: 1st (20%), 2nd (40%), 3rd (60%), 4th (80%), 5th

				95% Reference Interval
Neural Function				
19	Vanillmandelate	1.6	L	1.5 - 6.1
20	Homovanillate	1.1	L	1.3 - 15.2
21	5-Hydroxyindoleacetate	2.8		0.9 - 13.0
22	Kynurenate	0.9		<= 2.5
23	Quinolinate	4.5		<= 16.5
Detoxification				
24	Citrate	661		127 - 1,550
25	Cis-Aconitate	66		29 - 122
26	Isocitrate	71		36 - 130
27	2-Methylhippurate	< 0.1		<= 0.46
28	Orotate	0.8		<= 1.6
29	Glucarate	1.6		<= 11.9
30	a-Hydroxybutyrate	2.9	H	<= 2.2
31	Pyroglutamate	21		< 38
32	Sulfate	241		111 - 477

Georgia Lab Lic. Code #067-007
CLIA ID# 11D0255149

New York Clinical Lab PFI #4178
Florida Clinical Lab Lic. #800008124

Laboratory Directors: J. Alexander Bralley, PhD
Robert M. David, PhD

Functional Liver Detoxification

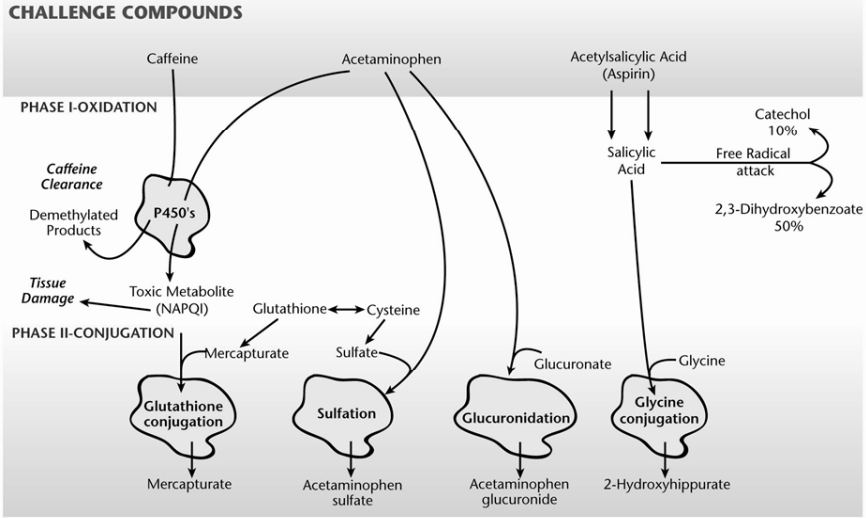
Test Procedure:

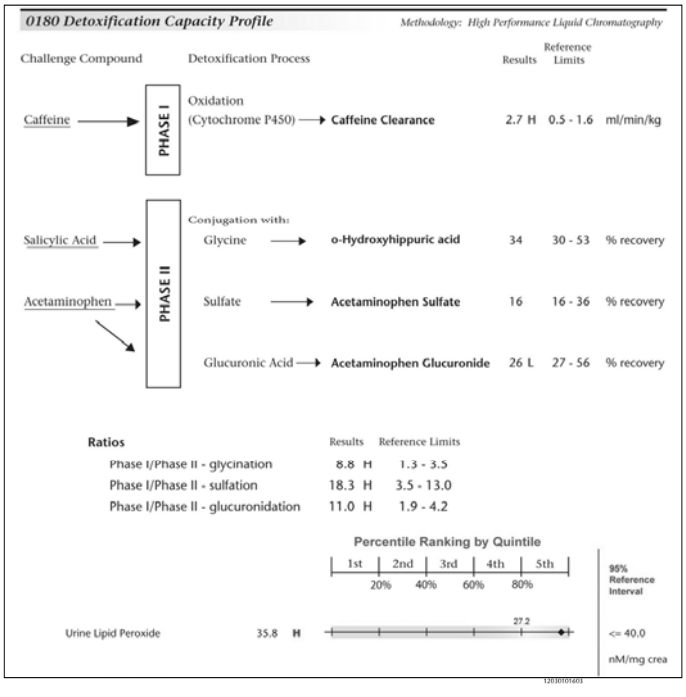
- **On Waking**
  - 200 mg caffeine

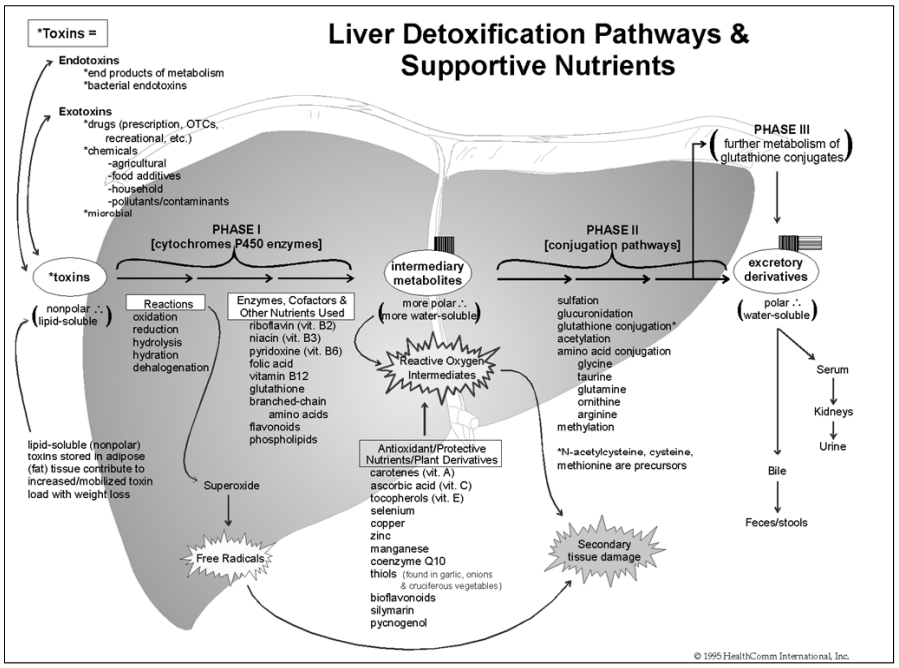
collect saliva sample 2 and 8 hours later

- **At Bedtime**
  - 650 mg aspirin
  - 650 mg acetaminophen

collect urine for the next 10 hours







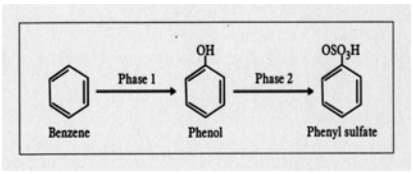
Sample Detox Support

	• Amounts per serving
• **Serving size**	• **6 capsules**
• **Number of servings per container**	• **180**
• **Alpha-Ketoglutarate**	• **200 mg.**
• **L-Glutamine**	• **500 mg**
• **L-Glycine**	• **500 mg**
• **N-Acetly-Cysteine**	• **250 mg**
• **Glutathione**	• **200 mg**
• **L-Methionine**	• **200 mg**
• **Ornithine**	• **200 mg.**
• **Taurine**	• **250 mg**
• **Calcium-d-glucarate**	• **200 mg**
• **MSM**	• **400 mg**
• Suggested Dose: **Take 3-6 capsules daily, between meals, or as directed by your health care practitioner.**	

•Sample Detox Powder	•Amounts per serving
•Serving size	•36 gm (2 scps)
•Number of servings per container	•21
•Calories	•145
•Total Carbohydrates	•11 gm.
• Sugars	• 0 gm.
• Sugar alcohol	• 7 gm.
•Dietary Fiber	• 2 gm.
•Protein	•15 g.
•Total Fat	• 3 g.
•Cholesterol	•0 mg
•Vitamins	
•Vitamin A (mixed carotenoids)	•5000 IU
•Vitamin D3 (cholecalciferol)	•50 IU
•Vitamin C (sodium ascorbate)	•500 mg.
•Vitamin E (high gamma-delta)	•20 IU
•Vitamin B1 (thiamin hydrochloride)	•3 mg.
•Vitamin B2 (riboflavin 5'phosphate)	•3mg
•Vitamin B3 (niacinamide)	•8 mg.
•Pantothenic Acid	•25 mg.
•Vitamin B6 (pyridoxal 5'phosphate)	•4 mg.
•Vitamin B12 (methylcobalamin)	•4 mcg.
•Biotin	•140 mg.
•Folate (Folic Acid)	•100 mcg.

