Metal exposure in the physically and mentally challenged children of Punjab, India

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ABSTRACT

We collected 149 hair samples at the Baba Farid Centre at Faridkot in Punjab, India to evaluate the trace and toxic metal concentration via ICP-MS. A total of 53 elements were tested. The hair of the children tested showed high values for Ba, Cd, Mn, Pb and U, signifying long-term exposure. Urine baseline testing supported hair analysis findings for all the elements listed above; a DMSA (Dimercapto Succinic Acid) challenge test raised urinary values for lead. Testing of six randomly selected water samples showed concentrations above the European maximum contaminant level for uranium (U) in three samples and lead (Pb) in one.

Research aim:
- a) To evaluate if hair analysis and/or urine provocation confirm or refute long term metal intoxication.
- b) To support or refute that hair mineral analysis confirms urine challenge test results.
- c) To support or refute that a DMSA urine challenge test provides a valuable treatment option for metal exposure in children.

Conclusion:

Our results documented that hair and urine mineral analysis results are supportive of each other, and are both useful diagnostic tools in chelation therapy. We also documented that a DMSA challenge test confirms long term exposure as detected through hair mineral analysis. This indicates that the chelating agent DMSA (Dimercapto succinic acid) provides a safe and valuable treatment option for lead overexposure.

Keywords: barium; cadmium; manganese; lead; uranium; urine analysis; hair analysis; water analysis; DMSA; chelation; Punjab children; India.

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BACKGROUND AND INTRODUCTION

By many, India is considered the toxic dumping ground of Europe and the United States. A BBC News Article from 11 Sept 2000 stated “More than 100,887 tonnes of hazardous and potentially hazardous wastes entered illegally into India in 1998-99, stated Greenpeace Asia Toxic Campaigner in India, Nityanand Jayaraman. (1) This was despite a 1997 Indian Supreme Court order banning the import of hazardous wastes. Among the waste products dumped are zinc ash, lead waste, used batteries, cadmium, cobalt and chromium.

A report, released Nov 4, 2003 by the New Delhi-based environment group, Centre for Science and Environment (CSE), states “imports of the toxic compound have gone up six times in the last seven years.” (2) While the richer nations such as US and Europe are forced to reduce toxic waste dumps in their own countries, selling toxic waste to India has become an enterprise.

The Indian Ministry of Environment & Forests sponsored a status report by the National Environmental Engineering Research Institute, Nehru Marg, Nagpur-440 020 (India in 2006) which concluded that “Hazardous waste management is a new concept for most of the Asian countries including India. The lack of technical and financial resources and the regulatory control for the management of hazardous wastes in the past had led to the unscientific disposal of hazardous wastes in India, which posed serious risks to human, animal and plant life.”

The safety of Indian ground water and drinking water deserves attention. An estimated 10 million Indians may be exposed to high arsenic. Other metals found in unsafe quantities were manganese, iron, lead, nickel and chromium. (3)

Uranium and uranium waste is another concern. Due to India’s geological formation, Uranium is found in water and soil of certain regions, posing a potential risk. At Jaduguda of the Western State of Jamshedpur uranium is mined. On April 11, 2007, the Hindu Business Line quoted Mr S.K. Jain, Chairman and Managing Director of Nuclear Power Corporation of India Ltd.(UCIL). It was said at the press conference that India had proven reserves of about 60,000 tons.

Indian mining and nuclear facilities operate quietly. When thousands of liter of radioactive waste spilled in a creek because of a pipe burst at the UCIL facility at Jaduguda, the accident neither made newspaper headlines nor did UCIL come to know of the disastrous leak till alerted by the local villagers. A rise in crippling diseases has since been noted in the area. (4)

Punjab is one of the smallest states of India with an area of 50362 sq km. It is situated in the North Western part of India, bordering Pakistan. A fertilizer plant was set up in Nangal, Punjab in 1961, a heavy water plant was commissioned in 1962. (5)

