(4B) Chemical Analyses in Bio-medical Investigations: the Foundation for Proving Possible Damage to Human Health

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The goal of this presentation:

• To open up discussion on the kind of bio-medical research programs that could uncover possible cause-and-affect “proof of damage” to public health in the town of Zakamensk.
Theoretical background for this proposed program

In accordance with accepted methodologies for establishing possible damage to public health, it is required that evidence be brought forward step by step, mainly by:

• Identifying Bio-Markers of Exposure

....and by

• Identifying Bio-Markers of Effect
F4. Measuring chemical substances (Markers of Exposure) in patients’ (or groups of patients’) bodies

F1. Evaluation of the objective situation. Identifying sources of exposure

F2. Evaluating the conditions of exposure

F3. Characterizing the risks to human health

F4. Measuring chemical substances (the markers of exposure) in (groups of) patients’ bodies

F5. Analysis of a series of laboratory, clinical, functional, and instrumental indicators of substantial doses (markers of response)

F6. Health diagnostics + evaluation of functional impacts on critical organs and bodily systems, as established during risk assessments

Harm is done
A working system of bio-monitoring can help substantiate that contact with certain materials can be hazardous to human health.

The latest advances in gas and liquid chromatography, as well as in atom-absorption spectral-photometry and chromato-mass spectrometry, now allow us to identify and quantify concentrations in blood, urine, breast milk, hair, bile, etc., for **more than 150 different chemical substances** and their various metabolites (**including heavy metals, aliphatic and aromatic hydrocarbons, alcohol compounds and aldehydes, as well as ketones, pesticides, dioxins, etc. etc.**)

![Mass spectrograph of a group of aliphatic hydrocarbons and their derivatives in blood](image1)

![Chromatogram of a sample of blood containing phenol as well as o-, p- and m-cresols—with an internal standard level of naphthalene](image2)
The rationale behind markers of exposure: confirming that humans have been in contact with some external impact factor

**Chemical analyses of both qualitative and quantitative exposure to chemical substances in the surrounding eco-sphere should be adequate to establish actual risks**

<table>
<thead>
<tr>
<th>Target groups to be studied</th>
<th>Means for chemical and analytical testing (35 methods in all)</th>
<th>Chemicals of risk (&gt; 50 classes of substances)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residents living in exposure areas, esp.:</td>
<td>Contents of the human body</td>
<td>Chemical substances</td>
</tr>
<tr>
<td>- Children, 0-14 in age,</td>
<td>- Blood plasma</td>
<td>- Metals</td>
</tr>
<tr>
<td>- Expecting mothers,</td>
<td>- Blood serum</td>
<td>- Aldehydes</td>
</tr>
<tr>
<td>- Women in their reproductive years.</td>
<td>- Urine</td>
<td>- Aromatic hydrocarbons</td>
</tr>
<tr>
<td>Workers who handle hazardous production materials</td>
<td>- Bile</td>
<td>- Aliphatic alcohols</td>
</tr>
<tr>
<td></td>
<td>- Gastric juices</td>
<td>- Aromatic amines</td>
</tr>
<tr>
<td></td>
<td>- Mother’s milk</td>
<td>- Saturated hydrocarbons</td>
</tr>
<tr>
<td></td>
<td>- Hair</td>
<td></td>
</tr>
</tbody>
</table>

Studies should be conducted in accordance with ethical principles put forward in the Helsinki Declarations (of 1975, 1983), whereby information is gathered with the full consent of the (volunteer) subject.

Populations exposed to cresols

Chromatogram of the blood of a baby living in an exposure zone shows high levels of m-, o-, and p-cresols

Chromatogram of the blood of a baby living outside of exposure zone shows normal (average) levels of m-cresols
The Rationale behind Markers of Exposure

The association between higher concentrations of formaldehyde in the blood and the overall dose level from chronic exposure ($R^2 = 0.64$, $p \leq 0.05$)

The association between higher concentrations of manganese in the blood and the overall dose level from chronic exposure ($R^2 = 0.55$, $p \leq 0.05$)

Air-borne components of exposure
- manganese
- chromium
- benzole
- formaldehyde
- methanol