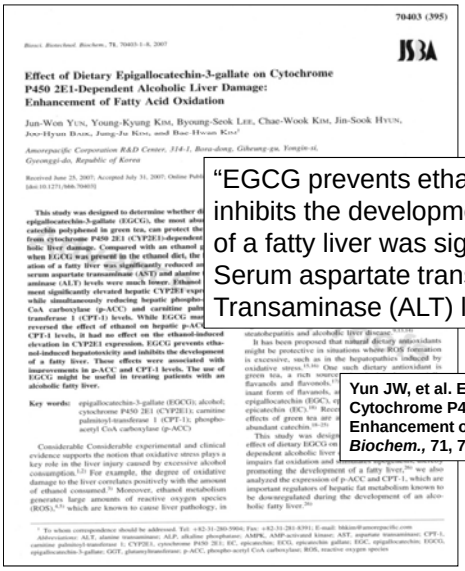
•Minerals	
•Sodium	• 235 mg
Molybdenum (glycinate)	50 mcg.
•Calcium (citrate and rice protein)	•200 mg.
•Magnesium (glycinate)	•200 mg.
•Phosphorus (rice protein and dipotassium phosphate)	•200 mg.
•Iodine (potassium iodide)	•50 mcg.
•Zinc (glycinate)	•7 mg.
•Copper (glycinate)	•1 mg.
•Manganese (glycinate)	•1 mg.
•Chromium (nicotinate-glycinate)	•50 mcg.
•Vanadium (nicotinate-glycinate)	•50 mcg.
•Selenium (selenomethionine)	•50 mcg.

•Specialty Nutrients	
•L-Glycine	1500 mg
•L-Lysine	•550 mg.
•L-Threonine	•550 mg.
•N-acetyl cysteine (NAC)	•100 mg.
•L-Cysteine	•250 mg.
•L-Glutathione	• 25 mg.
•L-Taurine	•200 mg.
•DL-Methionine	•100 mg
•Inositol	•100 mg
•Choline	•100 mg
•Sodium sulfate	•100 mg.
•MSM (methylsulfonymethane)	•100 mg.
•Calcium D-Glucarate	•250 mg.
•Silymarin (Milk thistle)	•200 mg.
•Dandelion Root (Taraxicum off.)	50 mg.
•Celandine (Chelidonium Majus)	50 mg
•Fringe Tree (Chionanthus virginicus)	50 mg.
•Quercitin	200mg.
•Catechins (green tea)	50 mg.



Rev. 6-16-06

EGCG from Green Tea and Detoxification



70403 (395)

BBA

Effect of Dietary Epigallocatechin-3-gallate on Cytochrome P450 2E1-Dependent Alcoholic Liver Damage: Enhancement of Fatty Acid Oxidation

Jun-Won Yun, Young-Kyung Kim, Byoung-Seok Lee, Chae-Wook Kim, Jin-Sook Hyun, Joo-Hyun Baik, Jung-Ja Kim, and Bae-Hwan Kim

Amorepacific Corporation R&D Center, 314-1, Bora-dong, Giheung-gu, Yongin-si, Gyeonggi-do, Republic of Korea

This study was designed to determine whether ... epigallocatechin-3-gallate (EGCG), the most abu... catechin polyphenol in green tea, can protect the ... from cytochrome P450 2E1 (CYP2E1)-dependent ... holic liver damage. Compared with an ethanol ... when EGCG was present in the ethanol diet, the ... ation of a fatty liver was significantly reduced a... serum aspartate transaminase (AST) and alanine ... aminase (ALT) levels were much lower. Ethanol ... ment significantly elevated hepatic CYP2E1 expr... while simultaneously reducing hepatic phospho... CoA carboxylase (p-ACC) and carnitine palm... transferase 1 (CPT-1) levels. While EGCG mo... reversed the effect of ethanol on hepatic p-AC... CPT-1 levels, it had no effect on the ethanol-induced elevation in CYP2E1 expression. EGCG prevents ethanol-induced hepatotoxicity and inhibits the development of a fatty liver. These effects were associated with improvements in p-ACC and CPT-1 levels. The use of EGCG might be useful in treating patients with an alcoholic fatty liver.

Key words: epigallocatechin-3-gallate (EGCG); alcohol; cytochrome P450 2E1 (CYP2E1); carnitine palmitoyltransferase 1 (CPT-1); phospho-acetyl CoA carboxylase (p-ACC)

Considerable Considerable experimental and clinical evidence supports the notion that oxidative stress plays a key role in the liver injury caused by excessive alcohol consumption.[1,2] For example, the degree of oxidative damage to the liver correlates positively with the amount of ethanol consumed.[3] Moreover, ethanol metabolism generates large amounts of reactive oxygen species (ROS),[4,5] which are known to cause liver pathology, in

“EGCG prevents ethanol-induced hepatotoxicity and inhibits the development of fatty liver. The formation of a fatty liver was significantly reduced and the Serum aspartate transaminase (AST) and alanine Transaminase (ALT) levels were much lower.”

Yun JW, et al. Effect of Dietary Epigallocatachin-3-gallate on Cytochrome P450 2E1-dependent alcholic liver damage: Enhancement of fatty acid oxidation. *Biosci. Biotechnol. Biochem.*, 71, 70403-1-8, 2007

Adjunctive Detox Therapies

Infrared Saunas

Additional Therapies:
Saunas and Detoxification

thermal stress

release of norepinephrine and growth hormone

increased lipolysis

release of xenobiotics from adipose tissue

Fix the biochemistry first !!!!

Exercise

- **How Does Exercise Support Detoxification?**
  - **Burst Training improves beta-oxidation, i.e. fat burning which is where many toxins are stored**
  - **Exercise improves endogenous antioxidant production**
  - **Exercise raises beta-endorphins**
  - **Exercise helps the body handle stress (short intense exercise)**
  - **Exercise raises core body temperature and causes sweating**
  - **Exercise improves immune function (short intense exercise)**
  - **Exercise improves circulation and supports the lymphatic system**
  - **Improves bowel regularity**
  - **Improves Sleep**
  - **Raises glutamine levels (short intense exercise)**
  - **Raises growth hormone (short intense exercise)**