Punjab’s natural eco system, rich in animal and plant life, is facing problems. Continuous use of herbicides, pesticides and large volumes of fertilizers are affecting the ecological quality of soil and water. While no uranium mining is reported in Punjab, coal mines may be a uranium source, InfoChange News & Features stated in an editorial on May 2009. Water testing by the Bhabha Atomic Research Centre (BARC) of Mumbai ruled out danger to health, although the uranium content in water samples was found to be in the range of 2.2-244.2 micrograms per liter, which translates into 0.002 to 0.244mg/l. The German Environmental Protection Agency (UBA) has recommended a maximum contaminant level of 0.01mg/l; American Environmental Protection Agency (EPA) of 0.03mg/l. The Atomic Energy Regulatory Board has set a limit of 60mcg/l, which is 0.06mg/l. This indicates that the definition of what is considered harmful leaves room for interpretation. (6)

Through the authors’ involvement in Clinical Metal Toxicology this study evolved. The South African neurotherapist Carin Smit, a Candidate of Clinical Metal Toxicology, alerted the authors to the problem children of the Baba Farid Centre at Faridkot in Punjab, India. Many of the young patients receiving treatment there suffer from a variety of health problems, and through this study, the authors set out to prove or disprove metal intoxication.

METHODOLOGY AND EXPLANATION

Phase 1

Phase 1 of this study consisted of a hair sample evaluation. A total of 149 samples were
collected at the Baba Farid Centre at Faridkot in Punjab, India. Carin Smit volunteered to oversee the sampling according to protocol. Samples were shipped to Micro Trace Minerals Laboratory Services in Germany. Testing was performed in cooperation with the analytical team of Labor Friedle of Regensburg. All samples were washed with a de-ionized detergent, rinsed 3 times with de-ionized water and dried before weighing. Samples were weighed in at around 100mg, with sample weights varying between 94mg and 116mg.

For sample digestion, certified metal-free acids were used. Digestion took place in a closed-vessel microwave digestion system. Ultrapure water was used for final sample dilution and testing was performed via inductively coupled plasma with mass spectrometry (ICP-MS) utilizing collision/reaction cell methods coupled with ion-molecule chemistry, a relatively new method for interference reduction. (7) Statistical evaluation of data included a comparison of test values to existing reference ranges for children and adults. Certified hair standards and in-house standards were used to validate results.

We used Hair mineral analysis (HMA) as a starting test to locate chronic exposure. While HMA received universal attention as a tool to evaluate long term metal exposure, recognition of this test as a valuable diagnostic tool largely remains with researchers, or practitioners of alternative medicine. While considerable research has documented HMAs validity (8-10), its value of diagnosing metal overexposure at an early stage before acute symptoms of metal intoxication is viewed controversial.

HMA can also be used as a diagnostic tool to determine chronic under-supplementation of nutrient metals before a state of acute deficiency is reached. Despite existing research, the medical establishment has difficulties in understanding and accepting this concept. (11) For instance, research confirmed that hair and serum zinc values confirm the zinc status of Ethiopian and Bangladesh children, (12,13) and that of vegetarian pregnant females. (14) Ilhan et al reported in 1999 that serum and hair analysis suggest that the changed element status (Zn, Mg, and Cu) in hair play an indicator role in the diagnosis of epileptic patients. (15) Bryne and Benedik of the University of Llubljana compared uranium levels of blood, hair and urine of occupationally exposed and non-exposed test persons and concluded that hair shows promise as an indicator of exposure. (16) Our research supports all of the above.

**Phase 2**

Hair analyses results provided a basis to ‘prepare’ our test group for the DMSA challenge test. All synthetic chelating agents, DMSA included, show an affinity to bind nutrient metals. Therefore, nutritionally deprived patients, especially ill children, should be nutritionally evaluated prior to chelation therapy. Nutritional needs must be met before any type of chelation is started.

We thus provided the Faridkot Centre with nutritional supplements including a multimineral-vitamin-amino acid complex in powder form, zinc gluconate, 15mg capsules, lecithin granules and probiotic capsules to support the digestive tract. Dr. Prithpal Singh, a naturopath and social activist who heads the Centre was in charge of distributing supplements and the children were on a supplemental program for several weeks prior to the DMSA urine challenge.

**Phase 3**

Prior to the DMSA urine challenge, all children were clinically evaluated. A blood chemistry was performed, including evaluation of:

- B. Urea
- S. Creatinine
- S. Bilirubin
- S. Alkaline Phosphatase
- SGOT
- SGPT
- Serum Calcium
- S. Sodium
- S. Potassium

Children admitted into the study showed no abnormal values. Again, Carin Smit volunteered her time to oversee Phase 3. Of the initial 149 test persons, we received a total of 55 baseline urine samples from patients age 6 to 12 years, and 55 DMSA provocation urines. (Note: To avoid contamination, all urine collection cups and tubes had been provided to the Centre by Micro Trace Minerals Laboratories of Germany.)

