Porphyrin testing and heavy metal toxicity: unresolved questions and concerns

by William Shaw, Ph.D.

The assertion by Nataf et al (1) that the Laboratoire Philippe Auguste could detect a specific porphyrin termed "precoproporphyrin" associated with mercury toxicity and autism was enthusiastically embraced by a large segment of the autism community, who saw the study as documentation of mercury involvement in autism and a better way to confirm mercury toxicity that might be undetected by typical metal tests of urine, blood, or hair.

Unfortunately, adequate scientific scrutiny may not have adequately applied to these claims. The Great Plains Laboratory started evaluating the claims of Nataf et al in order to replicate their findings and to be able to offer similar testing in the USA. Any important scientific claim needs to be replicated before it can be accepted as true. The following aspects of Nataf's claims were evaluated by multiple comparisons of testing performed by HPLC MS/MS and LabCorp, the laboratory marketed by The Great Plains Laboratory. The Great Plains Laboratory obtained access to additional chromatographic information and data not usually reported by LabCorp. The Great Plains Laboratory also performed creatinine testing to obtain porphyrin/creatinine ratios. In addition, samples of highly characterized porphyrins were obtained from a commercial laboratory specializing in porphyrin calibration, *Recipe Chemical + Instruments Labortechnik*, Munich, Germany. Rat urine from rats exposed to high concentrations of mercury was obtained from a generous gift from Dr. Wood's laboratory.

The areas that appear to be inadequately scientifically addressed by Nataf and Laboratoire Philippe Auguste which are scrutinized in this article are:

1. Urine sample preparation for porphyrin analysis by Laboratoire Philippe Auguste does not appear to eliminate interferences that may erroneously be reported as "precoproporphyrin".
2. Use of reference ranges for coproporphyrins by Laboratoire Philippe Auguste in which the age of normal controls does not match those of the autistic spectrum groups, even though previous studies indicate significant age-related changes.
3. Failure by Nataf or Laboratoire Philippe Auguste to scientifically identify in any way the compound termed "precoproporphyrin" or even to prove it is a single chemical entity.
4. Changing, without any adequate explanation, normal reference ranges for "precoproporphyrin" obtained in the Nataf published study (1) compared to those used commercially in the Laboratoire Auguste Philippe.

To evaluate the accuracy of the Laboratoire Auguste Philippe we sent a sample with known concentrations (Calibrator) of porphyrins from a laboratory (Recipe Chemicals + Instruments) specializing in providing porphyrin standards. Their contact information is included with the references at the end of this article.

Table 1. Porphyrin analysis on calibration standard

Urine Porphyrin Calibrator (nmol/L)

	Laboratoire Philippe Auguste	Great Plains Laboratory/ LabCorp	Target of porphyrin Laboratory
Uroporphyrin	**175.47**	**174.5**	**225**
7-CP	**36.19**	**43.2**	**51**
6-CP	**34.11**	**36.4**	**47**
5-CP	**47.01**	**42.9**	**43**
4-CP I, III	**700.56**	**675.1**	**588**

In general, both laboratories reported very similar results for all major porphyrins, indicating that any differences between the laboratories are not likely to be due to analytical differences in the testing for major porphyrin species when interferences are not present in the samples such as in calibration standards prepared in urine with minimal interferences. Values for both laboratories were significantly below target values for uroporphyrin, 7-CP, and 6-CP. Values for both laboratories were above the target value for total coproporphyrins (4-CP I, III). Both laboratories were very close to the target value for pentacarboxyporphyrin (5-CP) although the Laboratoire Auguste Philippe had the greatest deviation from the target value.

Correspondence in the measurement of samples with high concentrations of porphyrins with few interferences does not mean that correspondence will be present in patient samples with interferences and low concentrations of porphyrins. This correspondence also will not resolve issues regarding inappropriate age-related reference ranges. The rest of this article deals with these issues.

A brief history of mercury exposure and its relationship to porphyrins is in order.

Dr. James Woods et al (2) first reported the presence of a porphyrin compound (Figure 1) eluting between pentacarboxyporphyrin and coproporphyrins using high performance liquid chromatography (HPLC). This compound was termed “precoproporphyrin” although no evidence was presented in his published articles proving that the compound was a particular porphyrin or even that the compound was a porphyrin at all. Woods et al claimed that the compound might be a specific porphyrin termed ketoisocoproporphyrin but published **no characterization of this molecule.** It is much better to report the

compound as unknown substance or unknown peak. Two other research groups use the unknown peak designation (3, 4) which is much more appropriate. Thus, the term "precoproporphyrin" does not define a chemical compound but only the fact that a chemical with fluorescent properties is eluting at a certain time in a particular HPLC system.

The most significant finding of Woods et al (2) and two other research groups (3, 4) was that an unknown peak (that may or may not be the same compound found in each group) was found to be associated with heavy metal toxicity. Some found a relation with mercury toxicity but one (5) also found a correlation with lead exposure. In addition to this unknown peak, Dr. Woods showed that in the presence of mercury three other porphyrins (pentacarboxyporphyrin and coproporphyrins I, III) were significantly elevated by 3-4 folds (6) in dentists exposed to vapor mercury. Consequently, the presence of high amounts of this unknown peak **"**precoproporphyrin" appears to be inconsistent with mercury toxicity **unless** pentacarboxyporphyrin and coproporphyrins I, III are also elevated. Thus, unless these three porphyrins (pentacarboxyporphyrin and coproporphyrin I, III) are abnormally elevated with "precoproporphyrin", then it is likely that this unknown peak is not mercury-related. This research observation is important in the analysis of a split sample discussed later.