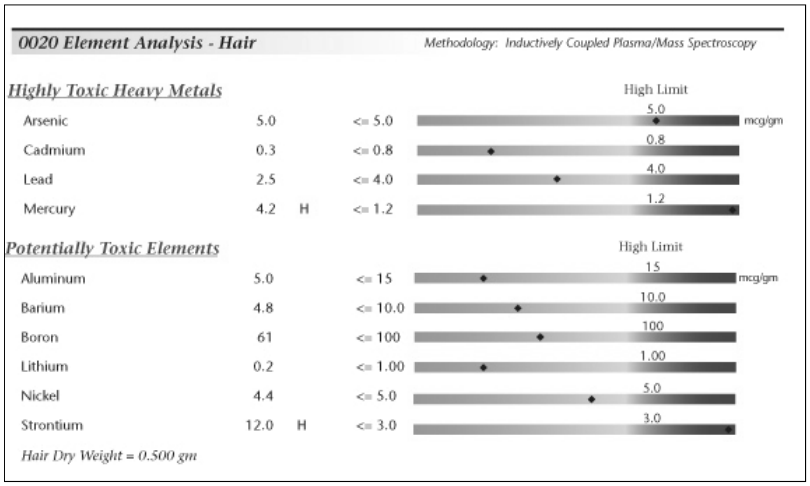
Heavy Metal Toxicity

Assessment and Treatment Options

Exposure

- Dietary
- Dental Amalgams
- Environmental

Hair Analysis



0020 Element Analysis - Hair — *Methodology: Inductively Coupled Plasma/Mass Spectroscopy*

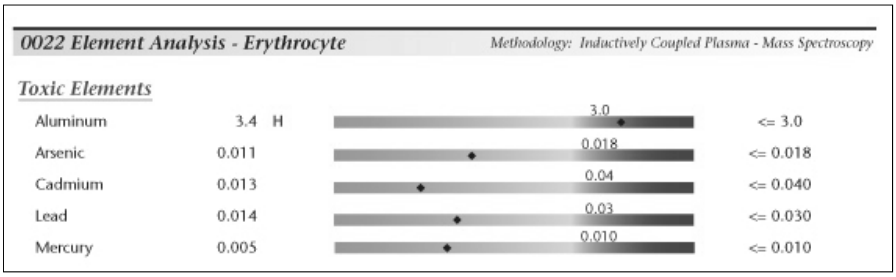
Highly Toxic Heavy Metals				High Limit
Arsenic	5.0		<= 5.0	5.0 mcg/gm
Cadmium	0.3		<= 0.8	0.8
Lead	2.5		<= 4.0	4.0
Mercury	4.2	H	<= 1.2	1.2

Potentially Toxic Elements				High Limit
Aluminum	5.0		<= 15	15 mcg/gm
Barium	4.8		<= 10.0	10.0
Boron	61		<= 100	100
Lithium	0.2		<= 1.00	1.00
Nickel	4.4		<= 5.0	5.0
Strontium	12.0	H	<= 3.0	3.0

Hair Dry Weight = 0.500 gm

•Good for organic forms only (i.e., fish)!
•Not valid for inorganic form (i.e., amalgams)!

RBC Membrane Analysis

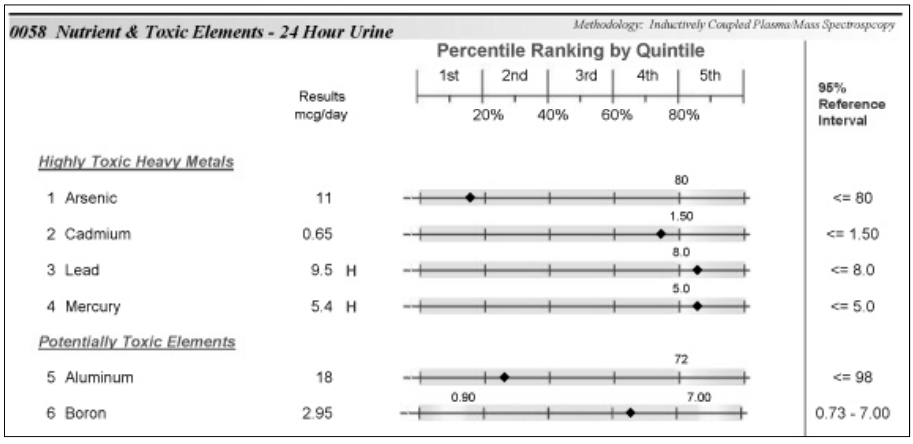


0022 Element Analysis - Erythrocyte — *Methodology: Inductively Coupled Plasma - Mass Spectroscopy*

Toxic Elements

Aluminum	3.4 H	3.0	<= 3.0
Arsenic	0.011	0.018	<= 0.018
Cadmium	0.013	0.04	<= 0.040
Lead	0.014	0.03	<= 0.030
Mercury	0.005	0.010	<= 0.010

Provoked Urinalysis



0058 Nutrient & Toxic Elements - 24 Hour Urine — *Methodology: Inductively Coupled Plasma/Mass Spectrospcopy*

Percentile Ranking by Quintile: 1st (20%) | 2nd (40%) | 3rd (60%) | 4th (80%) | 5th

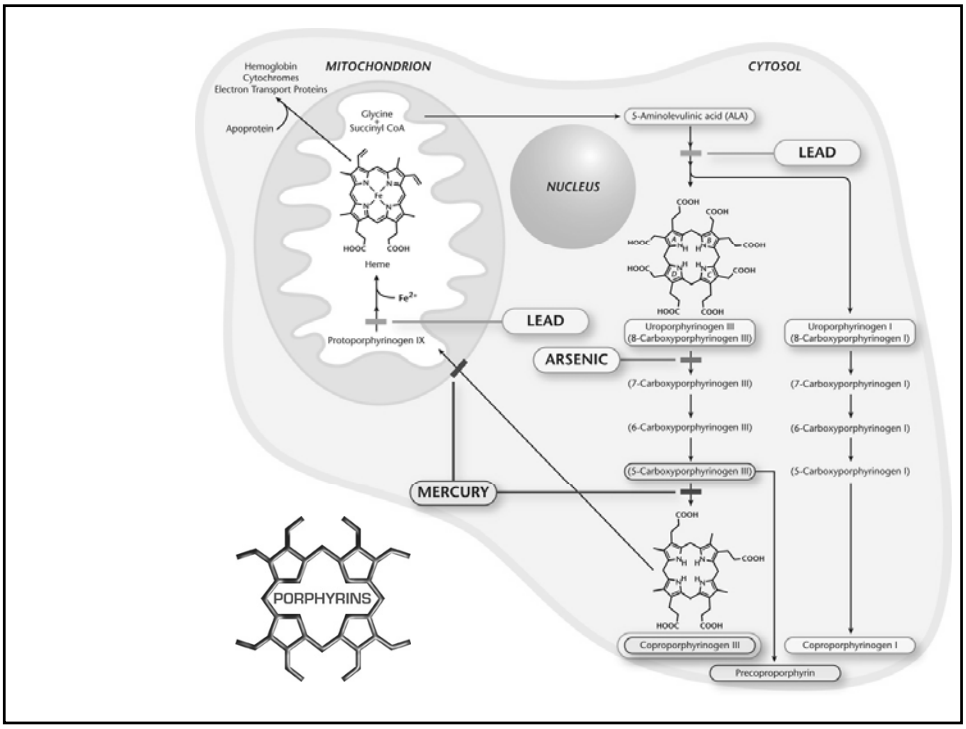
	Results mcg/day	95% Reference Interval
Highly Toxic Heavy Metals		
1 Arsenic	11	<= 80
2 Cadmium	0.65	<= 1.50
3 Lead	9.5 H	<= 8.0
4 Mercury	5.4 H	<= 5.0
Potentially Toxic Elements		
5 Aluminum	18	<= 98
6 Boron	2.95	0.73 - 7.00