- For baseline urine samples, the urine was collected in morning.
- Patients then received oral DMSA, 10mg/kg body weight. DMSA was given on an empty stomach.
• The provocation urine was collected after 4 hours.

Samples were acid-digested with certified metal-free acids involving closed vessel microwave digestion. For sample dilution ultrapure water was used. Testing was performed via inductively coupled plasma with mass spectrometry (ICP-MS) as outlined under Phase 1 (see hair mineral analysis). Certified urine standards and in-house standards were used to validate results.

Urine test values were compared with reference ranges as provided by the German Environmental Protection Agency (UBA). (18) Statistical data evaluation included a comparison of test values to existing reference ranges for children and adults.

Phase 4

While Dr. Singh had collected a number of water and soil samples, we randomly selected six water samples for testing. Water testing was performed utilizing ICP-MS as outlined under Phase 1 and Phase 3.

Phase 5

A. Friedle volunteered his time to validate test results and provided the initial evaluation of data.

E. Blaurock-Busch further validated and compared urine and hair data as shown below.

RESULTS

1. Hair Mineral Analysis (HMA)

The patient population at the Baba Farid Centre at Faridkot was randomly selected. All were mentally and/or physically impaired. Seizures were listed among a variety of health problems. For the 149 participating persons, the main symptoms listed were:

- Cerebral Palsy– 76 persons
- Cerebral Palsy and Mental Retardation– 24 persons
- Mental Retardation alone – 12 persons
- ADHS- 13 persons
- Autism- 4 persons

During Phase 1 of the research, we utilized hair analysis to evaluate the long-term metal exposure of 149 test persons, 114 of them were younger than 12 years of age. Since Environmental Protection Agencies provide no reference ranges for hair, Micro Trace Minerals Laboratories had developed reference ranges for adults and children, following standard procedures as recommended by American and German/European Laboratory Regulations as early as 1984. (18) From 1997 to 1998, E.Blaurock-Busch in cooperation with Prof. Helion Povoa of Rio de Janeiro, Brazil evaluated hair samples from over 10000 people, including children from the US, Brazil, and Germany and compared values to existing ranges. The last comprehensive hair reference range evaluation and update was in 2006. Cadmium ranges for children were amended in 2009.

For this study, we established a 95 Percentile of all test values (N=149) and compared these to adult reference ranges. See TABLE 1.

For the study, we established a 95 Percentile of all hair test values from children less than 12 years of age and compared that data to the appropriate reference range for children. We found deviation from established reference ranges for a number of metals.

The most significant changes are listed in TABLE 1&2.
Barium: For the adults, 9% exceeded the adult reference range; for the children's group 32% exceeded the lower reference range for children.

The 95 Percentile test value for the children group was 4.16mg/kg compared to a 95 Percentile Reference Range of 2.65mg/kg.

Barium is a heavy metal that enters the air during mining processes, refining processes, and during the production of barium compounds. It can also enter the air during coal and oil combustion. Some barium compounds that are released during industrial processes dissolve easily in water and are found in lakes, rivers, and streams. Barium levels may be higher at hazardous waste sites.

Exposure to barium occurs mostly in the workplace or from drinking contaminated water. Ingesting drinking water containing levels of barium above the EPA drinking water guidelines for relatively short periods of time can cause gastrointestinal disturbances and muscle weakness. Ingesting high levels for a long time can damage the kidneys. (21,22)

Cadmium: 25% of the children's group and 6% of the adults exceeded the reference range.

The 95 Percentile test value for the children group was 1mg/kg compared to a 95 Percentile Reference Range of 0.2mg/kg.

Eating food or drinking water with very high cadmium levels severely irritates the stomach, leading to vomiting and diarrhea. Eating lower levels of cadmium over a long period of time can lead to a build-up of cadmium in the kidneys. If the levels reach a high enough level, the cadmium in the kidney will cause kidney damage. Exposure to lower levels of cadmium for a long time can also cause bones to become fragile and break easily. Anemia, liver disease and nerve or brain damage have been observed in animals eating or drinking cadmium, but ASTDR has no good information that indicate what levels of cadmium people would need to eat or drink to result in these diseases, or if they would occur at all. (23)

Manganese: 87% of the children <12 years of age and 26% of the patients >13 years of age exceeded the appropriate reference range. The 95 Percentile test value for the children group was 7mg/kg compared to a 95 Percentile Upper Reference Range of 0.72mg/kg.

