

Safe and Efficacious Metal Detoxification

David Quig, PhD

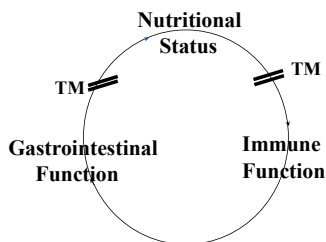
Basic Toxicology and Lab Support for Safe and Efficacious Metal Detoxification

David W. Quig, PhD
St. Charles, IL

May 3, Montreal Canada

Environmental Toxins

- W.H.O. 30-40% of childhood illnesses associated with environmental toxins
- C.D.C. “The epidemic of epidemics of CVD and immunological and neurological disease is likely associated with environmental toxins”



Nutritional Support

Monitor and optimize GI health & nutritional status!

Essential Elements (WB, RBC)
Comprehensive Stool Analysis
Amino Acids (urine, plasma)
Erythrocyte Glutathione

Dietary Protein and Mercury Excretion

- Mice fed normal (24%) or low (7.5%) protein diet for 7 days
- Oral MeHgCl (5 mg/kg) on day 6
- Low protein diet associated with:
30% lower urinary and fecal MeHg⁺ excretion

Amino Acids, Lubec & Rosenthal, (1992)

Glutathione

- λ glutamylcysteinylglycine
SH
- Intracellular Protective Functions
 - Antioxidant (redox state)
 - Conjugation of metals and chemicals
 - Regulation of immune function
 - Inhibit replication of retroviruses

Proc. Nat. Acad. Sci. (1997) **94** AIDS Res. Hum. Retrovir. (1992) **8**
Immunology (1967) **61**

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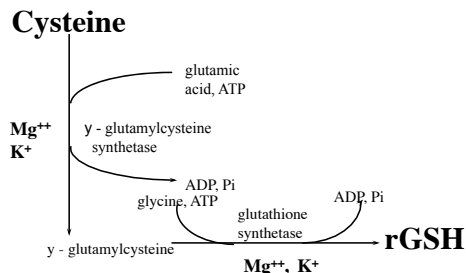
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Cellular Adaptation to Hg

- Initially, 2-3x upregulation of GSH synthesis and steady state levels (**kidney cells**)
- Increased activities of GSSH reductase and GSH peroxidase
- Decreased TBARS
- Neurons Do Not have such capacity**

Biochem Pharmacol (1995) J Biochem Toxicol (1996)
Toxicol Appl Pharmacol (1996) Neurotoxicology (1996)

Glutathione Biosynthesis



Metabolism of Sulfur Amino Acids & GSH in Autistic Children

- ASD (80) vs. control children (73), 6-7 yo
- ↓ plasma **methionine, cysteine, total GSH**
- ↑GSSH and GSSH:rGSH (oxidative stress)
- Normalized with folinic acid, betaine & Me-B-12*
- Similar % changes for GSSG and total GSH**

Am J Med Genet B Neuropsychiatr Genet (2006)141B:947-56 *Am J Clin Nutr (2004)80:1611-17 Horm Res (2006)66:182-8

Detoxification Potential



DETOX MARKERS	RESULT	REFERENCE RANGE	PERCENTILE				
			2.5 th	16 th	50 th	84 th	97.5 th
Methionine	4.9	8 - 50					
Cysteine	9	25 - 84					
Taurine	36	220 - 1420					
Glutamine	430	200 - 900					
Glycine	1490	500 - 4200					
Aspartate	11	8 - 33					

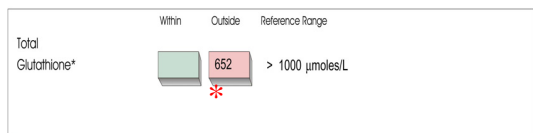
Severely compromised endogenous detoxification

Erythrocyte Total Glutathione

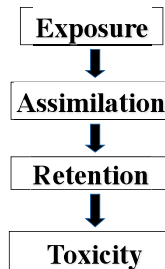


SEX: Female
AGE: 28

Total Glutathione; Erythrocytes



Basic Toxicology



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Basic Toxicology

Remove the source(s) of exposure!

