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

Olga Yurevna Ustinova

The Federal Scientific Centre for Preventative Medicine and the Advancement of Technologies for Managing Risks to Public Health —a division of the Federal Agency for Financing the Sciences.

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

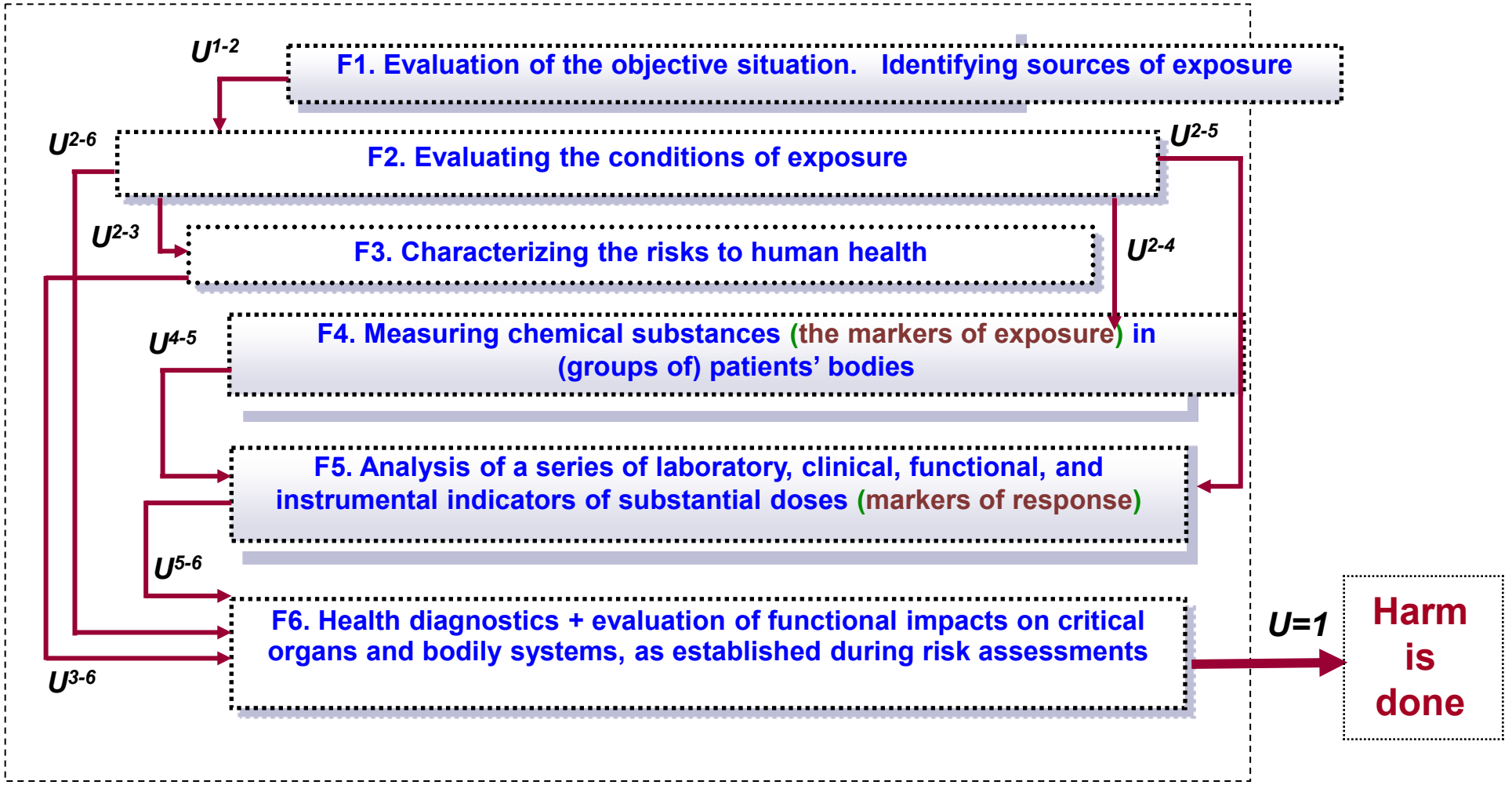
In accordance with accepted methodologies for establishing possible damage to public health, it is required that evidence be brought forward step by step, mainly by:

- **Identifying Bio-Markers of Exposure**

….and by

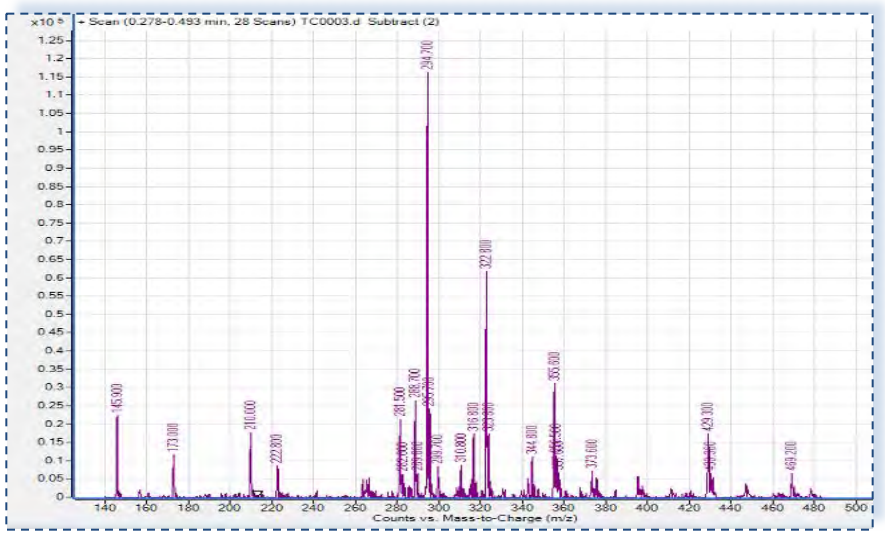
- **Identifying Bio-Markers of Effect**

F4. Measuring chemical substances (Markers of Exposure) in patients' (or groups of patients') bodies

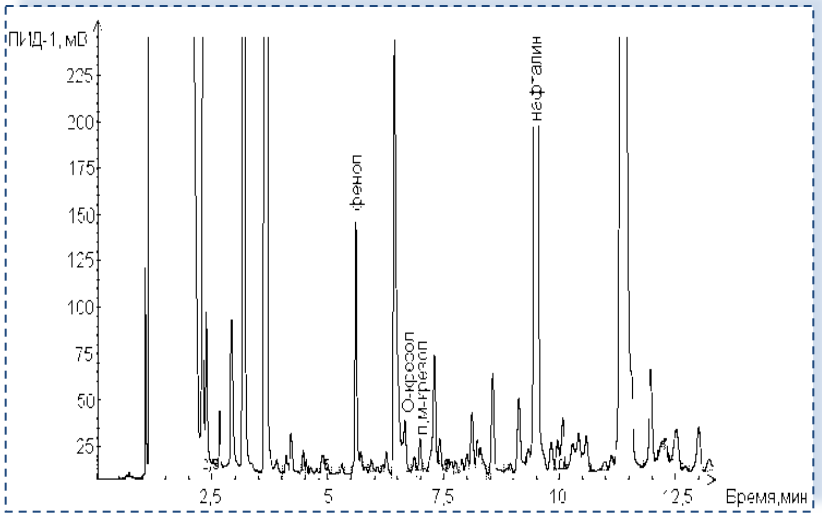


A working system of bio-monitoring can help substantiate that contact with certain materials can be hazardous to human health

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



Chromatogram of a sample of blood containing phenol as well as o-, p- and m-cresols—with an internal standard level of naphthalene

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

Target groups to be studied

- Residents living in exposure areas, esp.:
  - **Children, 0-14 in age,**
  - **Expecting mothers,**
  - **Women in their reproductive years.**
- **Workers who handle hazardous production materials**

Means for chemical and analytical testing (35 methods in all)

Contents of the human body

- **Blood plasma**
- **Blood serum**
- **Urine**
- **Bile**
- **Gastric juices**
- **Mother's milk**
- **Hair**