Manganese is an essential trace element and ingesting small amounts from food or water is needed to stay healthy. Exposure to excess levels of manganese may occur from breathing air, particularly where manganese is used in manufacturing, and from drinking water and eating food. At high levels, it can cause damage to the brain. (24)

Lead: 55% of the children <12 years of age and 29% of the adults exceeded the appropriate reference range.

The 95 Percentile test value for the group of 114 children was 16mg/kg compared to a 95 Percentile Reference Range of 3mg/kg.

Exposure to lead can happen from breathing workplace air or dust, eating contaminated foods, or drinking contaminated water, which may come from lead pipes in homes. Children can be exposed from eating lead-based paint chips or playing in contaminated soil. Lead is also a stable element of the uranium decay chain. Lead can damage the nervous system, kidneys and the reproductive system. The Centers for Disease Control and Prevention (CDC) recommends that children are tested as early as at ages 1 and 2 years. EPA limits lead in drinking water to 15µg per liter (=0.015mg/l). (25)

Uranium: 88% of the children <12 years of age and 85% of the patients >13 years of age exceeded the recommended reference range for children resp. for adults. The 95 Percentile for children was 1.9mg/kg compared to a hair reference range of 0.1mg/kg, exceeding levels 20 times.

Uranium is a naturally occurring chemical substance that is mildly radioactive. Everyone is exposed to low amounts of uranium through food, air, and water. Exposure to high levels of uranium can cause kidney disease. It is not known to cause cancer, but can decay into other radioactive materials that may. (26)

2. Baseline Urine Test Results versus DMSA challenge test results

Baseline urine results exceeded the reference ranges for many elements, indicating immediate metal exposure. The reference ranges listed are provided by the German Environmental Protection Agency or are developed by the laboratory according to standard laboratory regulations. (18)

DMSA has been approved by the US Food and Drug Administration for the treatment of lead intoxicated children, and the DMSA challenge indeed increased urinary excretion for
lead. The recommended dose is 10-30mg/kg body weight. Due to the severity of some of the children’s condition, we stayed with the lower dose.

Barium, cadmium, manganese and uranium were not affected by the DMSA challenge.

3. Water Metal Testing

Since urine baseline levels exceed the post chelation levels for the elements barium, cadmium, manganese and uranium, we did suspect immediate environmental exposure. Consequently, Dr. Prithpal Singh of the Center collected a number of water or soil samples, of which we randomly tested six.

Since water issues are widely discussed in Indian publications, we tested a number of potentially toxic elements as listed below. Concerning the sample sources listed, we, the analysts, have no knowledge where these places are and how these relate to the population of the Children’s Center.

In TABLE 5, water results are compared to reference ranges (RR), also called maximum contamination ranges as set by the European Water Regulation Boards.

Values highlighted in red exceed reference ranges as set by the German Environmental Protection Agency (UBA). For uranium, these ranges are lower than those set by the US Government which has set a maximum contaminant limit of 30µg/l = 0.003mg/l much representing our values. It would be of great interest to know what maximum contamination level (MCL) these values were compared to. (19)

## CONCLUSION

- ICP-MS Hair mineral analysis confirmed long-term metal exposure for Barium, Cadmium, Manganese, Lead, and Uranium, with the children’s hair values exceeding reference ranges more than the adult group. See Table 1, 2 and 3.
- Baseline Urine testing of the children’s group confirmed metal exposure for all elements tested. See Table 4: The very high concentration of uranium in 3 of the water samples supports the much-discussed problem of environmental exposure. See Table 5.
- The results of this study demonstrate that Hair Mineral Analysis can be used as a diagnostic tool to confirm long-term metal exposure of toxic and nutrient metals.
- Hair mineral results and baseline urine results provided diagnostic information in support of each other. This documents that both tests are valuable for the detection and treatment of metal intoxication, particularly if used in combination.
- Data documented that oral DMSA chelation is a useful and safe therapeutic approach to treat lead intoxication in children. Although some research list DMSA as a potential uranium antidote, (17) our data could not confirm this.