Markers of Exposure in the blood
- manganese $3x$ > than referent concentration Rfc
- chromium – 7 to 8.5 times > than Rfc
- benzole – 0.0012 to 0.01 microgram / liter
- formaldehyde – 4 to 6 times > Rfc
- methanol – 3.5 to 45 times > Rfc

A requisite stage for bio-monitoring is when we establish a direct tie between certain levels of chemical concentrations in body tissues (or bio-media) with specific levels of exposure ($p \leq 0.05$)
F1. Evaluation of the objective situation. Identifying sources of exposure

F2. Evaluating the conditions of exposure

F3. Characterizing the risks to human health

F4. Measuring chemical substances (the markers of exposure) in (groups of) patients' bodies

F5. Analysis of a series of laboratory, clinical, functional, and instrumental indicators of substantial doses (markers of response)

F6. Health diagnostics + evaluation of functional impacts on critical organs and bodily systems, as established during risk assessments

Harm is done
Chemical risk factors and incidence of disease in human populations

**Triggers (or precipitating factors)**
- The level of additional morbidity, including from environmentally determined diseases (i.e. early manifestations, or rapid progression of disease, or premature disabilities) 12-20%

**Primers (patho-morphoses)**
- The growth of certain types of environmentally determined pathologies (i.e., modified biological agents, goiter-related diseases, and diseases of the upper respiratory tracts and gastro-duodenal systems) is 30-35% higher than in uncontaminated sites

**Predictors (for direct and specific impacts)**
- Specific Diseases (i.e. Minimata mercury syndrome, etc.) 0.3-0.5%

**Somatic causal factors**
- (hereditary, autoimmune, and others)

**Etiological factors**
- Acute and chronic specific diseases

**Risk Factors in the Environment**
- Adaptation
- Incapacity
- Loss of adaptability and hypersensitivity to chemical toxicants
- The development of chronic pathological processes

**Average incidence of disease (at independent levels)**
- 65-80%
Effects from chemical substances in the natural and industrial environment

Disruption of homeostasis and the failure of adaptive mechanisms

Central Nervous Systems
Endocrine systems
Immune Systems
Systems of non-specific resistance
Cardio-vascular systems
Organs for metabolism and excretion
Hematopoietic and other blood systems

Level of impact

Molecular
Cellular
Body Tissues
Body Organs

Manifestations of Impact

Membrane and nucleotoxicity
Cytotoxicity
Dystrophy
Functional loss
Degeneration

Enzymopathy
Lack of nutrient balance
Neuro-endocrine dysregulation
Sensitization
Immunosuppression

Microbial Imbalance
Active peroxidation
Auto-immune processes

Pathological processes

Conditions for auto-immunity
Allergy-related, acute inflammatory, and degenerative processes
Chronic inflammatory and degenerative processes
Long-term affects:
- Fetal malformations
- Mutagenesis
- Carcinogenesis
- Gerontogenesis
Under normal conditions, where there is exposure of body tissues to even a certain amount of chemical toxicants, the response of each individual depends on a full variety of factors, such as:

1. The type of environmental exposure
2. The level of toxicant concentrations in the body
3. Genetic pre-conditions
4. The presence or absence of concurrent acute or chronic pathologies
5. Respond reaction for the individual
6. Lifestyle factors
7. The overall status and reserve capacity of the body’s ability to adapt
8. Socio-economic factors

Bio-markers of effect

• Epidemiological indicators
• Clinical data
• Results of research into the functional conditions of body organs and systems
• Laboratory findings
By detecting Markers of Response that are proven to be tied to specific Markers of Exposure, we are allowed to talk of the existence of separate impacts (including—at the level of the proteome—such things as cell apoptosis, or metabolic issues, or other disruptions and predictors of somatic and reproductive pathologies).