A prominent porphyrin researcher in the United Kingdom, using HPLC MS/MS has identified many new porphyrins that were not previously known. This researcher stated to us in an email:

"*There are many porphyrins that can elute between pentacarboxyporphyrin and coproporphyrin I. These are hydroxycoproporphyrin, hydroxyisocoproporphyrin, ketoisocoproporphyrin, formylcoproporphyrin and beta-ketopropionic acid pentacarboxylporphyrin. Beta-ketopropionic acid coproporphyrin eluted after coproporphyrin with our running conditions. This is because beta-ketocids are able to form stable intramolecular H-bond, thus making the compounds more hydrophobic and therefore longer retention.*

It is always dangerous to assume that a compound eluting between penta and copro is ketoisocopro. It could be any of the above, and, with inferior HPLC conditions, many of the above may even co-elute".

Thus, this researcher indicates that there is insufficient evidence to identify Wood's peak as **any particular compound**.

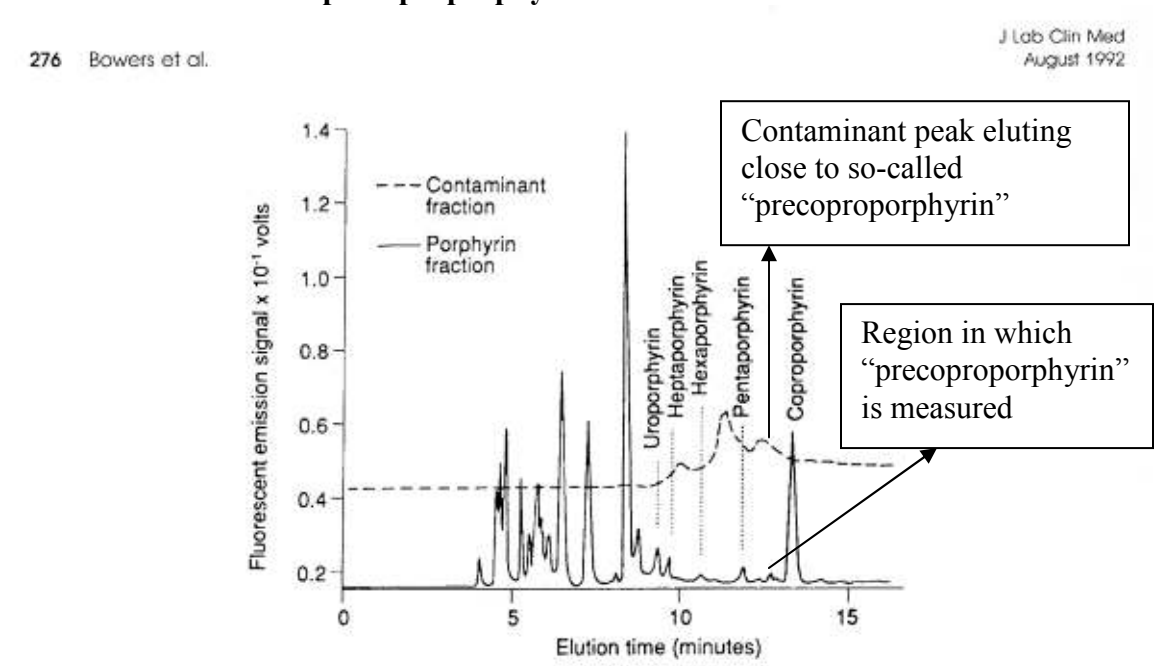
Following Dr. Woods' work, Dr. Nataf published the first scientific paper associating the *speculative* presence of the uncharacterized and an unvalidated "precoproporphyrin" with autistic spectrum disorder (ASD). The research group speculated that the presence of "precoproporphyrin" was due to mercury exposure. Yet, no urine mercury levels were reported in his paper.

The methodology used by Dr. Nataf's group failed to perform a critical step necessary to eliminate interferences that were used by Dr. Woods' group (4). This step was used for all of Dr. Woods research and is used to "clean up" interferences before running the quantification. Figure 1 from the Bower's paper (2) referenced by Nataf shows that between pentacarboxyporphyrin and coproporphyrins I,III there is a significant peak very close to the purported "precoproporphyrin" that is found in the clean up step named "Contaminant Fraction". Dr. Nataf's method DOES NOT include this critical step. The exact method of Nataf is given below:

"Porphyrin analysis was by an HPLC spectrofluorometric technique (Bowers et al., 1992). After centrifugation (3000×g, 5 min) 1 ml supernatant was acidified (40 µl HCl 37%w/v), recentrifuged, and 50 µl injected (Econosphere column C18, 5 µm particle size, 250×46mm;Alltech, Templemars, France)."

Consequently, it appears that this contaminant fraction is an interference in the Nataf study and in the reports of the Laboratoire Auguste Philippe. This contamination peak may be erroneously measured as "precoproporphyrin" when in fact it appears to be the contaminating material of the Bowers paper cited by Nataf. The contaminant fraction produces a significant peak that has some fluorescence characteristics like porphyrins but it is not a porphyrin.

Figure 1. Reproduction of porphyrin method showing presence of contamination that is removed by pre-chromatographic purification. Major contaminant is present near retention time of "precoproporphyrin".