ADHD / Tourette's Syndrome

- 10 y.o. male
- Learning problems, physical / verbal tics
- **Exposure:** hair lead **10 µg/gm** (99th percentile)
- Checked for neurotoxic metal **retention**
40 mg/kg glycine (oral)
DMSA (**10 mg/kg**)

DMSA/glycine challenge

LAB#: UD41110-0000-0

PATIENT: ADHD/TS

SEX: Male

AGE: 9

CLIENT#:

DOCTOR:

POTENTIALLY TOXIC METALS

METALS	RESULT µg/g CREAT	REFERENCE RANGE	WITHIN REFERENCE RANGE	ELEVATED	VERY ELEVATED
Aluminum	< dl	< 60			
Antimony	0.2	< 1.5			
Arsenic	24	< 13.0			
Beryllium	< dl	< 0.6			
Bismuth	0.4	< 2.0			
Cadmium	0.4	< 2			
Lead	110	< 5			
Mercury	2	< 5			
Nickel	1.7	< 15			
Platinum	< dl	< 1			
Thallium	0.4	< 1.1			
Thorium	< dl	< 0.5			
Tin	3.5	< 15			
Tungsten	< dl	< 1.5			
Uranium	< dl	< 0.2			

Pb = 110 µg/gm

Pb = 110 µg/gm

Glycine : Assisting Agent

- 40 mg/kg glycine orally ~ about 2 hrs. before provocation test
- **Use in CONJUNCTION** with agents
(logK- Hg; 9-13, Al; 8-10, Pb & Sb; 8-9)
- **Not** to be used alone

Envir Hlth Perspect (1986)65:363-411 Pangborn(1995), DDI/Bionostics
Quig, Townsend Letter, June 2007

The Source

- Soil in the yard
- Lead level: **7,108 mg/kg**
- E.P.A. safety level: **400 mg/kg**

Assessment of Exposure: Hair

- Hair is an excretory tissue that irreversibly binds *circulating* metals.
- **Concentrates** metals cumulatively
- Hair Me-Hg 200-300X > than blood
- Recommended by CDC for Me-Hg
EXPOSURE

Arch Environ Hlth (1980) 35:161-68 www.astdr.cdc.gov/HAC/
hairanalysis/6.2.htm

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Pubic Hair Metals: Machinist / Welder

TOXIC ELEMENTS	RESULT $\mu\text{g/g}$	REFERENCE RANGE	POTENTIALLY TOXIC ELEMENTS	
			PERCENTILE	
Aluminum	7.2	< 12	68 th	
Antimony	0.31	< 0.080		Sb, As
Arsenic	0.44	< 0.12		
Beryllium	< 0.01	< 0.020		
Bismuth	0.065	< 2.0		
Cadmium	0.58	< 0.15		Cd, Pb
Lead	14	< 2.0		
Mercury	0.30	< 1.1		
Platinum	< 0.003	< 0.005		
Thallium	0.001	< 0.010		
Thorium	0.001	< 0.005		
Uranium	0.007	< 0.060		
Nickel	0.32	< 0.40		
Silver	0.05	< 0.10		
Tin	0.38	< 0.30		
Titanium	0.95	< 1.0		
Total Toxic Representation				



Machinist/Welder(cont'd)

(Mn, Cr, Mo, Co, Fe)

ELEMENTS	RESULT $\mu\text{g/g}$	REFERENCE RANGE	ESSENTIAL AND OTHER ELEMENTS				
			PERCENTILE	2.5 th	16 th	50 th	84 th 97.5 th
Lithium	349	3.75-11.00					
Magnesium	73	40-140					
Sodium	470	24-180					
Potassium	350	20-80					
Copper	23	9.0-25					
Zinc	140	32.0-200					
Manganese	2.9	0.20-0.95					
Chromium	0.69	0.20-0.45					
Vanadium	0.087	0.018-0.055					
Molybdenum	0.21	0.040-0.10					
Barium	2.3	0.70-4.0					
Indium	1.9	0.25-1.3					
Lithium	0.037	0.008-0.040					
Phosphorus	332	20.0-100					
Selenium	1.4	0.95-1.7					
Strontium	1.3	0.5-6.0					
Sulfur	46800	4200-49000					
Barium	1.6	0.50-3.0					
Cobalt	0.11	0.013-0.050					
Iron	86	0.8-14					
Copper	0.043	0.045-0.065					
Rubidium	0.26	0.030-0.25					
Zinc	0.13	0.040-1.0					

Assessment of Exposure: Blood

Blood Metals

Very recent or ongoing exposure

Shortest half-life in body

Still “Gold Standard”