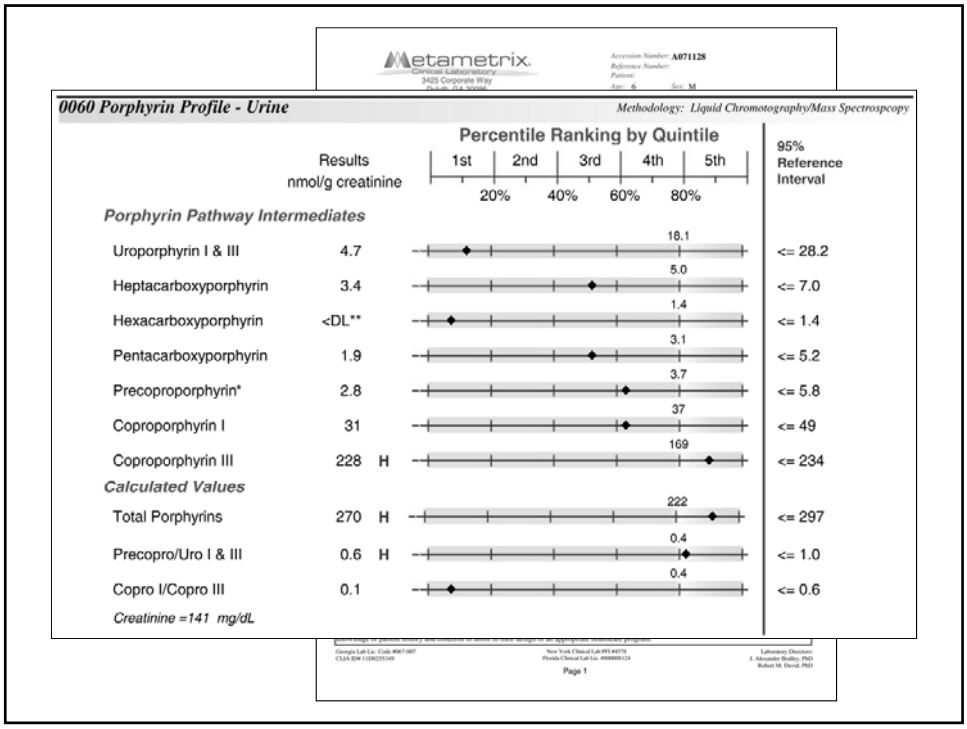
Non-Provoked Urine Mercury Studies

- Urine good for inorganic forms only (i.e., amalgams)!
- Not valid for organic form (i.e., fish)!
- Provocation changes things (both forms can be detected)

Why Evaluate Porphyrins?

- A functional test for heavy metals without DMSA, DMPS, and EDTA
- Normal results reduces (or eliminates) concern about current toxic mercury effects, even if mercury is detected in urine, hair or blood
- Monitor patients before and during chelation therapy
- Superior to hair or challenged urine
- Biochemical damage caused by toxicant exposure





Metametrix Clinical Laboratory
3425 Corporate Way
Duluth, GA 30096

Accession Number: A071128
Reference Number:
Patient:
Age: 6 Sex: M

0060 Porphyrin Profile - Urine

Methodology: Liquid Chromotography/Mass Spectrospcopy

	Results nmol/g creatinine	Percentile Ranking by Quintile (1st: 20%, 2nd: 40%, 3rd: 60%, 4th: 80%, 5th)	95% Reference Interval
Porphyrin Pathway Intermediates			
Uroporphyrin I & III	4.7	18.1	<= 28.2
Heptacarboxyporphyrin	3.4	5.0	<= 7.0
Hexacarboxyporphyrin	<DL**	1.4	<= 1.4
Pentacarboxyporphyrin	1.9	3.1	<= 5.2
Precoproporphyrin*	2.8	3.7	<= 5.8
Coproporphyrin I	31	37	<= 49
Coproporphyrin III	228 H	169	<= 234
Calculated Values			
Total Porphyrins	270 H	222	<= 297
Precopro/Uro I & III	0.6 H	0.4	<= 1.0
Copro I/Copro III	0.1	0.4	<= 0.6

Creatinine =141 mg/dL

Page 1

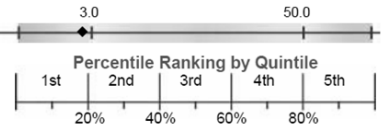
Interpreting a Urine Porphyrin Profile

	Mercury	Lead	Arsenic	Xenobiotics
Uroporphyrin I & III		(ALA)	↑	↑
Heptacarboxyporphyrin				
Hexacarboxyporphyrin				↑
Pentacarboxyporphyrin	↑			
Precoproporphyrin	↑↑			
Coproporphyrin I		↑	↑	↑
Coproporphyrin III	↑	↑	Low I/III ratio	↑
Pre / Uro	↑			

WHAT ABOUT ESTROGEN?

Primary Estrogen Risk Ratio

Hydroxyestrogen Ratio	Results		Reference Limits
2-OHE1 & E2 / 16a-OHE1	2.8 L	3.0 — 50.0	3.0 - 50.0



Case Presentation 1

- Donna 50 year old obese Native American female
- Personal history of total abdominal hysterectomy at age 40 due to excessive bleeding and fibroids
- Family history of estrogen-related problems including breast cancer and other female relatives who had hysterectomies at an early age due to excessive bleeding

0142 Estronex™ - 2/16 OH Estrogen Ratio in Urine

Methodology: Enzyme Immunoassay, Coorimetric Assay

	Results		Reference Limits
2:16 Ratio	1.00 L	2.00 — 8.00	2.30 - 0.00

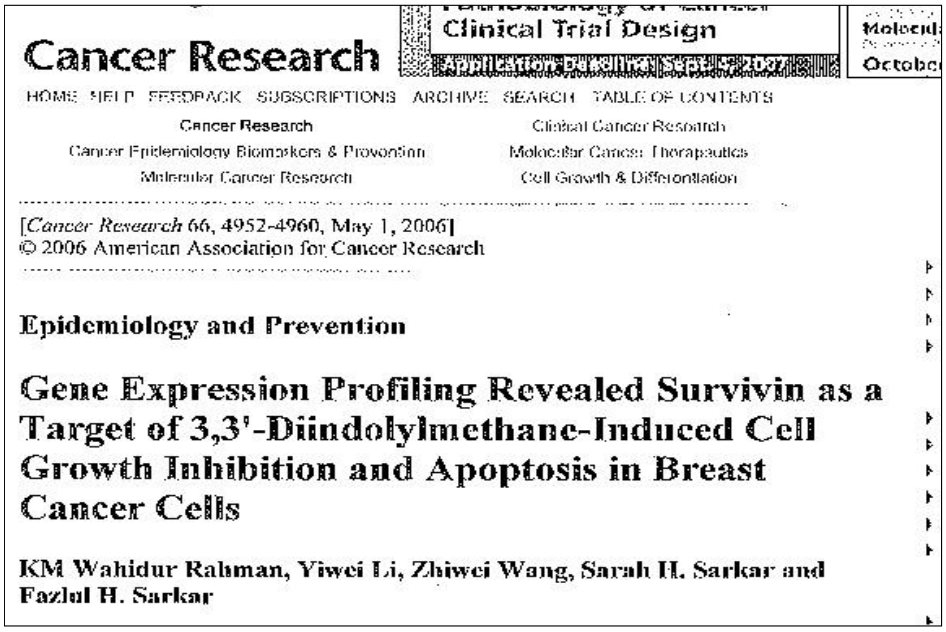
	Results	Normal Limits			
		Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	19.1	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	17.7	3 - 30	2 - 8	5 - 25	

Creatinine = 75 mg/dl

Note the level of total estrogens are above the normal limits for a woman who is post menopausal (surgical menopause in this case) without hormone replacement

Case Presentation 1 (continued)

- Rx: DIM 100 mg BID
- Patient requested this to be the only intervention at this time to be able to evaluate response
- Estrogen metabolites repeated in 4 months.
- Weight was the same pre- and post-DIM



Cancer Research

Clinical Trial Design

HOME HELP FEEDBACK SUBSCRIPTIONS ARCHIVE SEARCH TABLE OF CONTENTS

Cancer Research | Clinical Cancer Research
Cancer Epidemiology Biomarkers & Prevention | Molecular Cancer Therapeutics
Molecular Cancer Research | Cell Growth & Differentiation