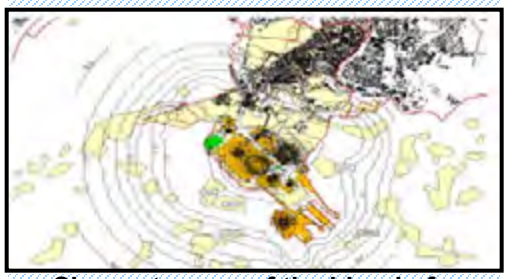
Chemicals of risk (> 50 classes of substances)

Chemical substances

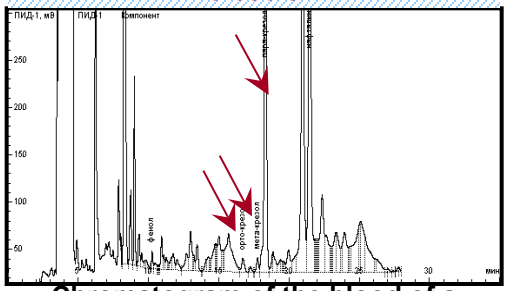
- **Metals**
- **Aldehydes**
- **Aromatic hydrocarbons**
- **Aliphatic alcohols**
- **Aromatic amines**
- **Saturated hydrocarbons**

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.

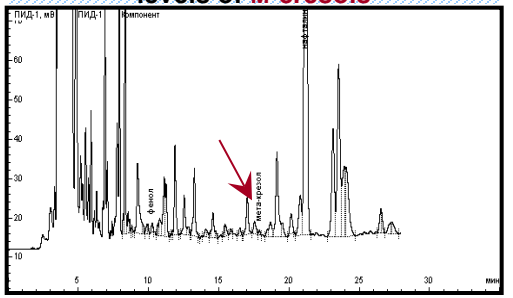
Populations exposed to cresols



Chromatogram of the blood of a baby living in an exposure zone shows high levels of м-, о-, and р-cresols

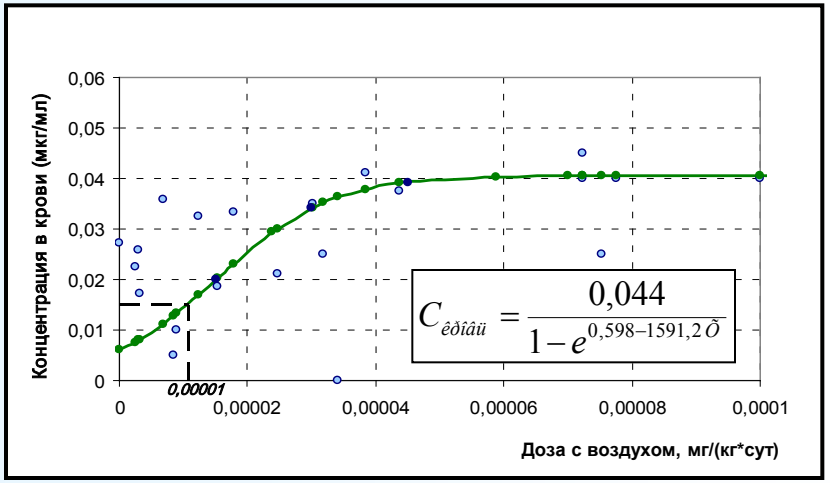


Chromatogram of the blood of a baby living *outside* of exposure zone shows normal (average) levels of м-cresols