## DISCUSSION

In clinical metal toxicology, it is a known fact that chronic metal exposure affects health.
Whether and when treatment is needed is discussed and argued among health professionals. It is clear, however, that a one time provocation treatment provides information regarding exposure and excretion ability, but is never sufficient to eliminate a problem of acute or long term exposure.

Compared to intravenous chelation, oral chelation has its special advantages and disadvantages. One of the advantages is the ease and relative safety of application. It is far easier to provide a child with an oral chelating substance such as DMSA, rather than using invasive procedures such as injections or infusions.

Depending on the point of view, the disadvantage of oral chelators is its way of action. Oral chelators first detoxify the digestive tract. Unless the digestive tract is relatively metal-free (which is unlikely in those exposed to metal-rich foods or people suffering from digestive disorders), a first challenge with an oral chelator will first bind metals found in the digestive tract, increasing metal excretion via fecal matter. Consequently, urinary excretion may be secondary i.e. comparatively minor.

The reason is simple. Every chelating substance has a limited binding ability which can be mathematically defined based on the molecular weight of the substance. If the oral chelator’s binding capacity is largely saturated by metals found in the digestive tract and the remaining activity is limited. For the same reason, urinary metal concentration will be limited as well.

This study was funded and supported by individuals. It was carried out over 2-3 years, challenging us in many ways. Overall, this study provides valuable information that hopefully paves the way for follow-up studies, which are much needed.

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**TABLE 5.** Test results of randomly selected water samples from Faridkot area, Punjab. Results are in mg/l

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>Ag</th>
<th>Al</th>
<th>As</th>
<th>Ba</th>
<th>Bi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Range</td>
<td>0.005</td>
<td>0.2</td>
<td>0.01</td>
<td>1</td>
<td>no RR</td>
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<tr>
<td>Limit of Detection (LOD)</td>
<td>0.0008</td>
<td>0.005</td>
<td>0.0005</td>
<td>0.0001</td>
<td>0.00003</td>
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<tr>
<td>VPO Kalli Mandi-Near budha Nala’s railway bridge</td>
<td>0.027</td>
<td>4.112</td>
<td>0.008</td>
<td>0.436</td>
<td>n.d.</td>
</tr>
<tr>
<td>diksha rani-Kotakpura-drinking water 28.07.09</td>
<td>0.001</td>
<td>0.016</td>
<td>0.003</td>
<td>0.016</td>
<td>n.d.</td>
</tr>
<tr>
<td>Baba. Fario- Ctr Special Children Faridkot</td>
<td>0.001</td>
<td>0.018</td>
<td>0.001</td>
<td>0.119</td>
<td>n.d.</td>
</tr>
<tr>
<td>bathinda thermal canal-Punjab</td>
<td>0.001</td>
<td>0.194</td>
<td>0.002</td>
<td>0.065</td>
<td>n.d.</td>
</tr>
<tr>
<td>harpartika kaur house-ground water-faridkot punjab</td>
<td>0.002</td>
<td>0.037</td>
<td>n.d.</td>
<td>0.009</td>
<td>n.d.</td>
</tr>
<tr>
<td>kashish setia house-drinking ground water ferdzerpur</td>
<td>0.001</td>
<td>0.007</td>
<td>0.001</td>
<td>0.086</td>
<td>n.d.</td>
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<thead>
<tr>
<th>Sample Source</th>
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<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
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<td>2</td>
<td>0.2</td>
<td>0.001</td>
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<tr>
<td>Limit of Detection (LOD)</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.0005</td>
<td>0.001</td>
<td>0.0004</td>
</tr>
<tr>
<td>VPO Kalli Mandi-Near budha Nala’s railway bridge</td>
<td>0.003</td>
<td>2.603</td>
<td>0.152</td>
<td>12.7</td>
<td>0.001</td>
</tr>
<tr>
<td>diksha rani-Kotakpura-drinking water 28.07.09</td>
<td>n.d.</td>
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<td>0.004</td>
<td>0.062</td>
<td>n.d.</td>
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<tr>
<td>Baba. Fario- Ctr Special Children Faridkot</td>
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<td>0.047</td>
<td>0.102</td>
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<tr>
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<td>0.015</td>
<td>0.359</td>
<td>n.d.</td>
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<tr>
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<td>0.006</td>
<td>0.089</td>
<td>n.d.</td>
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<tr>
<td>kashish setia house-drinking ground water ferdzerpur</td>
<td>n.d.</td>
<td>0.007</td>
<td>0.025</td>
<td>0.007</td>
<td>n.d.</td>
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<table>
<thead>
<tr>
<th>Sample Source</th>
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<th>Ni</th>
<th>Pb</th>
<th>Sr</th>
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</tr>
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<tr>
<td>Reference Range</td>
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<td>0.02</td>
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<td>Limit of Detection (LOD)</td>
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<td>0.0005</td>
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<td>0.00003</td>
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<td>0.124</td>
<td>0.178</td>
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<td>0.02</td>
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<tr>
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<td>0.002</td>
<td>0.017</td>
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<tr>
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<td>0.002</td>
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<td>0.009</td>
<td>2.395</td>
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<td>0.018</td>
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<td>0.004</td>
<td>0.331</td>
<td>0.003</td>
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<tr>
<td>harpartika kaur house-ground water-faridkot punjab</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
<td>0.020</td>
<td>n.d.</td>
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<tr>
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<td>0.001</td>
<td>0.002</td>
<td>0.001</td>
<td>6.919</td>
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</table>
It should be noted that during the past 3 years, the laboratory team scored near 100% in all governmental round robins scheduled for laboratory licensing requirements. When we started our research, we were well prepared for the task that involved people around the globe and received attention long before we were ready to take a stand. Preliminary information was provided to the media by anxious parties, causing concern among the people of Punjab and among governmental agencies. We regret that. Nevertheless, our hair sample results clearly indicate long term uranium exposure, and urine concentrations point towards environmental exposure. We validated data with extreme care. Double testing did occur. We also tested batches of samples at different times.