Not indicative of body burden

Environ Hlth Perspect (1995)103:1048-1052

Fish 3X / week

WHOLE BLOOD ELEMENTS							
LAB: B08077-0120-1							
PATIENT: Jan							
ID: MEN TSA-J-00001							
AGE: 59							
CLIENT: 29466							
DOCTOR: Kevin Y. Sun, MD							
Integrative Wellness Center							
4 Higgins # 100							
Irvine, CA 92618							
ELEMENTS	RESULT / UNIT	REFERENCE RANGE	NUTRIENT ELEMENTS				
			PERCENTILE	2.5 th	16 th	50 th	84 th 97.5 th
Calcium	9.9 mg/dL	4.5-5.3					
Magnesium	3.5 mg/dL	2.0-4.4					
Copper	70 $\mu\text{g/dL}$	61-128					
Zinc	679 $\mu\text{g/dL}$	420-810					
Manganese	9 $\mu\text{g/L}$	0-10					
Lithium	1.0 $\mu\text{g/L}$	0.3-2.0					
Selenium	382 $\mu\text{g/L}$	130-340					
Strontium	30 $\mu\text{g/L}$	8-38					
Molybdenum	0.8 $\mu\text{g/L}$	0.2-4.0					
TOXIC ELEMENTS	RESULT / UNIT	REFERENCE RANGE	ESSENTIALLY TOXIC ELEMENTS				
			PERCENTILE	95 th	99 th		
Arsenic	19 $\mu\text{g/L}$	< 20					
Barium	< 0.2 $\mu\text{g/L}$	< 10.0					
Cadmium	0.9 $\mu\text{g/L}$	< 4.0					
Cobalt	0.3 $\mu\text{g/L}$	< 4.0					
Lead	1.9 $\mu\text{g/dL}$	< 3.5					
Mercury	18 $\mu\text{g/L}$	< 5.0					**
Nickel	< 3 $\mu\text{g/L}$	< 15					
Platinum	< 0.2 $\mu\text{g/L}$	< 5.0					
Silver	0.1 $\mu\text{g/L}$	< 5.0					
Thallium	< 0.1 $\mu\text{g/L}$	< 1.0					
Uranium	< 0.1 $\mu\text{g/L}$	< 1.0					

Chronic Metal Toxicity

- Not acute poisoning, “treatment NOT necessary”

“Sub clinical metal toxicity”

Chronic Metal Toxicity

- “Sub-clinical” metal toxicity = **sub-threshold**
- For a given *individual*, toxicity is exhibited when the level of **net retention exceeds physiological tolerance**.

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Net Retention

- Relative rates of *assimilation* and *excretion*.
- Excretion is **highly variable** and determined by protein expression (MT, GSH), nutritional status, antibiotic use, life style, and **total toxic load**.

Unprovoked Urine: As Exposure

- Organic As rapidly excreted for ~ 48 hrs. after consumption of **shellfish**
- **PREVENT ALARMISM !:** Do **pre-** and **post** urinalysis initially, and abstain from fish and shellfish prior to provocative challenges

Occup Environ Med (1999)56:825-32 Environ Res (1994)66:44-51
Circulation (1995)91:646-55



Unprovoked 1st AM Urine

POTENTIALLY TOXIC METALS				
METALS	RESULT µg/g CREAT	REFERENCE RANGE	WITHIN REFERENCE RANGE	ELEVATED VERY ELEVATED
Aluminum	< dl	< 60		
Antimony	< dl	< 1.5		
Arsenic	1930	< 130		
Beryllium	< dl	< 0.6		
Bismuth	< dl	< 20		
Cadmium	0.4	< 2		
Lead	0.5	< 5		
Mercury	< dl	< 5		
Nickel	9.5	< 15		
Platinum	< dl	< 1		
Thallium	< dl	< 1.1		
Thorium	< dl	< 0.5		
Tin	0.4	< 15		
Tungsten	0.1	< 1.5		
Uranium	< dl	< 0.2		

As = 1,930 µg/gm

“All You Can Eat Crab Fest” night before

Provoked Urinary Metals.gov

The measurement of lead excreted in urine following an injection of Ca-Na₂- EDTA has been used to detect elevated body burden of lead in adults (2,3,4,5) and children (6,7), and is **considered to be a reliable measure of the potentially toxic fraction of the lead body burden** (8).

www.atsdr.cdc.gov/toxprofiles/tp13.html#

Assessment of Retention

- **The precedent has been set**, assess the net retention of **metals** using EDTA, DMPS or DMSA
- **Pre- vs. Post Provocation urinary metals**
 - (1) Objective, differential DATA
 - (2) legal/medical board considerations