**Detailed mass-spectrum of peptides in children’s plasma samples, in the range of 58.1 to 58.7 min.**

**Phenotypes and genotypes by flow-cytometry and polymerase chain reactions in real time**
### Chemical Elements, Critical Organs & Body Systems, with Referent Levels

<table>
<thead>
<tr>
<th>Substance</th>
<th>CAS</th>
<th>RfC, mg/m³</th>
<th>Critical Organs and Systems</th>
<th>RFD, mg/kg</th>
<th>Critical Organs and Systems</th>
<th>Sfi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (Cu)</td>
<td>7440-50-8</td>
<td>2.00E-05</td>
<td>Respiratory Organs</td>
<td>0.019</td>
<td>Digestive tract and liver</td>
<td>–</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>7440-66-6</td>
<td>0.0009</td>
<td>Respiratory organs, immune systems, blood</td>
<td>0.3</td>
<td>Blood, bio-chemicals (superoxide dismutase)</td>
<td>–</td>
</tr>
<tr>
<td>Arsenic (As)</td>
<td>7440-38-2</td>
<td>3.00E-05</td>
<td>Development (i.e., fetal development.), nervous &amp; cardio-vascular systems, respiratory organs, cancer</td>
<td>0.0003</td>
<td>Skin, central nervous, immune, &amp; cardio-vascular systems, hormones (diabetes), digestive tract</td>
<td>15</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>7439-92-1</td>
<td>0.0005</td>
<td>Central nervous system, blood, development, reproductive and hormonal systems, kidneys</td>
<td>0.0035</td>
<td>Central nervous system, blood, biochemical balance, development, reproductive &amp; hormone systems</td>
<td>0.042</td>
</tr>
<tr>
<td>Molybdenum (Mo)</td>
<td>7439-98-7</td>
<td>0.012</td>
<td>–</td>
<td>0.005</td>
<td>Kidneys</td>
<td>–</td>
</tr>
<tr>
<td>Tungsten (W)</td>
<td>7440-33-7</td>
<td>0.1</td>
<td>Respiratory organs</td>
<td>0.0025</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>7440-43-9</td>
<td>2.00E-05</td>
<td>Kidneys, respiratory organs, hormone system, cancer</td>
<td>0.0005</td>
<td>Kidneys, hormonal system</td>
<td>6.3</td>
</tr>
<tr>
<td>Antimony (Sb)</td>
<td>7440-36-0</td>
<td>0.0004</td>
<td>Respiratory organs</td>
<td>0.0004</td>
<td>Biochemical balance. (glucose + cholesterol in blood), death</td>
<td>–</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>7440-48-4</td>
<td>2.00E-05</td>
<td>Respiratory organs</td>
<td>0.02</td>
<td>Blood</td>
<td>9.8</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>7439-96-5</td>
<td>5.00E-05</td>
<td>Central and overall nervous systems, respiratory organs</td>
<td>0.14</td>
<td>Central nervous system, blood</td>
<td>–</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>7439-97-6</td>
<td>0.0003</td>
<td>Central nervous system, hormones, kidneys</td>
<td>0.0003</td>
<td>Kidneys, reproductive, immune + central nervous systems, hormones</td>
<td>–</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>7440-47-3</td>
<td>0.0001</td>
<td>Respiratory organs, liver, kidneys, immune systems, digestive tract</td>
<td>0.005</td>
<td>Liver, kidneys, digestive tract, mucous</td>
<td>42</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>7440-02-0</td>
<td>5.00E-05</td>
<td>Respiratory organs, blood, immune and central nervous systems, cancer</td>
<td>0.02</td>
<td>Liver, cardio-vascular system, digestive tract, blood, body mass</td>
<td>0.84</td>
</tr>
</tbody>
</table>

*Elements, the presence of which can be used to designate an area as an Environmental Disaster Zone in Russia*
Epidemiological Studies

• Analysis of the dynamics, structure, and tempo in which disease grows among the residents in the area under study

• Comparative study of the dynamics and structure of specific diseases between the target area and an analogous “relatively uncontaminated” area—or with data for the entire country

• Determining classes of high-priority diseases and disorders

• Epidemiological analysis of the target group itself

• Making correlations between the identified priority substances and the various factors of risk
Clinical data regarding the onset and development of bronchial asthma (BA) in children, as well as goiter issues in the presence of environmental conditions that are subject to man-made impacts.