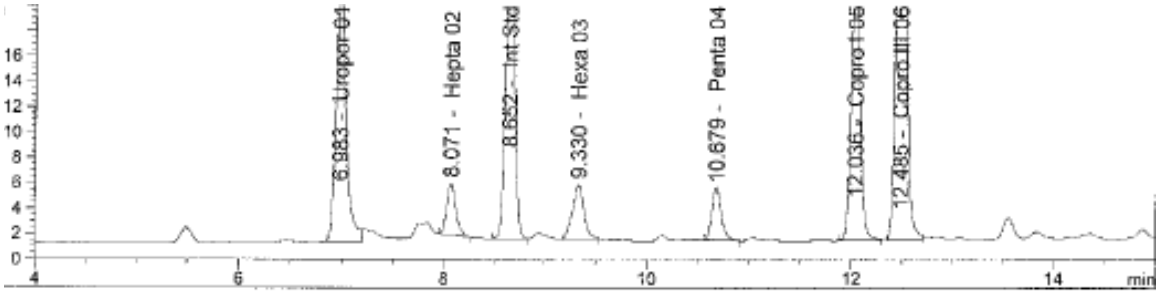
This contamination peak could be a vitamin, a drug, or an environmental chemical. The lack of adequate identification is an even more severe deficiency since the Nataf laboratory uses a method that employs no purification (cleanup step) prior to chromatography. Any fluorescent compound might coelute and give a false positive

result. The Nataf article does not indicate that even a single drug, vitamin, or other chemical was evaluated for interference in their test. Many children with autism take high doses of vitamins such as riboflavin that are highly fluorescent and which might interfere in this test. Nataf presents no mass spectra evidence of identification, no type of spectral identification, no ultraviolet light absorption spectrum, no infrared absorption spectrum, and no fluorescence spectrum of "precoproporphyrin". These deficiencies could be overlooked if an authentic calibration standard were used.
However, no such calibration standard was used because "precoproporphyrin" has never even been chemically characterized, much less synthesized. To summarize, Nataf et al. reported finding a compound that is elevated in urine samples of those on the autistic spectrum. The Nataf work **does not offer any** evidence that this substance is even a porphyrin. It would be much more appropriate to identify this compound as unknown fluorescent peak or compound eluting between pentacarboxy- and copro- porphyrins.

To validate the ability of The Great Plains/LabCorp method to detect peaks associated with true mercury toxicity, urine from mercury exposed rats was evaluated (Fig. 2B). A porphyrin calibration standard is shown below for comparison (Fig. 2A). The chromatogram of rat urine from rats exposed to high mercury doses indicated the presence of two additional peaks between pentacarboxyporphyrin and coproporphyrin I not usually found in human or rat urine samples. The retention time of these peaks were 11.021 and 11.196 minutes. The Great Plains Laboratory now reports certain mercury-associated compounds as mercury associated peaks unknown 1 and 2. Note how the pentacarboxyporphyrin is markedly elevated in the urine samples of the mercury treated rats. The third chromatogram (2C) is a urine sample from a person who may have heavy metal intoxication and had extreme elevation of porphyrins using appropriate age-related ranges. In this sample coproporphyrins are over five-times the upper limit of normal (Table 2). Coproporphyrins I and III as well as pentacarboxyporphyrin are markedly elevated, consistent with possible heavy metal poisoning. Even in this person, the peaks in the human urine sample for pentacarboxyporphyrin and the 2 peaks eluting after pentacarboxyporphyrin (peaks associated with mercury toxicity in the rat) are much lower than the corresponding peaks from the mercury treated rat urine, indicating that porphyrin testing is only likely to be useful in more extreme cases of metal toxicity, not in cases of mild or moderate exposure. In this person, the pentacarboxyporphyrin peak is much larger than the mercury associated peaks. Thus, the pattern in this person is consistent with the pattern reported by Dr. Woods in urine samples from mercury treated rats or mercury-exposed dental workers.

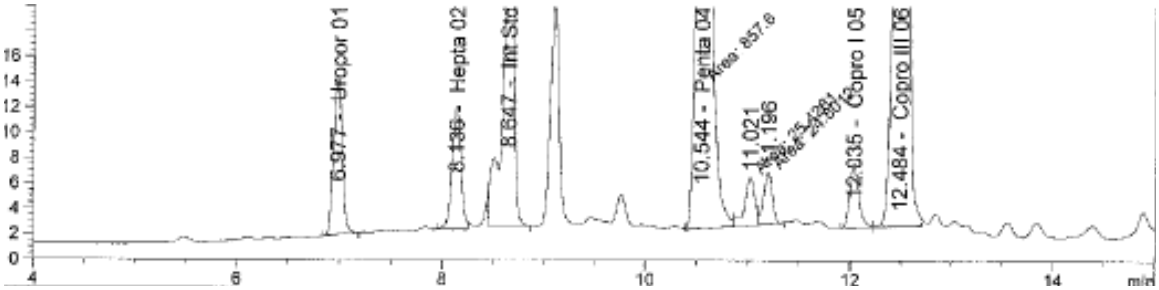
Figure 2. Effect of mercury and/or other substances on porphyrin metabolism.

2A.



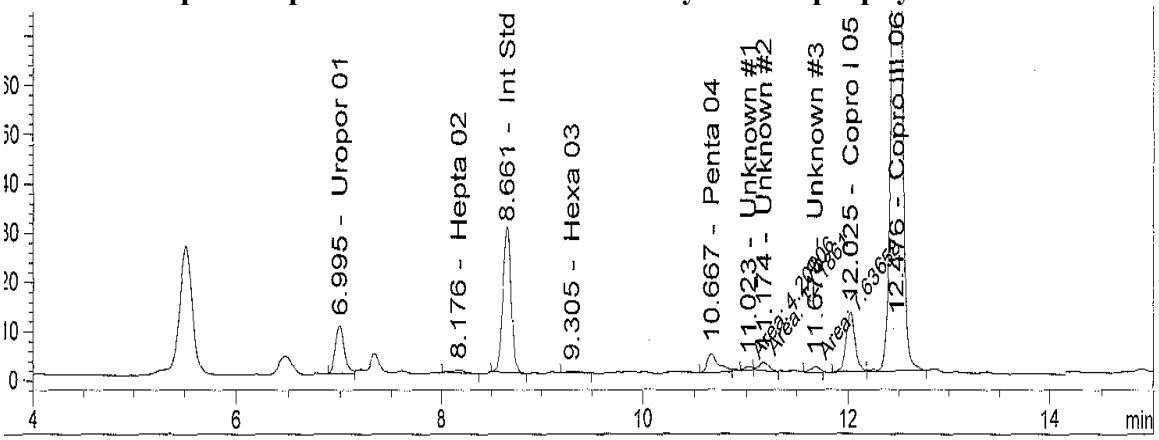
Urine porphyrin calibrator tested by The Great Plains Laboratory/LabCorp