[*Cancer Research* 66, 4952-4960, May 1, 2006]
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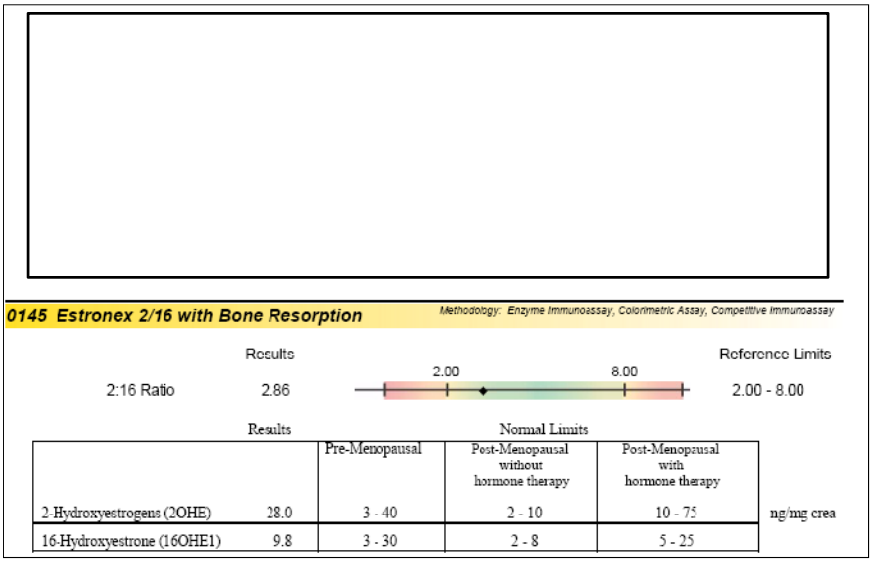
Epidemiology and Prevention

Gene Expression Profiling Revealed Survivin as a Target of 3,3'-Diindolylmethane-Induced Cell Growth Inhibition and Apoptosis in Breast Cancer Cells

KM Wahidur Rahman, Yiwei Li, Zhiwei Wang, Sarah H. Sarkar and Fazlul H. Sarkar

Cancer Research 66(9), 4952-60: 2006



0145 Estronex 2/16 with Bone Resorption *Methodology: Enzyme Immunoassay, Colorimetric Assay, Competitive Immunoassay*

	Results	Reference Limits
2:16 Ratio	2.86	2.00 - 8.00

	Results	Normal Limits: Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	28.0	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	9.8	3 - 30	2 - 8	5 - 25	

4 months post DIM introduction

. Notice change in ratio of 2:16a

0142 Estronex™ - 2/16 OH Estrogen Ratio in Urine *Methodology: Enzyme Immunoassay, Colorimetric Assay*

	Results		Reference Limits
2:16 Ratio	1.08	L	2.00 - 8.00

	Results	Normal Limits: Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	19.1	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	17.7	3 - 30	2 - 8	5 - 25	

Creatinine = 75 mg/dl

37.8 total estrogens

0145 Estronex 2/16 with Bone Resorption *Methodology: Enzyme Immunoassay, Colorimetric Assay, Competitive Immunoassay*

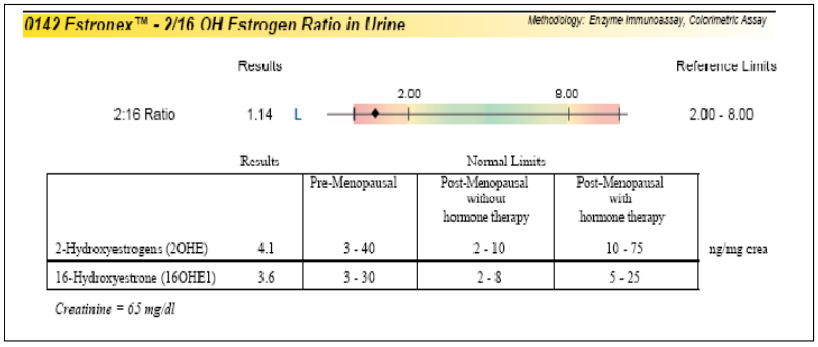
	Results	Reference Limits
2:16 Ratio	2.86	2.00 - 8.00

	Results	Normal Limits: Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	28.0	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	9.8	3 - 30	2 - 8	5 - 25	

36.8 total estrogens-4 month F/U on DIM without wt loss

Case Presentation 2

- Nancy, 60 year old obese white female, with history of right breast cancer treated with surgical mastectomy and chemotherapy.
- Discovered a lump in the left breast during chemo (had previous negative mammogram of left breast less than one year earlier).
- Left mastectomy performed with additional chemotherapy and radiation



0142 Estronex™ - 2/16 OH Estrogen Ratio in Urine

Methodology: Enzyme Immunoassay, Colorimetric Assay

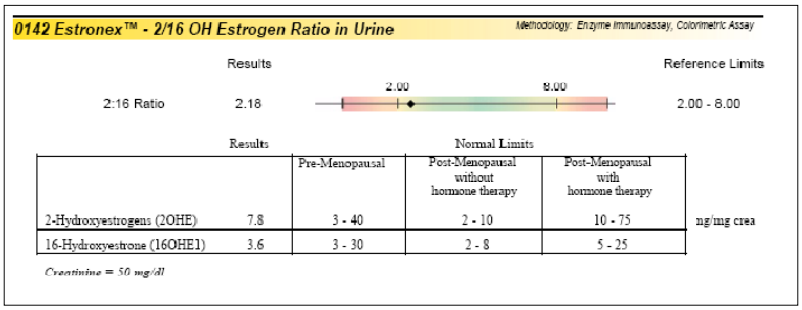
	Results		Reference Limits
2:16 Ratio	1.14	L	2.00 - 8.00

2.00 9.00

	Results	Normal Limits: Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	4.1	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	3.6	3 - 30	2 - 8	5 - 25	

Creatinine = 65 mg/dl

Nancy at baseline



0142 Estronex™ - 2/16 OH Estrogen Ratio in Urine

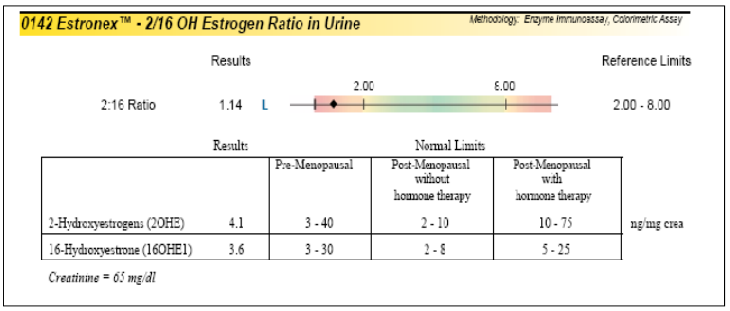
Methodology: Enzyme Immunoassay, Colorimetric Assay

	Results	Reference Limits
2:16 Ratio	2.18	2.00 - 8.00

	Results	Normal Limits			
		Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	7.8	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	3.6	3 - 30	2 - 8	5 - 25	

Creatinine = 50 mg/dl

Nancy after 5 months on DIM 100 mg BID



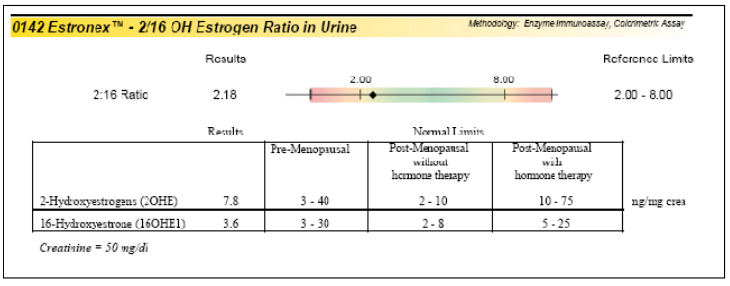
0142 Estronex™ - 2/16 OH Estrogen Ratio in Urine

Methodology: Enzyme Immunoassay, Colorimetric Assay

	Results		Reference Limits
2:16 Ratio	1.14	L	2.00 - 8.00

	Result	Normal Limits			
		Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	4.1	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	3.6	3 - 30	2 - 8	5 - 25	