**Early onset of allergies in children with BA and goiter**

- **Infants Ages 0-1**
  - Allergic Contact Dermatitis – 22.7%
  - Eczema - 28%
  - Infant Atopic Dermatitis AD – 50.7%

- **Babies Ages 1-3**
  - Childhood form of AD
  - Bronchial Asthma in association with AD 41.4%

- **Children Ages 4 – 7**
  - Disabling forms of Asthma 17.3%
  - Related Ears/Nose/Throat Pathologies: recurrent sinusitis, otitis, adenoiditis, and chronic tonsillitis 77.8%

- **Older Children Ages 8 or older**
  - Bronchial Asthma w/o accompanying AD 23.9%
  - Bronchial Asthma w/o accompanying AD 3.4%

- **Late onset of BA 4.0%**
  - First symptoms from 9-11 years of age

**Recurrence of Obstructive Bronchitis**

- **Infants Ages 0-1**
  - Bronchial Asthma w/o accompanying AD 3.4%
  - Disabling forms of Asthma 17.3%

- **Babies Ages 1-3**
  - Bronchial Asthma in association with AD 41.4%
  - Related Ears/Nose/Throat Pathologies: recurrent sinusitis, otitis, adenoiditis, and chronic tonsillitis 77.8%

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Functional disorders of the central and peripheral nervous systems, (i.e., disturbances in the brain’s bio-rhythms, asthenic-neurotic disorders, and ↓ dopamine, ↓ serotonin)

Issues related to parasympathetic autonomic regulation (vagotonic variations to autonomic tone or functioning, hyper-sympathetic reactions— such as bradycardia and shortness of breath )

Biliary dysfunction (as detected from ultra-sound scanning that shows ↑ in alkaline phosphatase)

Issues related to the development of motor functions of the stomach and duodenum, reflux (from ultra-sound screenings)

Deterioration of the liver (i.e., reactive changes in liver tissues, enlargement of the liver, and increased levels of Aspartate Aminotransferase Enzymes )

Manganese (R²=0.37 - 0.42)

Manganese and Chloroforms (R²=0.36-0.77)

Chloroforms (R²=0.39)

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Manganese (R²=0.52 – 0.61)

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Elevated levels of manganese and chloroforms in the blood

Manganese and Chloroforms (R²=0.33 – 0.63)

Elevation of Manganese and Chloroforms in the blood

Ingestion of manganese and large amounts of chlorine in food and drinking water

The presence of genetic predisposing factors (pathological alleles CPOX & Cytochrome 1A1 genes, polymorphism of SULT1A1 gene)

10-15% of the population

Manganese, Chloroforms (R²=0.37-0.77)

Deterioration of the liver (i.e., reactive changes in liver tissues, enlargement of the liver, and increased levels of Aspartate Aminotransferase Enzymes )

Chloroforms (R²=0.44)

Manganese (R²=0.52 – 0.61)

Manganese (R²=0.25 – 0.52)

Chloroforms (R²=0.44)

Reflux of duodenal contents back into the stomach

Functional disorders of the central and peripheral nervous systems, (i.e., disturbances in the brain’s bio-rhythms, asthenic-neurotic disorders, and ↓ dopamine, ↓ serotonin)

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Manganese, Chloroforms (R²=0.36-0.77)
Analytic assessments of the role played by toxic metals in the onset of patho-genetic, immuno-allergic and thyroid issues in children with bronchial asthma and goiter, all in the face of man-made impacts.
Inter-systemic connections of bone metabolism markers and other clinically and laboratory-derived indicators (within children) that can be traced to man-made impacts

- N-osteo-calcinosis
  - CH$_3$CHO D=0.50
    - HCHO, Va, Mn IgM (D=0.28)
    - Eosinophilia (D=0.50)
    - CH$_3$CHO Mn T$_4$ (D=0.35)
  - Bone iso-enzyme with alkaline phosphatase
    - Cr IgA, IgM (D=0.32)
    - MDA (D=0.64)
    - CH$_3$CHO T$_4$ (D=0.22)
    - HCHO, Mn Carbohydrate metabolism (D=0.54)
  - C-terminal telopeptide
    - Tartrate resistant acidic phosphatase
      - HCHO-IgE, IgM (D=0.30)
      - Va, Mn-IgM (D=0.20)
      - Ni-IgE (D=0.63)
      - Ni IgA (D=0.32)
- Humoral Immunity (IgE, IgA, IgM)
- Non-specific resistance and anti-oxidant defense mechanisms (phagocytosis, eosinophilia, anti-oxide activity, MDA or lipid peroxidation)
- Adaptive Thyroid Activity (T$_4$)
  - HCHO, CH$_3$CHO Mn Mineral exchange (D=0.27)
  - HCHO, Va, Cr Proteo-metabolism (D=0.29)
  - CH$_3$CHO, Mn Mineral exchange (D=0.64)
- The main types of exchange (mineral, protein, carbohydrate)
  - HCHO, Mn Hb (D=0.20)
  - Pb Fe (D=0.27)
  - Pb Bilirubin (D=0.24)
- Pigmentary exchange (Hb, Fe, Bilirubin, Coproporphyrin)
  - HCHO, Mn Bilirubin (D=0.20)
  - Pb Bilirubin (D=0.55)
Basis for Determining Markers of Effect