Pharmacological Provocations

- **Extracellular, aqueous** compartments
- Significant kidney “flush”
- Do **NOT** appreciably cross a **healthy** BBB
- Toxic elements excreted through the kidneys

J Pediatr(1997)130:966-71 Occup Environ Med (1995)52:13-19 Fund Appl Toxicol(1995)25:233-40 J Pharmacol Exp Ther (1987)243:804-3

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Evaluations Prior to Provocations

- Blood chemistries (e.g. CBC w/ differential, creatinine, BUN, liver enzymes)
- Low LBM: Creatinine Clearance Test (6 hr.)
- Hydration

Don't Forget the Kidneys!

- Many toxic metals are **nephrotoxic**
- Ca-EDTA, DMPS and DMSA : metals primarily to the kidneys for excretion
- Dosage of agent proportional to relative efficiency of creatinine clearance rate
- Avoid acute renal failure!

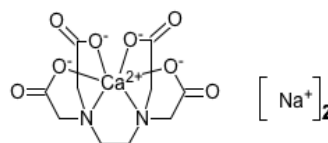
Assessment of Glomerular Filtration

- Serum creatinine (Cockcroft-Gault Equation)
- Elderly and others with very low muscle mass, serum creatinine alone **inadequate/insensitive** for detection of compromised GF
- Creatinine clearance: **timed** urine (6 h), and blood draw

Arch Intern Med (2003)163:356-60

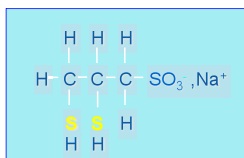
Common Agents : Ca-EDTA

- **Ca-Na₂-EDTA**: FDA approved in the 50s (Pb)



DMPS

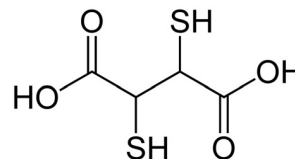
- **DMPS**: Not FDA approved, available from compounding pharmacies



(Informed consent)

DMSA

- **Chemet™**: FDA approved for Pb “poisoning” in children in 1990
2,3-meso-dimercapto-succinic acid



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Ca- Na₂-EDTA (cont'd)

- No biotransformation
- Contraindicated when BPb>80µg/dL
- Different application vs Na₂-EDTA, **SLOW DRIP, NEVER EVER PUSH!**
- Orally only 5-10 % absorbed
- Suppositories

Ca-Na₂-EDTA

- Recent introduction of Ca-EDTA slow push/fast drip
- 50 mg/kg, ≤ 3 gms
- T^{1/2} about 30-45 minutes
- 6 hr. urine collection

DMPS

- T^{1/2} iv < **1 hr.**
- T^{1/2} oral ~ **9 hr.**
- ~ 55 % absorbed (peak at 2 hrs.)

Fund Appl. Toxicol (1990)14:598-607

DMPS Challenges

- **IV:** 3-5 mg/kg (250 mg max), **slow** push (>5 min.)
- **Oral:** 10 mg /kg BW (5 mg/kg children), empty stomach (empty bladder)
- With hold food about 2 hrs.
- Encourage ~ 0.5L fluid over next few hrs.
- Collect all urine for 6 hrs.

Urinary Hg Before and After DMPS Challenge

ug Hg / 6h

	<u>Before</u>	<u>After</u>
Dental techs (10)	5 ± 1	424 ± 85
Dentists (5)	3 ± 1	162 ± 52
Controls (13)	1 ± 0.2	27 ± 3

(300 mg DMPS oral)

J Pharmacol Exp Ther (1995)272:264-74

DMPS “Frontfire.com”

- 31 y.o.f., ventricular tachycardia, demyelinating neuropathy with ↑ weakness
- DMSA 15 days, no ↑ UAs
- Progressive neuropathy (face, quadriplegic), ventilator dependent
- Started **iv** DMPS @ 250mg q4hrs for 12 days

J Toxicol Clin Toxicol (2000) 38:777-780

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DMSA General

- Does **NOT** cross a **healthy** human BBB
- Does **NOT** ↑ brain Pb or Hg levels
- ↓ brain Pb and Hg levels (animal studies)
- Increases urinary Pb, Hg and As in humans, but **NOT** U, Al or TI

Arch. Toxicol.(2002)76:437-31 Toxicol(1989)54:323-33 Toxicol (2002)177:186-97 Envir. Toxicol.(2001)9:173-84 Toxicol Appl Pharm (1999)161:283-93