2B.



Urine from mercury-treated rats by The Great Plains Laboratory/LabCorp

2C. An example of a patient with elevated mercury-related porphyrins



An example of a patient with elevated mercury-related compounds by The Great Plains/LabCorp method. Unknown peaks #1 and #2 in chromatogram 2C appear to correspond to the same peaks found in urine of mercury exposed rat in chromatogram 2B.

Table 2. Urine porphyrin results from patient in Figure 2C.

Urine Porphyrins					
	Results		Range	Normal	High
	nmol/ L	nmol/g CRT	nmol/g CRT		
Uroporphyrins (UP)	75.2	63.8	0 - 24		H
Heptacarboxy (7-CP)	4.8	4.1	0 - 13	N	
Hexacarboxy (6-CP)	2.3	1.9	0 - 4	N	
Pentacarboxy (5-CP)	24.2	20.5	0 - 10		H
Mercury associated peaks 1,2 total	14.0	11.9	0 - 9.0		H
Coproporphyrins I, III (CP)	982.4	832.6	0 - 153		H

CRT=creatinine (118 mg/dL)

To evaluate the ability of Laboratoire Philippe Auguste to test children with lower levels of porphyrins, a urine sample was collected from a child previously diagnosed with autism who had completely recovered and was attending public school in a normal class without any aides. The child had had six months of chelation therapy with the chelating agent DMSA. No elevated mercury had been detected in the urine in two separate samples collected after DMSA treatment. The sample was divided into 2 aliquots. One aliquot was sent to Laboratoire Philippe Auguste by regular USA mail, and the second was sent to LabCorp.

The huge discrepancy (Table 3) of two of the porphyrins (uroporphyrin and pentacarboxyporphyrin) between The Great Plains Laboratory results and Laboratoire Philippe Auguste results is not based on lack of accuracy because both labs provided similar results from the analysis of the calibrator. This lack of correlation is most likely due to some compounds that co-elute with the analytes of interest in the Laboratoire Auguste Philippe test. This condition is evident when you compare chromatograms between The Great Plains Laboratory/ LabCorp and Laboratoire Philippe Auguste. The Great Plains Laboratory/LabCorp uses HPLC conditions that separate Coproporphyrin I and III. Laboratoire Philippe Auguste uses a different HPLC condition that is unable to separate them. It seems possible Laboratoire Philippe Auguste is also unable to separate compounds that coelute with uroporphyrin and pentacarboxyporphyrin.

Table 3. Porphyrin test results on recovered child who had previously been chelated with DMSA

Porphyrin nmol/ g CRT	Laboratoire Philippe Auguste	The Great Plains Laboratory/ LabCorp	% difference Laboratoire Philippe Auguste vs. The Great Plains Laboratory/ LabCorp**
Uroporphyrins	16.71	3.1	539
Heptacarboxy-	3.04	2.8	8.6
Hexacarboxy-	1.09	0.0	*
Pentacarboxy-	3.22	1.0	322
Precoproporphyrin(Philippe) Or Mercury assoc. peaks (Great Plains)	18.37	0.0	*
Coproporphyrins I,III	228.39	251	9.0

*Unable to calculate. Division by zero.

** % difference was calculated by dividing the difference between the mean Laboratoire Philippe Auguste porphyrin value by The Great Plains Laboratory /LabCorp value and multiplying the net result by 100.

A chromatogram of urine from mercury-treated rats showed two significant peaks (Figure 3A) next to the pentacarboxyporphyrin peak which are not present in a chromatogram (Figure 3 B) of urine from the DMSA-treated child in The Great Plains/LabCorp(Figure 3B) test. These peaks associated with mercury toxic rats are essentially missing from the LABORATOIRE PHILIPPE AUGUSTE chromatogram (Figure 3C). This same chromatogram indicates a huge “precoproporphyrin” peak, about 6 times bigger than the pentacarboxyporphyrin. The interpretation of the Nataf laboratory indicates that the porphyrin pattern is consistent with mercury toxicity.

In reality, this pattern is **inconsistent** with the research done by Dr Woods. It appears that the “precoproporphyrin” identified by the Laboratoire Auguste Philippe test in the urine of the recovered child with no current evidence of mercury toxicity is due to a combination of unresolved large interfering peaks that coelute with mercury associated peaks (if indeed such peaks are even present). Earlier in the manuscript, it was shown (Figure 1) that significant interfering peaks occur very near to that of “precoproporphyrin” when a prepurification step is omitted as it is in the LABORATOIRE PHILIPPE AUGUSTE method. Based on Woods’ research pentacarboxyporphyrin, “precoproporphyrin", and coproporphyrins I, III are **all elevated** in mercury-toxic rats and humans. The lack of elevation of pentacarboxyporphyrin in the LABORATOIRE PHILIPPE AUGUSTE test of the urine of the recovered child also indicates that the elevated peak at the retention time

of “precoproporphyrin” is not, in fact, the same compound measured by Woods but is probably the interfering substances that were not removed prior to analysis.