1. The study of how the body responds to elevated internal levels of target substances, where these elevated levels are caused by exposure

- List of indicative data sources for research – the data bases of WHO, US-EPA, the US Agency for Toxic Substances and Disease Registry (ATSDR), and the Russian On-line Information Retrieval System for “Hazardous Substances”

High-standard analytical equipment

Indicators of response on body systems and separate organs, as well as on cellular and molecular levels

Research analyses methods for various bio-substrates

- Electro-phoretic
- Bio-chemical
- Immuno-genetic
- Molecular cyto-genetic
- Chromato-Mass-Spectrometric
- Morphological
A list of diagnostic indicators for detecting response: overall principles and rationale, plus the adequacy of these indicators at given levels of exposure

<table>
<thead>
<tr>
<th>Factors of Exposure</th>
<th>Non-specific</th>
<th>Specific</th>
<th>Types of exposure</th>
</tr>
</thead>
</table>
| Majority of toxicants | - Malondialdehyde  
- Superoxide dimutase  
- Glutathione peroxidase  
- Lipid hydroperoxide & catalase  
- Antioxidant activity  
- 8-hydroxy-2-deoxyguanosine  
- Enzymes and pigments  
- Spectrum of lipids  
- Nitrogen compounds  
- Eosinophils abs.  
- Eosinophilis lymphatic  
- Immunoglobulin E  
- Erythrocytes—Hb, Ht  
- Platelets, leukocytes  
- Duration of bleeding  
- Blood clotting time  
- C-reactive proteins  
- Immunoglobulin A  
- Carcinoembryonic antigen, C-125  
- Alpha-fetoprotein, Ferritin  
- Karotypes  
- Micronucleus test | - Levels of Coproporphyrin  
- Δ-amino-levulinic acids  
- Erythrocytes with Basophil granulocytes  
- Ceruloplasmin  
- Copper levels  
- Hemoglobin  
- Carboxyhemoglobin  
- Heinz bodies in blood  
- Gamma-aminobutyric acid  
- Glutamate  
- Beta-2 microglobulin  
- Specific Immunoglobulin E  
- Osteo-calcin  
- Tartrate resistant phosphatase | - Lead, Benzol  
- Copper  
- Aniline, carbon monoxide  
- Manganese  
- Cadmium  
- Formaldehyde, manganese, nickel, chromium  
- Strontium |
Analysis of proteomes: identifying basic new markers of effect

Factors of Exposure—metals (Ni)

1. 2D electrophoretogram of blood plasma in children

In an area with no exposure:
Ni in the blood at level of 1 RL

In an area with exposure:
Ni in the blood at level of 2 RL

HTPR_HUMAN (a haptoglobin-related protein) ensures the normal metabolism of hemoglobin

Damage to the structure and functioning of hemoglobin

Protein
HTPR_HUMAN
- target impact from Nickel

Molecular Level
Cytogenetic analysis techniques: used for the purpose of determining markers of effect where conditions point to impacts from chemical mutagens or reproductive toxicants from the man-made environment

**Factors for exposure - mutagens, reproductive toxicants**

**Chromosomal anomalies** (chemical factors of risk for populated areas, odds ratio >7, factors of risk at production, odds ratio >10: Congenital malformations, infertility, miscarriages)

**Nuclear anomalies in cells** (chemical factors of risk for populated areas, odds ratio >5, factors of risk at production, odds ratio >13)

- **Mother** – polymorphism in 14 and 21 chromosomes
  - Formaldehyde in blood – 10RL