Clinical Pharmacology: DMSA

- Only 20-25% absorbed
- Peak plasma ~ 3 hrs., rate U excretion 3- 4 hrs.
- **90% excreted as mixed disulfides with 2 cysteines (1:2)**

(cys → rGSH_{Intracellular})

J Nutr Envir Med(1998)8:219-31 PDR(2005) Toxicol(1995)97:23-38 J Pharmacol Exp Therap(1993)267:12-21

Bolus DMSA Challenge

- **20-30** mg DMSA/kg BW as oral bolus on empty stomach (≤ 2 gms)
- With hold food about 2 hrs.
- Encourage ~ 0.5L fluid over next few hrs.
- Collect all urine for 6 hrs.

J Nutr Envir Med (1998) 8:219-231

Dysbiosis and Sulfur Compounds

- N-AC, ALA and DMSA exacerbate GI symptoms
- Urease+ bacteria (e.g. Citrobacter freundii) produce H₂S and NH₄ from cysteine (N-AC)
- Assess GI health/integrity: Clean up GI tract **BEFORE** using oral SH- compounds

Fungal Methylation of Hg

- C. albicans and S. cerevisiae incubated with HgCl₂
- Both species transformed Hg⁺⁺ to MeHg in proportion to Hg⁺⁺ added
- **Survival mechanism for yeast, increased toxicity to host (95% absorption)**

Appl. Environ. Microbiol.(1991)57:245-7



Microbiology / Mycology

Comprehensive Stool Analysis / Parasitology x2

MICROBIOLOGY					
Bacteriology Culture					
Beneficial flora		Imbalances		Dysbiotic flora	
Bifidobacterium	2+	Gamma strep	4+	Citrobacter freundii	1+
E. coli spp.	4+	Providencia alcalifaciens	1+	Staphylococcus aureus	4+
Lactobacillus spp.	4+				
Enterococcus spp.	0+				
Mycology (Yeast) Culture					
Normal flora			Dysbiotic flora		
			Candida coliculosa		4+

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Yeast : Culture, Speciate and Challenge It

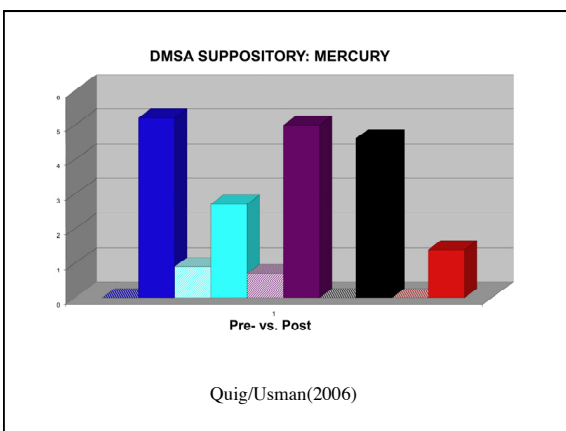
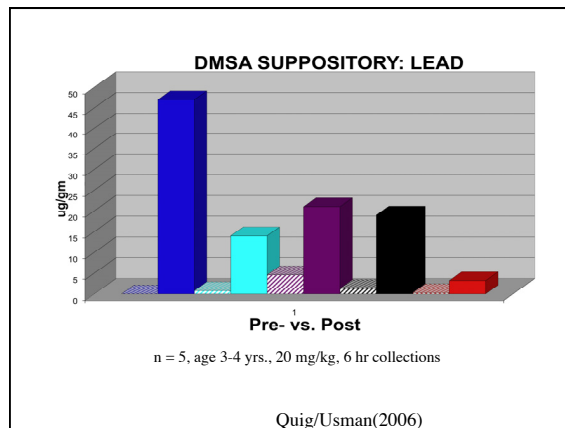
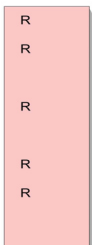
Natural agents

Berberine
Black Walnut
Caprylic Acid
Cats Claw
Citrus Seed Extract
Goldenseal
Oregano
Uva Ursi

Sensitive



Resistant



Mobilization of Metals

- Determined by:
 1. Relative **affinities** (stability constants)
 2. **Mass competition**
 3. Competition with **endogenous ligands**
(Hg^{++} binds protein-SH- at $\log K \sim 10^{40}$)