In the chromatogram of The Great Plains/LabCorp test (Figure 3A), uroporphyrin is a very small peak with 2 large interfering peaks eluting very closely to uroporphyrin. In the LABORATOIRE PHILIPPE AUGUSTE chromatogram (Figure 3C), these interfering peaks are not apparent, probably because they are not resolved **at all using the LABORATOIRE PHILIPPE AUGUSTE method. Thus, i**t is likely that the 2 large peaks next to uroporphyrin in the chromatogram of in The Great Plains/LabCorp (Figure 3B) test appear as a single peak in the chromatogram from LABORATOIRE PHILIPPE AUGUSTE, falsely elevating the uroporphyrin result. Coproporphyrins I and III are not separated at all in the LABORATOIRE PHILIPPE AUGUSTE chromatogram (Figure 3C) while they are separated completely in The Great Plains/LabCorp chromatogram (Figure 3B).Pentacarboxyporphyrin is also much higher in the analysis of LABORATOIRE PHILIPPE AUGUSTE compared to that of The Great Plains/LabCorp method. An examination of the LABORATOIRE PHILIPPE AUGUSTE chromatogram indicates that one or more additional peaks appear to coelute with pentacarboxyporphyrin, resulting in a falsely elevated value for this compound. Overall, the resolving ability of the LABORATOIRE PHILIPPE AUGUSTE chromatography system appears to be inferior to that of The Great Plains/LabCorp method.

A number of other samples were split with the LABORATOIRE PHILIPPE AUGUSTE with outcomes similar to the one above, indicating that the sample analysis described above is likely representative of a substantial portion of the testing of the LABORATOIRE PHILIPPE AUGUSTE.

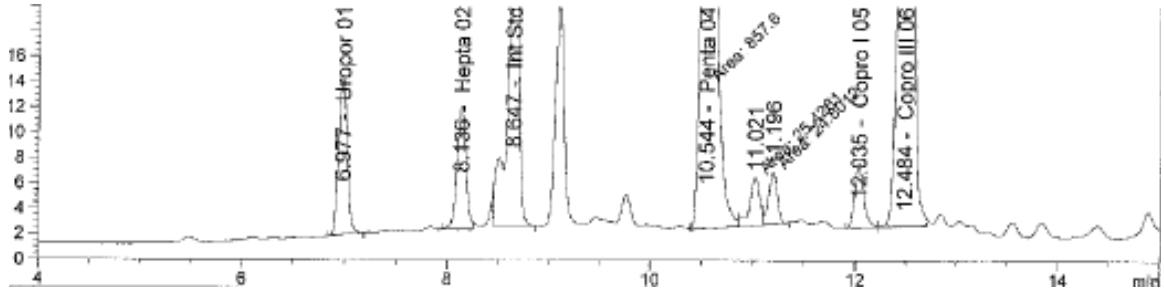


Figure 3A. Urine from mercury-treated rats by Great Plains Laboratory/LabCorp method

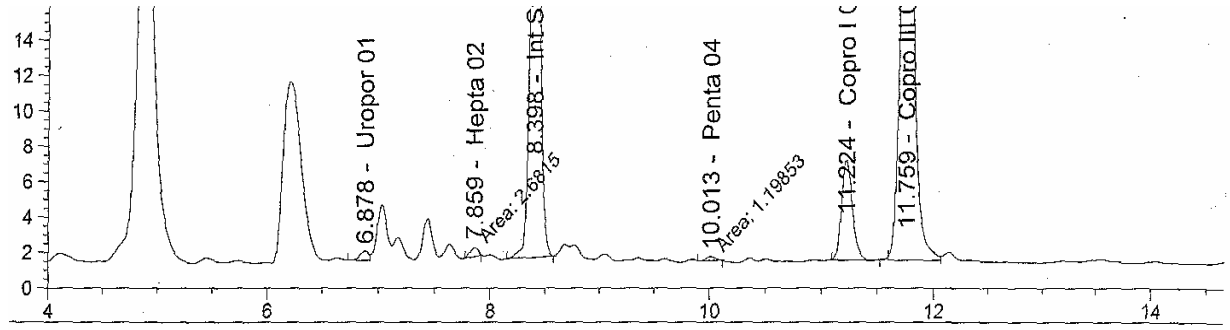


Figure 3 B. Urine from DMSA-treated recovered child tested by Great Plains Laboratory / LabCorp