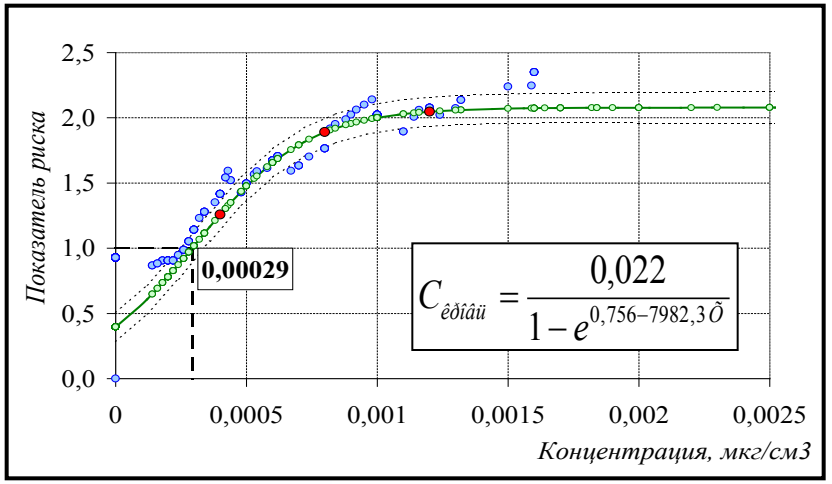


The Rationale behind Markers of Exposure

The association between higher concentrations of formaldehyde in the blood and the overall dose level from chronic exposure (R^2 = 0,64, p≤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≤0,05)



Air-borne components of exposure

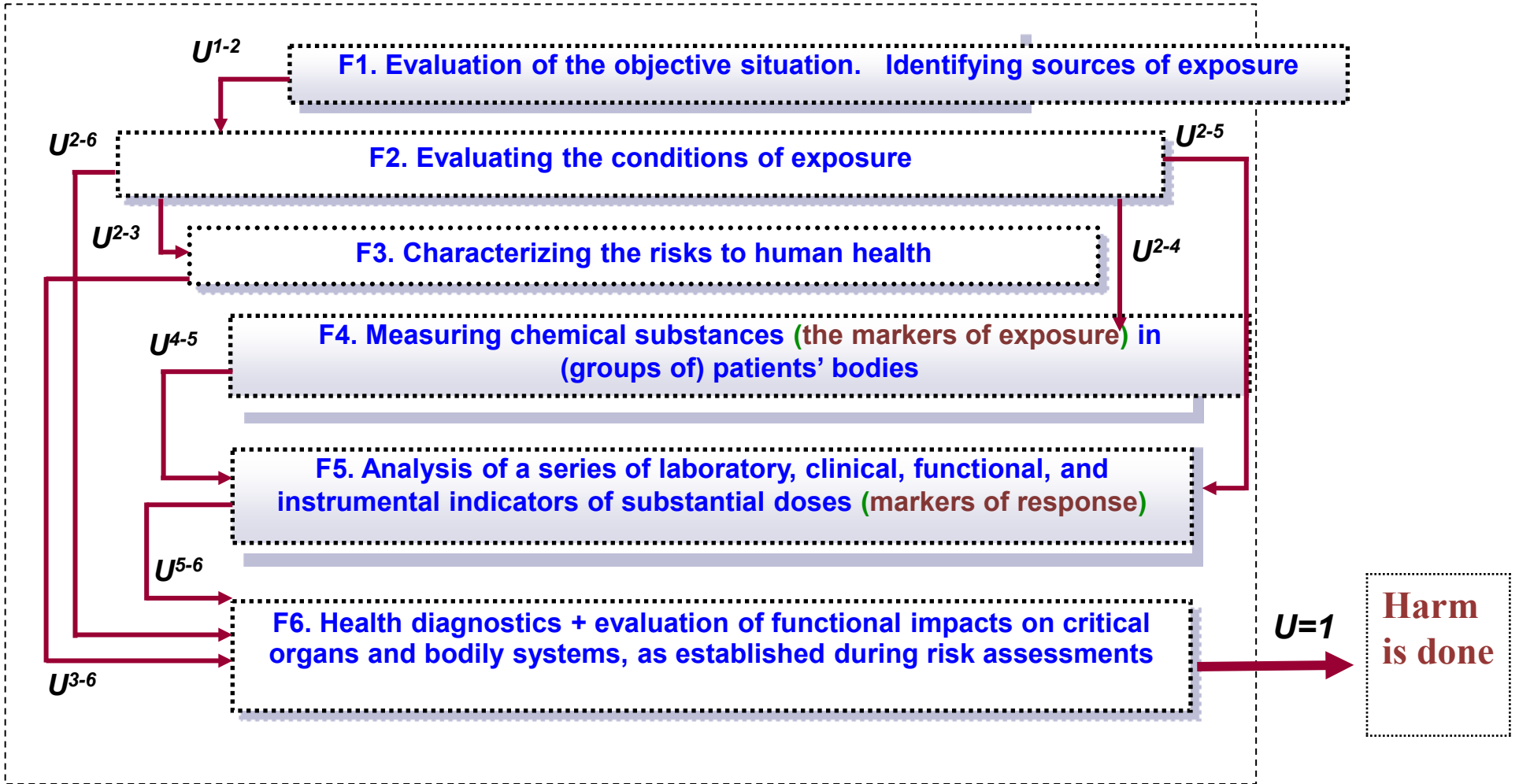
- manganese
- chromium
- benzole
- formaldehyde
- methanol