- **Offspring** – chromosomal pathology (Down Syndrome)
  - Formaldehyde in blood – 5RL

- **Mother** – cell nucleus.
  - Benzol in blood – 0.03 mg/dm³

- **New-Born** – cells with multiple nucleuses
  - Benzol in blood – 0.02 mg/dm³
Establishing and assessing causal relationships between "markers of exposure – markers of response"
A full array of response markers, in combination with the results from clinical studies, make it possible to verify that certain diseases or disorders are connected to a specific exposure.
## MEDICAL-BIOLOGICAL CRITERIA FOR IDENTIFYING POOLS OF CHILDREN THAT COULD UNDERGO PREVENTATIVE MEASURES FOR LOWERING THEIR CHANCES OF CHRONIC GLOMULAR AND TUBULO-INTERSTITIAL DISEASE OF THE KIDNEYS, RELATED TO INHALATION EXPOSURE TO CADMIUM AND PHENOL

<table>
<thead>
<tr>
<th>№№/№№</th>
<th>CRITERIA</th>
<th>MINIMAL CHANGE (or URINE) SYNDROME (International disease class 10: R80-R82)</th>
<th>GLOMULAR AND TUBULO-INTERSTITIAL KIDNEY DISEASE (International disease class: N14.3, N15.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>4-7 years old</td>
<td>Older than 7</td>
</tr>
<tr>
<td>2</td>
<td>Genetic factors</td>
<td>Polymorphism of homozygous and heterozygous gene variants known as: CYPOX, RCYT 450; SULTA1</td>
<td>Polymorphism of homozygous and heterozygous gene variants known as: CYPOX, RCYT 450; SULTA1</td>
</tr>
<tr>
<td>3</td>
<td>Hereditary factors</td>
<td>History of kidney pathologies</td>
<td>History of kidney pathologies</td>
</tr>
<tr>
<td>4</td>
<td>Possible perinatal risk factors</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Abnormalities in the urinary system</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Relapse rates</td>
<td>2-3 times per year</td>
<td>3 or more per year</td>
</tr>
<tr>
<td>7</td>
<td>Duration of relapses</td>
<td>Up to 1 month</td>
<td>Up to 1.5 or 2 months</td>
</tr>
<tr>
<td>8</td>
<td>Dysfunctions in urination</td>
<td>Disruption of the circadian rhythm of urination (where ratio of nighttime to daytime production of urine is – 1 : 2.5)</td>
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</tr>
<tr>
<td>8</td>
<td>Polyuria</td>
<td>Absent</td>
<td>During flare-up periods</td>
</tr>
<tr>
<td>8</td>
<td>Pressure in lower back region</td>
<td>Absent</td>
<td>During flare-up periods</td>
</tr>
<tr>
<td>8</td>
<td>Reaction to temperature</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>8</td>
<td>Symptoms similar to intoxication</td>
<td>Absent</td>
<td>During flare-up periods</td>
</tr>
<tr>
<td>8</td>
<td>Arterial hypertension</td>
<td>Absent</td>
<td>Rare</td>
</tr>
</tbody>
</table>
### MEDICAL-BIOLOGICAL CRITERIA FOR IDENTIFYING POOLS OF CHILDREN THAT COULD UNDERGO PREVENTATIVE MEASURES FOR LOWERING THEIR CHANCES OF CHRONIC GLOMULAR + TUBULOINTERSTITIAL DISEASE OF THE KIDNEYS RELATED TO INHALATION EXPOSURE TO CADMIUM & PHENOL