Stability Constants: DMPS

<u>Metal ion</u>	<u>logK₁</u>	<u>logK₂</u>
Hg ²⁺	27	36
Ag ²⁺	25	35
CH ₃ -Hg ¹⁺	21	31
Cu ²⁺	18	29
Cd ²⁺	18	26
Pb ²⁺	17	25
Zn ²⁺	15	25

Heyltex Corp. (1997)

Stability Constants: EDTA

	<u>LogK</u>
Hg ²⁺	21
Pb ²⁺	18.4
Cu ²⁺ , Ni ²⁺	18.3
Cd ²⁺ , Al ³⁺ , Zn ²⁺	16.1
Fe ²⁺	14.4
Mn ²⁺	13.4

Chemistry of Metal Chelate Compounds (1978)

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Efficacy of Agents to Remove Hg

- Rabbits exposed to HgCl_2
- Kidneys slices incubated with “chelators” at equivalent concentrations

Reduction in tissue Hg/ μg DNA

DMPS	86%*	DTT	55%
DMSA	65%*	Lipoic acid	35%
D-Pen	60%	EDTA	20%

*($p < 0.05$) Toxicol. (1997) 116: 67-75

Slow Push $\text{Ca-Na}_2\text{-EDTA}$: Urinary Metals

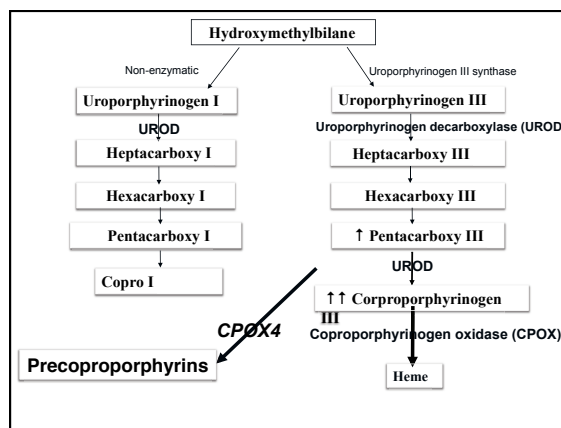
	Increase
Lead	147-X
Zn	32-X
Manganese	15-X
Iron	7.4-X
Cadmium	7-X
($p < 0.05$, $n = 14$) Antimony	4.4-X

Quig, Filidei, Whitaker (2002)

Mercury Specific Porphyrins

- $\uparrow$ penta, precopros, & copro III in urine
- MeHg-fed rats: dose, duration, urine Hg. $\downarrow$ w/ **DMPS treatment**
- Correlated w/ severity of ASD & oxidative stress,
- Dentists: UHg, neurological deficits. Most extreme w/ CPOX4 polymorphism

J Toxicol Env Hlth(1993)40:235 Can J Physiol Pharm(1996)74:210
Toxicol Sci(2001)61:234 Toxicol Appl Pharmacol(2006)214:99,
(2005)206:113 Porphyrins(2005)14:93 J Neurol Sci(2008)Epub
Toxicol Lett(2006)161:159



Porphyrins Affected by Toxins

- Hg: $\uparrow$ penta, precopros, & copro III
- As: $\uparrow$ uros, high **ratio of copro I : copro III**
- Pb: $\uparrow$ copro III, (Zn-protoporphryn, ALA)
- Hexachlorobenzene, dioxin: $\uparrow$ uros
- Methyl chloride, PVC, PBbiphenyl: $\uparrow$ copros

Should not be used *alone* to assess metal retention/
body burden

Summary: Diagnostic Tests

- Exposure**
Blood and unprovoked urine: very recent or ongoing
Hair: longer temporal window
- Net retention:**
Comparison of **pre** and **post** provoked urine
Urinary Porphyrins (very high/acute)

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Take Home Thoughts

- Use basic principles of toxicology in a **modern, preventive context**.
- Remove **source(s)** of ongoing exposure.
- Apply toxicokinetic / pharmacokinetic **facts**.
- Evaluate liver, kidney and GI function / integrity.
- Optimize nutritional status and GSH to optimize endogenous detoxification.

<u>Metal</u>	<u>1st Choice</u>	<u>2nd Choice</u>
Hg	DMPS or DMSA	
Pb	DMSA/EDTA	DMPS
As	DMPS/EDTA	DMSA
Cd	EDTA	EDTA
Sb	DMPS/DMSA	EDTA
Sn	DMPS,DMSA	EDTA
Pt, Ti	DMPS	EDTA
Fe, Al	EDTA	DFO

Toxicol (1995)97:23-38