- 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

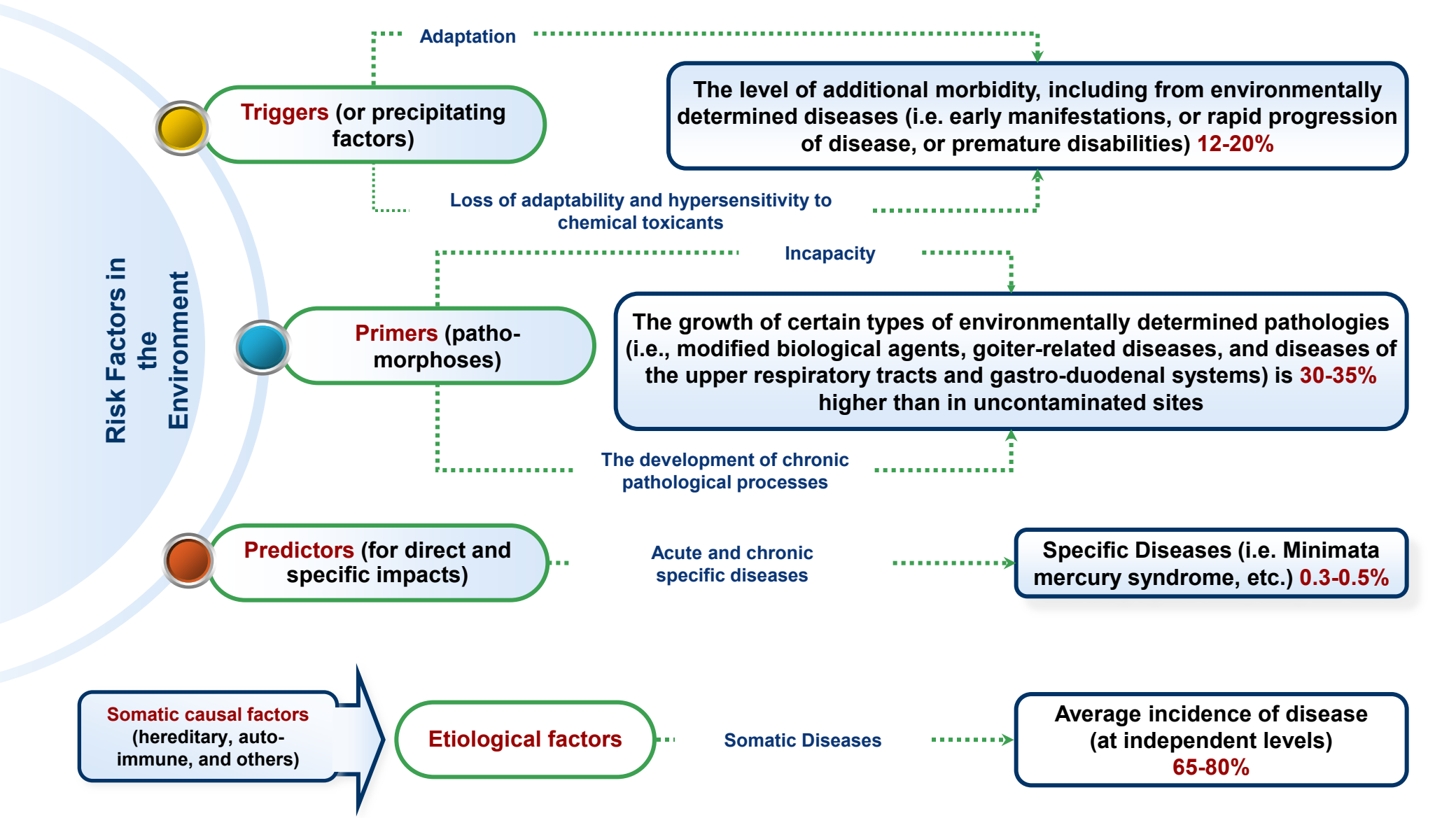
Markers of Exposure in the blood

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 ≤0.05)