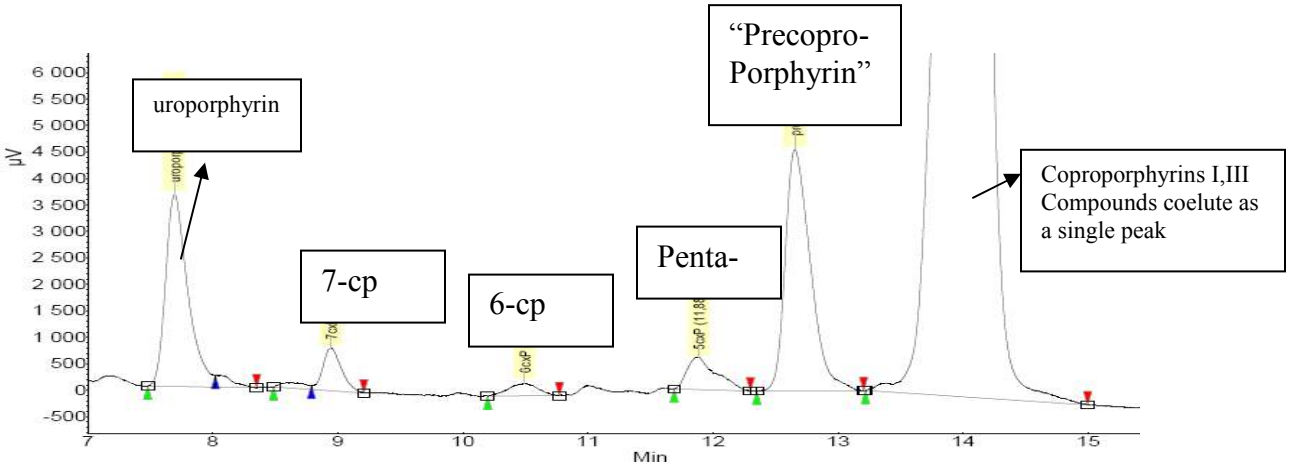
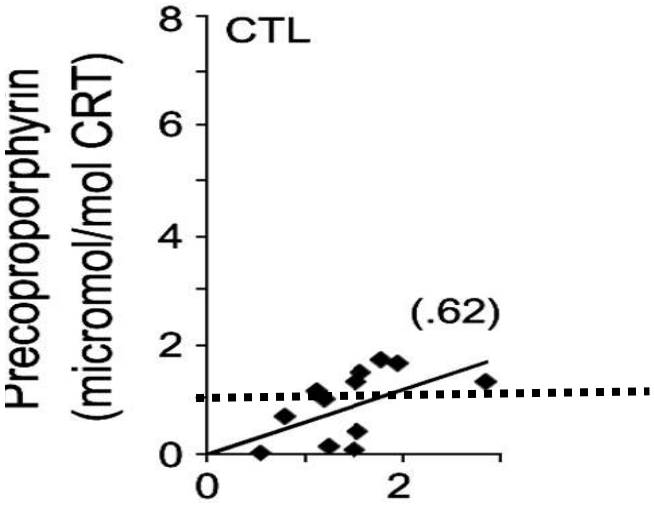


Figure 3C. Urine from DMSA-treated recovered child tested by LABORATOIRE PHILIPPE AUGUSTE

In addition, a second urine sample from the recovered child was sent to **an England lab** for mass spectrometry testing by HPLC/MS/MS. **This well known porphyrin researcher** failed to detect any of the ions that have been proposed for ketoisocoproporphyrin, the putative chemical proposed to be "precoproporphyrin".

What is the normal range for "precoproporphyrin"?
Inconsistency of normal ranges also seems to be a severe problem for the Nataf laboratory. Reproduced below is the normal range values reported in Nataf's article. We have modified the graph by drawing a straight line across from the value of "precoproporphyrin" at a concentration of 1.0 micromol/mol creatinine (CRT), a value equal to 8.85 nmol/mol creatinine. This value is nearly identical to the upper limit of normal reported by the Laboratoire Philippe Auguste which is 9.0 nmol/mol creatinine. There are 12 data points with 6 points clearly above 1.0 and 5 data points below 1.0 with 3 data points above at or above 1.5 micromol/mol creatinine. One data point is at or near 1.0. However, the reference range used commercially by Nataf's laboratory is 0.565-1.017 micromoles/mol creatinine, after converting the Laboratoire Philippe Auguste units (5-9 nmol/g CRT) to the same units found in the Nataf paper.* With 50% of the normal samples exceeding 1.0 micromoles/mol creatinine and 25% of the normal samples exceeding 1.5 micromoles/mol creatinine, the normal ranges of the Laboratoire Philippe Auguste are inconsistent with the previous research paper and require explanation. It would appear that the normal range should be 0-2.0 micromoles/mol creatinine or 0-17.7 nmol/g CRT.

* The conversion factor was calculated as follows:
nmol porphyrin/mol creatinine X 113 g creatinine/mol creatinine X 1 micromol/10^3 nmol =0.113



Reproduction of the figure from the Nataf paper of normal control urine porphyrin results. A straight line has been introduced at the concentration of 1.0 micromoles/mole CRT, the approximate upper limit of normal currently reported by the Laboratoire Auguste Philippe.

Are Laboratoire Philippe Auguste values for other porphyrin compounds appropriate for the age groups tested?

Coproporphyrin reference ranges: failure to provide adequate age-matched controls
Laboratoire Philippe Auguste reports use total coproporphyrin normal reference range between 100 – 200 nmol / g creatinine (CRT) for children. These units (nmol/g CRT) can be expressed as 11.36 – 22.72 micromol/mol creatinine. The values are reflected in fig. 2 of the Nataf paper reproduced below in which the control mean is approximately 10 micromol/mol creatinine (CRT) and the mean plus 2 standard deviations is approximately 20 micromol/mol creatinine (CRT). In the same figure, the mean value for autistic children is approximately 22 micromol/mol creatinine. Thus, superficially it appears reasonable to conclude that total coproporphyrins are significantly higher in the group with autism compared to the controls. However, Table 1 of the same Nataf paper indicates a major research flaw in reaching such a conclusion. The average age of the autism group is 6.4 years compared to an average age of 10.3 years in the control group.