<table>
<thead>
<tr>
<th>№/№</th>
<th>CRITERIA</th>
<th>MINIMAL CHANGE (or URINE) SYNDROME</th>
<th>GLOMULAR AND TUBULO-INTERSTITIAL KIDNEY DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Characterization of Renal functions</td>
<td>Decreases in the amplitude of changes in the specific weight of urine over the course of 24 hours</td>
<td>Up to 0.006 conventional units (CU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreases in tubular reabsorption</td>
<td>Up to 90-95%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β2-micro-globulin in urine</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>Renal function of Re-absorption</td>
<td>Blood in urine ((hematuria))</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Excess protein in urine ((proteinuria))</td>
<td>0.033‰</td>
</tr>
<tr>
<td></td>
<td>Renal function of Filtration</td>
<td>Abacterial leukocytes in urine</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycosuria</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Excess excretion of uric acid</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Excess oxalates in urine</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Excess phosphorous in urine</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Excess calcium crystals in urine</td>
<td>Absent</td>
</tr>
<tr>
<td>10</td>
<td>Diagnostic Data</td>
<td>From ultrasound scanning of the kidneys</td>
<td>Shows up as reduction of blood flow during color-Doppler imaging of renal sub-capsular zones; also as deviation from standard spectrograph values during pulsed-wave Doppler (for blood-flow velocity, and where resistance index is less than 0.6 CU., and pulsation index is less than 1.1 CU., along with a systolic-diastolic index with increases in the range of resistance from the core to the peripheral arteries up to 0.04 to 0.05 CU); also as increased echogenic quality of the functional part of the kidneys.</td>
</tr>
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<td>№/№</td>
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<td>-----</td>
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<td>-----------------------------------------------</td>
</tr>
<tr>
<td>11</td>
<td>The state of oxidation and anti-oxidation processes</td>
<td>Increase in the total anti-oxidant activity of the blood, and increases in the amount of superoxide dismutase, glutathione peroxidase, and lipid hydroperoxide</td>
<td>Fluctuations in the total antioxidant activity of the blood, as well as in the content of superoxide dismutase, glutathione peroxidase, &amp; catalase—also increases in lipid hydroperoxides &amp; malondialdehyde</td>
</tr>
<tr>
<td>12</td>
<td>The state of nonspecific resistance factors</td>
<td>Increased rates of phagocytic activity in blood</td>
<td>Decreases in phagocytic activity in blood</td>
</tr>
<tr>
<td>13</td>
<td>State of specific sensitization (Specific IgE to Chromium, and IgG to cadmium, lead, &amp; phenol)</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>14</td>
<td>State of mineral metabolism</td>
<td>Unchanged</td>
<td>Reduction in the concentration of sodium, potassium, chloride</td>
</tr>
<tr>
<td>15</td>
<td>Concentration of chemical substances in the blood</td>
<td>Cadmium concentrations exceed normal levels by a factor of 1.4 - 2.0; Lead concentrations exceed normal levels by a factor of 1.2 – 1.5; Chromium concentrations exceed normal levels by a factor of 1.2 – 1.7; Phenol concentrations exceed normal levels by a factor of 1.3 – 4.0.</td>
<td>Cadmium concentrations exceed normal levels by a factor of 2.0; Lead concentrations exceed normal levels by a factor of 1.5; Chromium concentrations exceed normal levels by a factor of 1.7; Phenol concentrations exceed normal levels by a factor of 4.0.</td>
</tr>
</tbody>
</table>

Laboratory Data

- The state of nonspecific resistance factors
- State of specific sensitization (Specific IgE to Chromium, and IgG to cadmium, lead, & phenol)
- State of mineral metabolism
- Concentration of chemical substances in the blood

Chemical Analyses

- Cadmium concentrations exceed normal levels by a factor of 1.4 - 2.0;
- Lead concentrations exceed normal levels by a factor of 1.2 – 1.5;
- Chromium concentrations exceed normal levels by a factor of 1.2 – 1.7;
- Phenol concentrations exceed normal levels by a factor of 1.3 – 4.0.
Research Design
(using the triad method)

Groups under observation:

- Children who live in the immediate impact zone (120 subjects)
  - Preschool age 4-7 yrs old (60 subjects)
  - School age 7-14 yrs old (60 subjects)
- Parent pairs (100 subjects)
- Second generation direct relatives to the children (i.e., grandparents: 100 subjects)
  - Those who have had direct professional contact with the source of contamination
  - Those who have had NO direct professional contact with the source of contamination

Comparative Groups:

- Children who live under relatively healthy or environmentally clean conditions (80 subjects)
  - Preschool age 4-7 yrs old (40 subjects)
  - School age 7-14 yrs old (40 subjects)
- Parent pairs (60 subjects)
- Second generation direct relatives to the children (i.e., grandparents: 50 subjects)
  - Those who have had NO professional contact with a source of contamination
Target organs and body systems
(as associated with this list of important toxicants)