F5. Analyses of a combination of clinical, laboratory, functional, & instrumental indicators that show human impact (Markers of Response)



Chemical risk factors and incidence of disease in human populations



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

Microbial Imbalance

Lack of nutrient balance

Active peroxidation

Neuro-endocrine dysregulation

Auto-immune processes

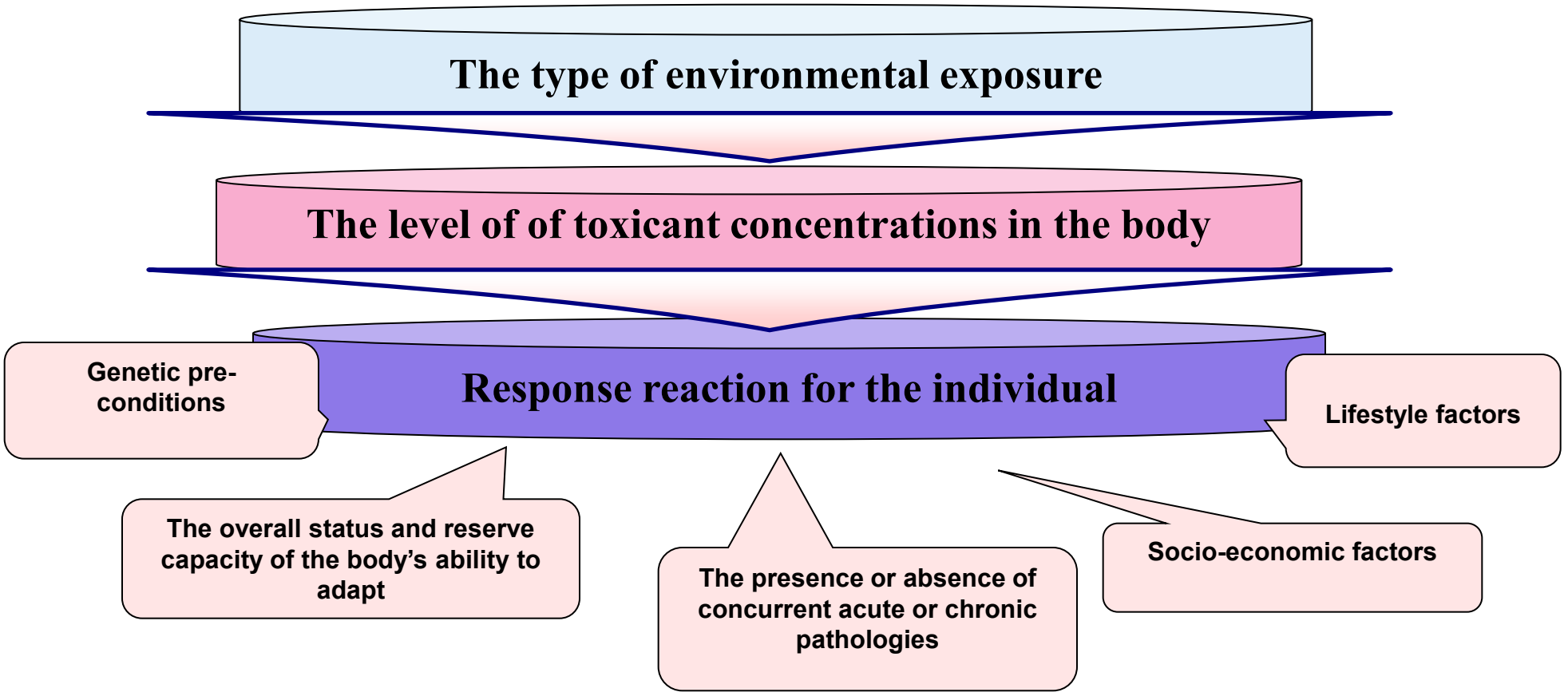
Sensitization

Immunosuppression

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: :