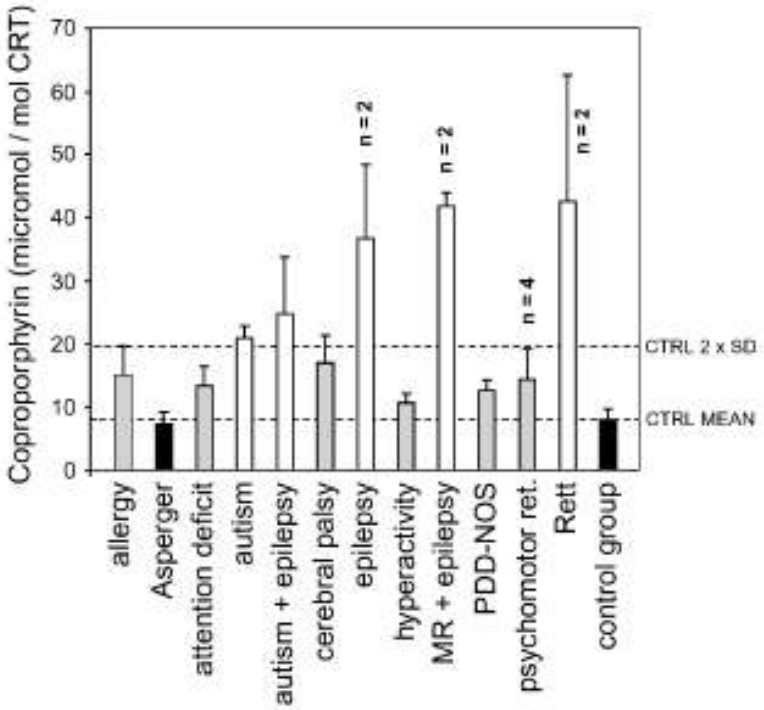


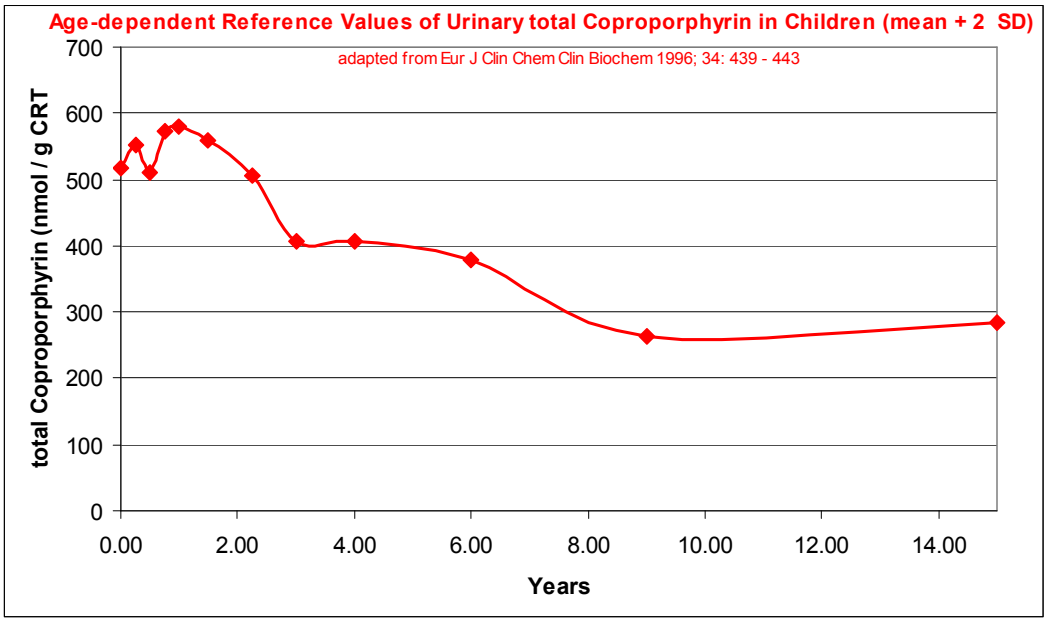
Fig. 2. Coproporphyrin levels in urines of children with neurodevelopmental and related disorders (Table 1 for details); the control group comprised children with unrelated conditions. Error bars are standard errors of the mean. Horizontal dashed lines indicate the control group (CTRL) mean and the mean plus 2 x standard deviation (SD). N values are indicated for groups with less than 8 subjects. MR, mental retardation; PDD-NOS, pervasive developmental disorder not otherwise specified.

Table 1 from the Nataf paper.