The Atomic Energy Regulatory Board also tested hair samples taken from Baba Farid Centre for Special Children in Faridkot, Punjab. VD Puranik of the Atomic Energy Regulatory Board stated that their results indicate that concentrations were within limit. We don’t know what limits i.e. reference ranges were used by the Atomic Energy Regulatory Board. To clarify this statement, it would be necessary to compare methodology and reference ranges. (20)

Due to the fact that we acted from a distance and had no medical doctors present in Punjab to supervise patient care, we chose oral DMSA chelation. FDA and other governmental agencies consider this chelating substance safe for children. Indeed, no unusual health problems were noted following the challenge test. Once again, this supports statements such as the one from the German Federal Environmental Agency declaring DMSA a valuable and safe chelating agent.

The metal concentration of baseline urines exceeded urine challenge levels, and we suspect immediate exposure through water or other sources may be responsible for this unusual occurrence. We have reasons to believe that the children’s ability to detoxify needs to be supported. As pointed out previously, oral chelation involves the digestive system. In fact, when a chronically intoxicated person is chelated with an oral chelating agent such as DMSA, the chelating substance first detoxifies the digestive tract. Since all chelating agents act on the mass principle, binding of easily-available metals in the digestive tract takes priority. While we had provided probiotic supplements to the Center to prepare the children for the oral DMSA challenge, we did not perform stool metal testing on this patient group.

We realize that a one-time challenge can only provide information regarding the patient’s potential toxic burden and his/her ability to detoxify. Thus, a one-time challenge test is only the first part of the diagnostic evaluation. Treatment can start thereafter. In other words, a one-time challenge should not be mistaken for a detoxification program. It is only the start from where detoxification treatment takes place.

We are aware that different toxic metals require different antidotes, and metal overloads found in the children necessitate further inquiry and more specific treatment, including nutritional support which can prevent excessive uptake of environmental toxins.

From our results we conclude that metal overexposure was detected for our test groups. Chronic and immediate uranium exposure was detected, but we cannot conclude that the health problems found in these children are due to the uranium exposure as has been indicated in media coverage. To further support and prove such a link, follow-up studies are required. Of particular interest would be a comparative study on healthy Punjab children.

**Acknowledgement**

Many thanks to Carin Smit of South Africa and Dr. Prithpal Singh of Punjab, India for donating their time to this project.

I am most indebted to Dipl Ing Albrecht Friedle www.labor-friedle.de and Yvette Busch www.microtrace.de. Without their substantial financial and personal support this project would not have been possible. Their engagement exceeded all expectations.

Many thanks for the financial support of Dr. Michael Godfrey, MD New Zealand.
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