Central Nervous System
Peripheral Nervous System
Respiratory Organs
Digestive Tract
Liver

Kidneys
Cardio-vascular System
Immune System
Blood
Endocrine System

Mo
Cd
Cr
Ni
Pb
Reproductive System
Arrested Development
The Main Manifestations of Impact

- Sensitization
- Immuno-toxicity
- Mutagenic
- Carcinogenic
- Immune Suppression
- Active peroxidation
- Manifestation of autoimmune processes
- Microbial imbalances
- Arrested physical development
- Cytotoxicity
- Enzymatic pathologies
- Birth Defects
- Embryo-toxicity
- Irregularities in the neuro-endocrine systems
- Interruptions in the basic modes of exchange
- Allergic responses
- Vegetative dysfunctions
- Arrested neuro-psychological development

Mo, Cd, Cr, Ni, Pb
Clinical-Laboratory Program Study on Non-Adult Populations

- **Epidemiological study of comparable sites** (looking at disease patterns, death-rates, birth statistics, frequency and amplitude of congenital defects, etc.—covering the last 25-35 years; using standardized format 12 and data as prescribed by the Federal Fund for Compulsory Medical Insurance)

- **Socio-Medical survey** with the use of specialized survey questions

- **Epidemiological study of target groups** (looking for patterns of chronic somatic diseases, as well as infectious diseases that also account for vaccination patterns)

- **Somato-metric studies** (assessing various indicators of the physical development of children and their maturity in biological terms)

- **Clinical studies** (by pediatricians, ear-nose-throat (ENT) doctors, neurologists, gastroenterologists, endocrinologists) evaluating the condition state of the:
  - Musculo-skeletal system
  - Cardio-vascular system
  - Respiratory system
  - Autonomic nervous system
  - Overall cognitive functions
Clinical and Laboratory Program for Surveying Non-Adult Populations

- **Functional tests**
  - EKGs
  - Spirography or pneumography
  - Rhinomanometry
  - Cardio-interval measurements
  - Ultra-sounds of the liver, bile tract, and pancreas
  - Ultra-sound tests of the thyroid gland
  - Ultra-sound of the kidneys to determine blood flow

- **Lab tests:**
  - Chemical analyses of the blood
  - Nasal swabs
  - General analyses of the urine, and specific analyses of urine using Nechiporenko methods
  - Erythrocyte indices in detail; platelets; leukocyte levels
  - Bio-chemical indices of the blood – anti-oxide activity; malondialdehyde (MDA) plasma; superoxide dismutase; glycerophosphate oxidase; the glucose, total protein, and cholesterol levels; both high- and low-density lipoproteins; triglycerides; alkaline phosphatase; urea content; creatinine; ionized calcium; alanine & aspartate aminotransferase; and Gamma-glutamyl
  - Hormone profiles – adrenocorticotropic hormones; thyroid-stimulating hormones and free T4; dopamine; serotonin; cortisol; adrenaline; norepinephrine
  - β2- micro-globulin
  - Energy Exchange – Cyclic adenosine and guanosine monophosphates
  - Genetic tests
  - Immunological tests
Survey program in clinics and laboratories of adult populations

- **Socio-medical survey questions based on similar specialized surveys**
- **Epidemiological studies of target groups** (for patterns of chronic somatic diseases)
- **Clinical studies** (by internists, cardiologists, ENT doctors, neurologists, gastroenterologists, endocrinologists) evaluating the condition of the:
  - Cardio-vascular system
  - Respiratory system
  - Central nervous system and autonomic nervous system
  - Kidneys
  - Gastro-intestinal tract
  - Endocrine system
Clinical and Laboratory Program for Surveying Adult Populations

- **Functional tests**
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  - Hormone profiles – adrenocorticotropic hormones; thyroid-stimulating hormones and free T4; dopamine; serotonin; cortisol; adrenaline; norepinephrine
  - β2- micro-globulin
  - Genetic tests
  - Immunological tests
Survey program in clinics and laboratories for close relatives (separated by no more than 2 removes from each other)

- Epidemiological study of comparable groups (in search of disease patterns)
- Socio-medical survey questions
  - Genetic studies of these groups
Thank you!