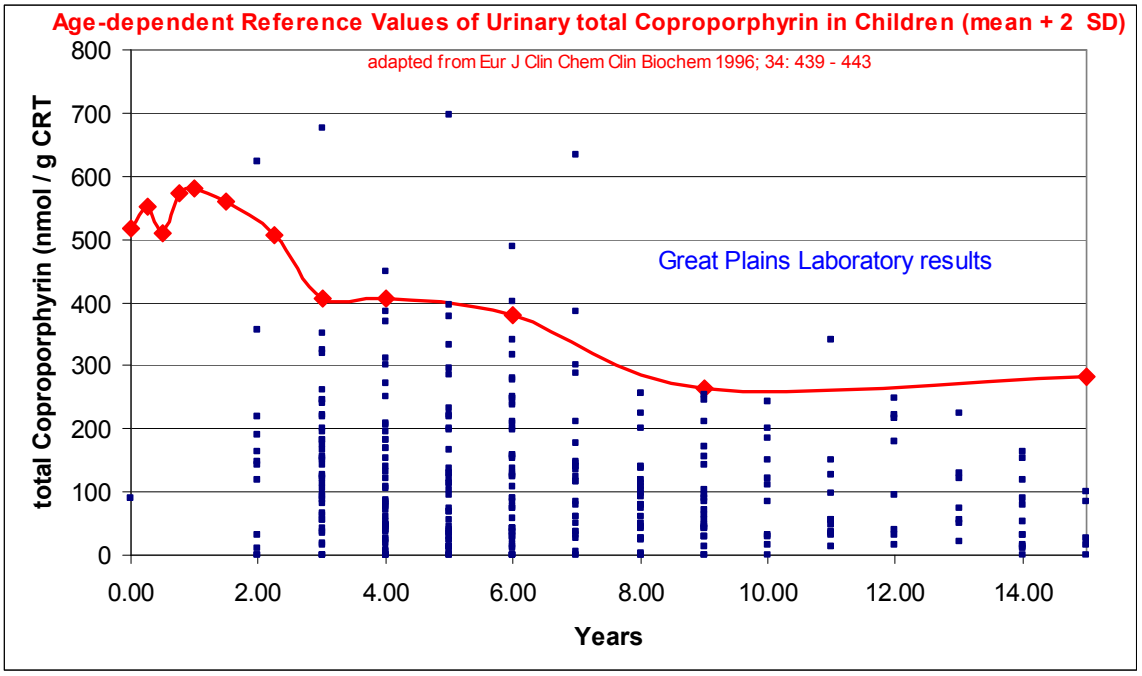
Table 1
Study subjects

Condition/diagnosis	M	F	Total	Mean age (years)	M/F	% total	% ASD group	
Allergy	5	3	8	7.3	1.67	3		ASD =
Asperger	10	1	11	10	10	4.1	5.8	71% of
Attention deficit	2	7	9	9.4	0.29	2.3		total
Autism (autistic disorder)	79	27	106	6.4	2.9	39	55.5	sample
Autism + epilepsy	7	2	9	9.3	3.5	3.3	4.7	(M/F = 3.34)
Cerebral palsy	6	6	12	8.3	1	4.4		
Epilepsy	2	0	2	10	na	0.7		
Hyperactivity	27	2	29	9.1	13.5	10.7		
MR + epilepsy	1	1	2	6	1	0.7		
PDD-NOS	51	12	63	6.6	4.3	23.4	33	
Psychomotor retardation	1	3	4	7.3	0.33	1.5		
Rett	0	2	2	2.5	0	0.7	1	
Control group	7	5	12	10.3	1.4	4.4		
TOTAL	198	71	269	7.4	2.8			

A Swiss study 'Age-dependent Reference Values of Urinary Porphyrins in Children' (7) consisting of 198 normal children ages 0.25 to 15 years old, showed that the normal upper range for total coproporphyrin (mean+ 2SD) for a 6 year old was as high as 42.86 micromol/mol CRT, indicating that **all** values for the autism group in fig. 2 of Nataf's paper are within the age-appropriate normal ranges when **age-appropriate** control values are used. A graph of this study is reproduced below.



Furthermore, The Great Plains Laboratory created a graph of 322 patients with autistic spectrum disorder in which coproporphyrin values obtained by The Great Plains Laboratory/Labcorp method were plotted using the appropriate age-related normal values. As shown in the graph, only a few patients (n=9 ; 2.8%) with autism were outside the age appropriate reference range.



Conclusions:

1."Precoproporphyrin" has not been adequately characterized by Nataf, the Laboratoire Philippe Auguste, or any other laboratory. There is no evidence that this compound is even a porphyrin at all. The chromatography in the Bowers paper indicates a contaminating peak in the same region as "precoproporphyrin". Bowers group (which includes Dr. Woods) took special efforts to remove this contaminant. The Laboratoire Philippe Auguste does not appear to take such special efforts based on the method description in the Nataf paper.

2. Two peaks associated with mercury exposure in rats were identified by The Great Plains Laboratory /LabCorp test but it was rare to find elevations of these peaks unless several other porphyrins were also very abnormal. These mercury associated peaks were missing from The Great Plains Laboratory/LabCorp chromatogram of a urine sample of a child recovered from autism who had been previously chelated and who had no evidence of current mercury toxicity. An aliquot of the same sample was **incorrectly** identified as consistent with mercury toxicity by the analysis of Laboratoire Philippe Auguste, which found a normal pentacarboxyporphyrin in this sample while simultaneously finding an elevated "precoproporphyrin" even though such patterns have never been identified by Woods in any of his publications.

The evidence is overwhelming that the values of "precoproporphyrin" reported by Laboratoire Auguste Philippe are falsely elevated due to chromatography with deficient resolution combined with the lack of prepurification by Laboratoire Auguste Philippe in contrast to Woods' work on "precoproporphyrin".

3. Unexplained inconsistencies of "precoproporphyrin" reference ranges exist between the published Nataf paper and the reference ranges used currently by Laboratoire Philippe Auguste.

4. Reference ranges of the Laboratoire Philippe Auguste or the Nataf paper are not adequately controlled for age-related changes in porphyrin values. Younger children, in general, have much higher porphyrin values than older children. After correction for differences due to age, differences between normal and autistic children do not appear to be highly significant.

5. A small percentage (2.8 %) of patients on the autistic spectrum screened by The Great Plains Laboratory has abnormal porphyrin results. It appears to me that a chelation challenge test is the most effective way to screen for heavy metal toxicity but the appearance of certain mercury associated peaks may be an indicator of severe mercury (or perhaps other heavy metal) toxicity.

6. Calibration standards tested by the two laboratories are very similar indicating the two laboratories have similar analytical values when calibration standards with few interferences are present. Significant differences in certain porphyrin values were found in the urine sample of a patient recovered from autism and in a group of additional samples probably due to lower resolving ability of the Laboratoire Auguste Philippe chromatography system. Higher values for "precoproporphyrin", pentacarboxyporphyrin, and uroporphyrin by Laboratoire Auguste Philippe compared to The Great Plains Laboratory/Labcorp appear to be due to the erroneous measurement of multiple coeluting peaks as if they were a single compound.

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8. Source of porphyrin calibration standard:

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