Creatinine = 65 mg/dl



0142 Estronex™ - 2/16 OH Estrogen Ratio in Urine

Methodology: Enzyme Immunoassay, Colorimetric Assay

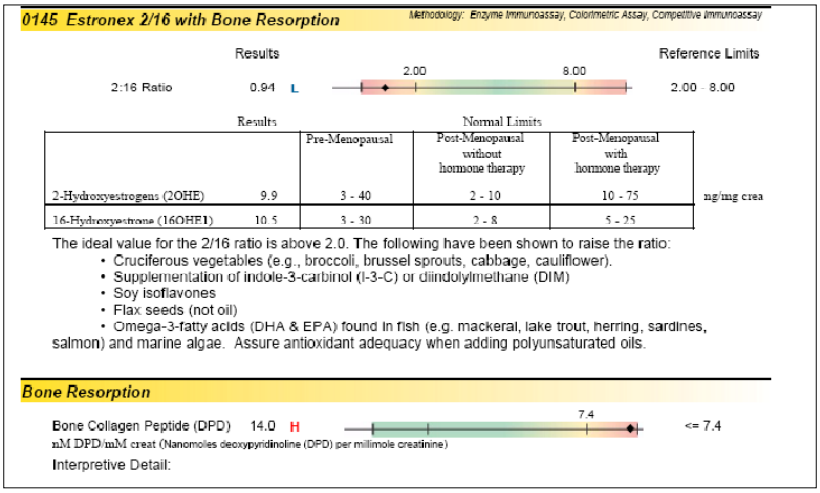
	Results	Reference Limits
2:16 Ratio	2.18	2.00 - 8.00

	Results	Normal Limits			
		Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	7.8	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	3.6	3 - 30	2 - 8	5 - 25	

Creatinine = 50 mg/dl

Case Presentation 3

- Sharon, 52 year old white female with c/o post menopausal symptoms
- History of recent mammogram showing a breast mass left breast diagnosed as benign
- Estrogen Metabolites done along with bone turnover markers due to concern regarding risk for osteoporosis



0145 Estronex 2/16 with Bone Resorption *Methodology: Enzyme Immunoassay, Colorimetric Assay, Competitive Immunoassay*

	Results		Reference Limits
2:16 Ratio	0.94 L	2.00 — 8.00	2.00 - 8.00

	Results	Normal Limits: Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	9.9	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	10.5	3 - 30	2 - 8	5 - 25	

The ideal value for the 2/16 ratio is above 2.0. The following have been shown to raise the ratio:
- Cruciferous vegetables (e.g., broccoli, brussel sprouts, cabbage, cauliflower).
- Supplementation of indole-3-carbinol (I-3-C) or diindolylmethane (DIM)
- Soy isoflavones
- Flax seeds (not oil)
- Omega-3-fatty acids (DHA & EPA) found in fish (e.g. mackeral, lake trout, herring, sardines, salmon) and marine algae. Assure antioxidant adequacy when adding polyunsaturated oils.

Bone Resorption

Bone Collagen Peptide (DPD)	14.0 H	7.4	<= 7.4

nM DPD/mM creat (Nanomoles deoxypyridinoline (DPD) per millimole creatinine)

Interpretive Detail:

Sharon - Baseline Testing

Bone Metabolism

			7.4	
Bone Collagen Peptide (DPD)	13.4	H		<= 7.4

nM DPD/mM creat (Nanomoles deoxypyridinoline (DPD) per millimole creatinine)

Interpretive Detail:

A DPD value above the Reference Limit indicates that resorption of bone is occurring faster than normal.

A DPD value equal to or below the Reference Limit is considered a normal result. In general, the lower the result the better to avoid development and/or progression of osteoporosis.

Case Presentation 3 (continued)

- Rx: DIM 100 mg BID
- Also started on a bone formula 2 caps BID
- Repeated testing after 6 months

Total Bone Support Forula

Supplement Facts

Serving Size 6 capsules
Servings Per Container 30

Amount Per Serving		% Daily Value
Vitamin C (as Calcium Ascorbate)	100 mg	167%
Vitamin D (as Cholecalciferol)	2000 IU	500%
Vitamin K (as Vitamin K1 Phytonadione 1000 mcg; Vitamin K2 Menaquinone-7 50 mcg)	1050 mcg	1313%
Calcium (as Di-Calcium Malate)	800 mg	80%
Magnesium (as Di-Magnesium Malate)	300 mg	75%
Zinc (TRAACS® Zinc Glycinate Chelate)	5 mg	33%
Copper (TRAACS® Copper Glycinate Chelate)	1 mg	50%
Manganese (TRAACS® Manganese Glycinate Chelate)	2 mg	100%
Potassium (as Potassium Glycinate Complex)	50 mg	1%
Tissue Regeneration Factor† (Cyplexinol™ containing Bone Morphogenetic Proteins)	50 mg	*
Milk Basic Protein (MBP®)	40 mg	*
Boron (as Bororganic Glycine)	4 mg	*

*Daily Value not established.

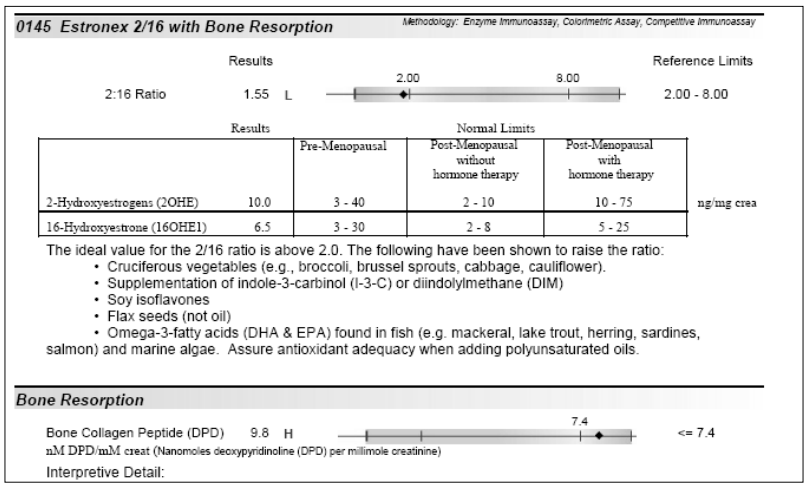
Aggressive Total Bone Support Formula

Supplement Facts

Serving Size 6 capsules
Servings Per Container 30

Amount Per Serving		% Daily Value
Vitamin C (as Calcium Ascorbate)	100 mg	167%
Vitamin D (as Cholecalciferol)	2000 IU	500%
Vitamin K (as Vitamin K1 Phytonadione 1000 mcg; Vitamin K2 Menaquinone-7 50 mcg)	1050 mcg	1313%
Calcium (as Di-Calcium Malate)	800 mg	80%
Magnesium (as Di-Magnesium Malate)	300 mg	75%
Zinc (TRAACS® Zinc Glycinate Chelate)	5 mg	33%
Copper (TRAACS® Copper Glycinate Chelate)	1 mg	50%
Manganese (TRAACS® Manganese Glycinate Chelate)	2 mg	100%
Potassium (as Potassium Glycinate Complex)	50 mg	1%
Milk Basic Protein (as MBP®)	40 mg	*
Boron (as Bororganic Glycine)	4 mg	*

*Daily Value not established.



0145 Estronex 2/16 with Bone Resorption

Methodology: Enzyme Immunoassay, Colorimetric Assay, Competitive Immunoassay

	Results	Reference Limits
2:16 Ratio	1.55 L	2.00 - 8.00

	Results	Normal Limits: Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	10.0	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	6.5	3 - 30	2 - 8	5 - 25	

The ideal value for the 2/16 ratio is above 2.0. The following have been shown to raise the ratio:

- Cruciferous vegetables (e.g., broccoli, brussel sprouts, cabbage, cauliflower).
- Supplementation of indole-3-carbinol (I-3-C) or diindolylmethane (DIM)
- Soy isoflavones
- Flax seeds (not oil)
- Omega-3-fatty acids (DHA & EPA) found in fish (e.g. mackeral, lake trout, herring, sardines, salmon) and marine algae. Assure antioxidant adequacy when adding polyunsaturated oils.

Bone Resorption

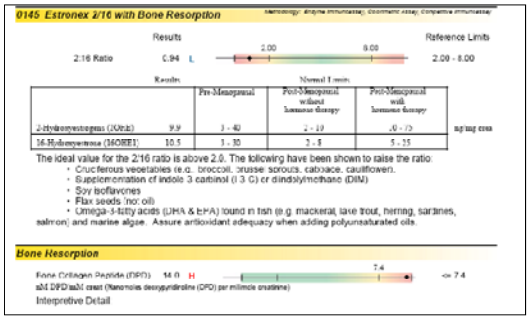
Bone Collagen Peptide (DPD)	9.8 H	7.4	<= 7.4

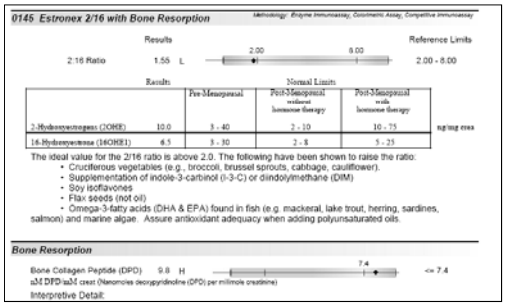
nM DPD/mM creat (Nanomoles deoxypyridinoline (DPD) per millimole creatinine)

Interpretive Detail:

Sharon, 6 months on DIM and bone formula

F/U mammogram excellent





Case Presentation 4

- Debra, 54 year old postmenopausal female, with complaints of breast tenderness
- Family history of breast cancer
- Estrogen Metabolite testing done

0142 Estronex™ - 2/16 OH Estrogen Ratio in Urine

Methodology: Enzyme Immunoassay, Colorimetric Assay

	Results		Reference Limits
2:16 Ratio	1.19 L	2.00 — 8.00	2.00 - 8.00

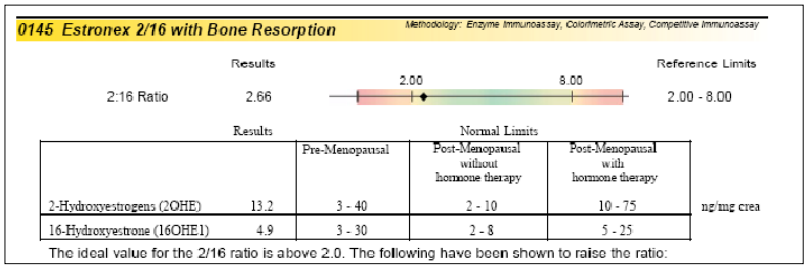
	Results	Normal Limits			
		Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	8.8	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	7.3	3 - 30	2 - 8	5 - 25	

Creatinine = 71 mg/dl

Debra: 54 yr, History of breast tenderness

Case Presentation 4 (continued)

- Rx: DIM 100 mg BID
- Estrogen metabolites testing repeated in 6 months at which time she stated she no longer experienced any breast tenderness



0145 Estronex 2/16 with Bone Resorption

Methodology: Enzyme Immunoassay, Colorimetric Assay, Competitive Immunoassay

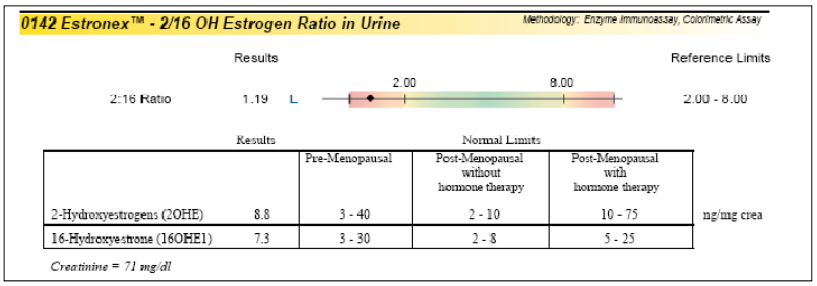
	Results	Reference Limits
2:16 Ratio	2.66	2.00 - 8.00

2.00 8.00

	Results	Normal Limits			
		Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	13.2	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	4.9	3 - 30	2 - 8	5 - 25	

The ideal value for the 2/16 ratio is above 2.0. The following have been shown to raise the ratio:

Debra: 6 months on DIM and feeling great

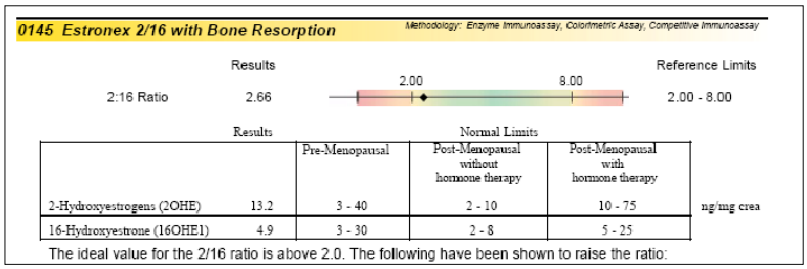


0142 Estronex™ - 2/16 OH Estrogen Ratio in Urine *Methodology: Enzyme Immunoassay, Colorimetric Assay*

	Results	Reference Limits
2:16 Ratio	1.19 L	2.00 - 8.00

	Results	Normal Limits: Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	8.8	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	7.3	3 - 30	2 - 8	5 - 25	

Creatinine = 71 mg/dl



0145 Estronex 2/16 with Bone Resorption *Methodology: Enzyme Immunoassay, Colorimetric Assay, Competitive Immunoassay*

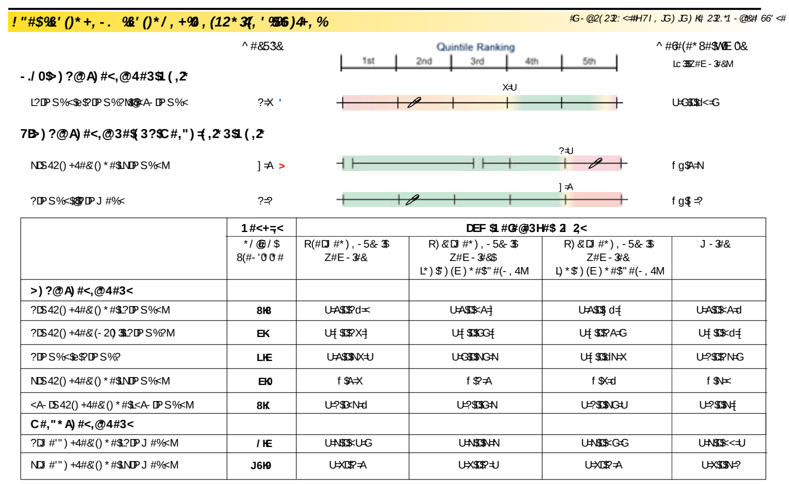
	Results	Reference Limits
2:16 Ratio	2.66	2.00 - 8.00

	Results	Normal Limits: Pre-Menopausal	Post-Menopausal without hormone therapy	Post-Menopausal with hormone therapy	
2-Hydroxyestrogens (2OHE)	13.2	3 - 40	2 - 10	10 - 75	ng/mg crea
16-Hydroxyestrone (16OHE1)	4.9	3 - 30	2 - 8	5 - 25	

The ideal value for the 2/16 ratio is above 2.0. The following have been shown to raise the ratio:

Case Presentation 5

- Karen, 55 year old postmenopausal female, with suspicious breast lesion
- No history of breast cancer
- Mammography and thermography
- Biopsy/lumpectomy recommended and denied by patient
- Estrogen Metabolite testing done



Case Presentation 5 (continued)

- Rx: DIM, Myomin, Sulphorophane-SGS, B12, folate, TMG, misc. immune support and antioxidants
- Estrogen metabolites testing repeated in 3 and 9 months
- Major improvement in 2/16 ratio and methylation markers

Sulforaphane-Glucosinolate

Supplement Facts

Serving Size 1 capsule

Amount Per Serving	% Daily Value
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]

*Daily Value not established.

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0145 Estronex™ Estrogen Metabolites with Bone Resorption - Urine

Methodology: UPLC/MS/MS, Colorimetric Assay

	Results	Quintile Ranking (1st 2nd 3rd 4th 5th)	Reference Limits (All Females)
2/16 Hydroxyestrogen Ratio			
(2-OHE1 + 2-OHE2) / 16a-OHE1	28.0 †	7.0	0.3 - 81.3
4-Hydroxyestrone and Methylation Ratio			
4-Hydroxyestrone (4-OHE1)	3.3 H	2.0	<= 6.4
2-OHE1 / 2-OMeE1	UC*	5.6	<= 9.2

	Results	95% Reference Limits			
	ng/mg creatinine	Pre-Menopausal Females	Post-Menopausal Females (no hormone therapy)	Post-Menopausal Females (on hormone therapy)	Males
Hydroxyestrogens					
2-Hydroxyestrone (2-OHE1)	**<0.6**	0.6 - 28.1	0.6 - 16.5	0.6 - 58.9	0.6 - 16.8
2-Hydroxyestradiol (2-OHE2)	**5.6**	0.9 - 27.5	0.9 - 33.9	0.9 - 26.3	0.9 - 18.9
2-OHE1 + 2-OHE2	**5.6**	0.6 - 47.0	0.3 - 43.4	0.9 - 84.7	0.2 - 24.3
4-Hydroxyestrone (4-OHE1)	**3.3**	< 6.7	< 2.6	< 7.8	< 4.1
16a-Hydroxyestrone (16a-OHE1)	**<0.2**	0.2 -14.8	0.2 - 3.4	0.2 - 43.0	0.2 - 4.9
Methoxyestrogens					
2-Methoxyestrone (2-OMeE1)	**<0.4**	0.4 - 10.3	0.4 - 4.4	0.4 - 13.3	0.4 - 11.0
4-Methoxyestrone (4-OMeE1)	**<0.7**	0.7- 2.6	0.7 - 2.0	0.7- 2.6	0.7 - 4.2

Creatinine = 45 mg/dL

Estrogen Metabolite Testing

1. Is the 2/16 ratio low? If so, then...
   - There may be an increased risk for cancer in estrogen-sensitive tissue.
   - The ratio may be increased by adding brassica vegetables or supplementing with I3C or DIM. Soy isoflavones, omega-3 fatty acids or flax seed (not oil) may also have favorable effects.
2. Is the 4-hydroxyestrone level abnormally high? If so, then...
   - This is another result that may be associated with increased cancer risk.
   - Methylation factors may be evaluated (vitamin B12, folate, COMT SNPs, methyl donor supply).
3. Is the 2-OHE1/2-OMeE1 ratio high? If so, then...
   - Catecholestrogen methylation status is poor.
   - Methylation ratios may be improved (lowered) by adding cofactors (vitamin B12 or folate) and methyl donors (such as betaine or DMG). Testing functional need for vitamin B12 or folate is recommended.

The 2/16 Ratio Range

Numerous studies have established that the relative risk of cancer in estrogen-sensitive tissues is increased for individuals with 2/16 ratios less than 2.0 when hydroxyestrogens are assayed by an immunoassay method (1). The UPLC/MS-MS analytical method now used at Metametrix gives superior analytical results and allows additional metabolites to be determined (2). The 2/16 ratio cutoff value of 7.0 shown on this report is the point at which the percentage of low results is equivalent to that for the immunoassay method.

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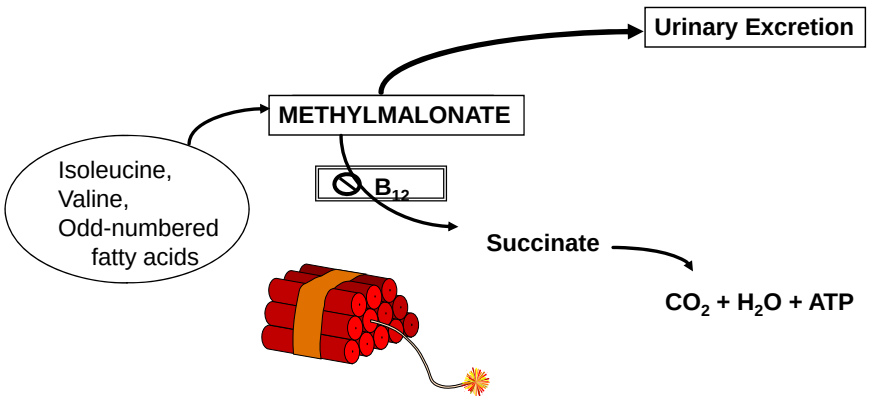
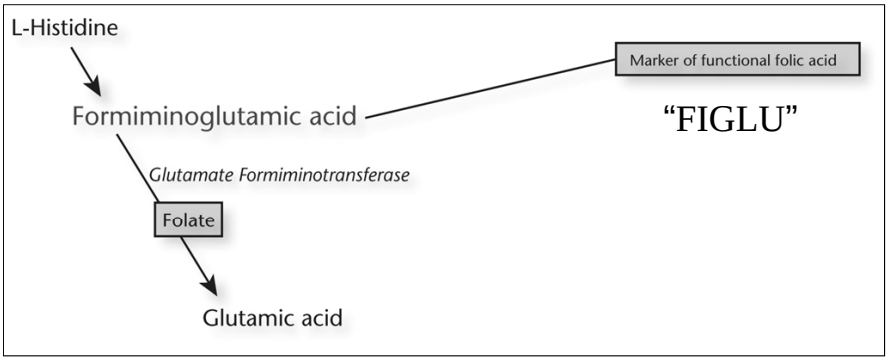
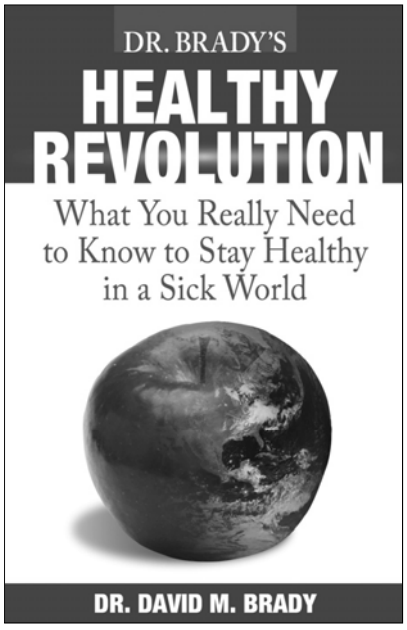
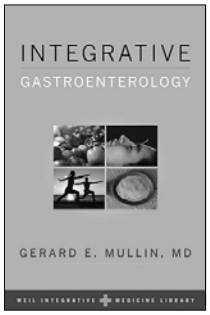
Methylmalonate
A Vitamin B12 Deficiency Marker

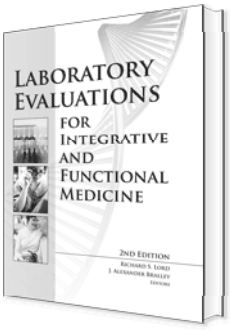


Functional Folic Acid Marker









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