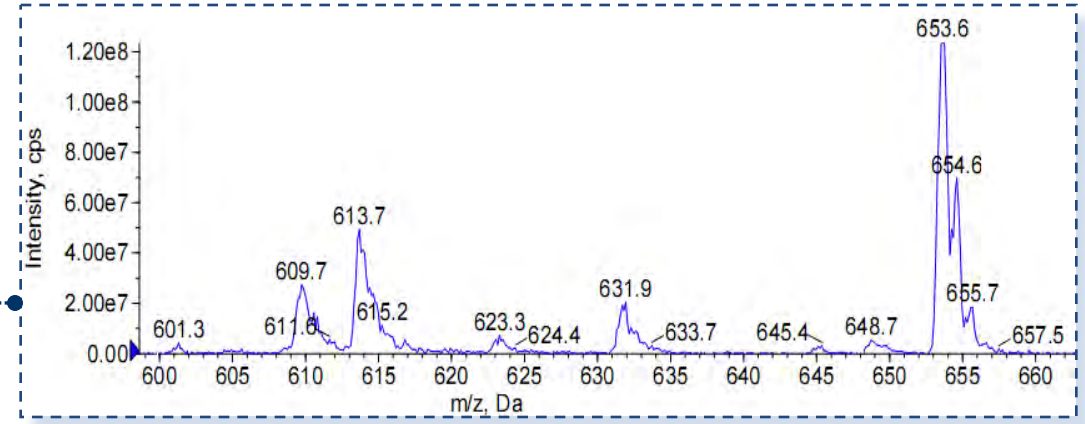
From Baikan, Maklakova, Luzhetski, Rumlyantsev in "Fundamental Research" – 2013. - № 11. – C. 74-78, and
From Maklakova, Ustinova, Luzhetski, Baidan in "Reports from the Samar Scientific Center – 2013. – Volume 15 №3(6). - C. 1845-1849
And from Zaitsev: "Environmentally determined disease in gastro-duodenal systems of children" Perm .- 2009. – 320 p.

Bio-markers of effect

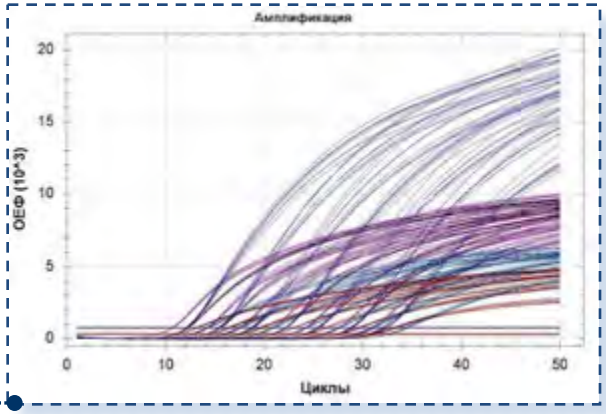
- **Epidemiological indicators**
- **Clinical data**
- **Results of research into the functional conditions of body organs and systems**
- **Laboratory findings**

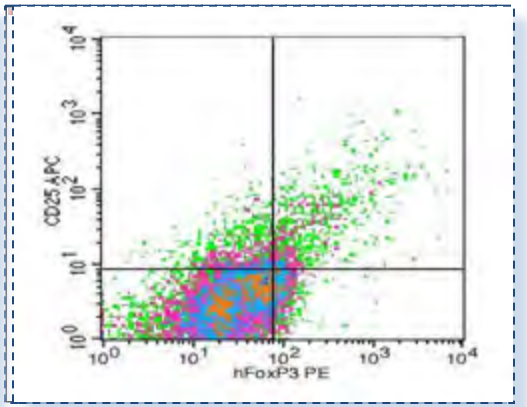
By detecting Markers of Response that are proven to be tied to specific Markers of Exposure, we are allowed to talk of the existence of separate impacts (including—at the level of the proteome—such things as cell apoptosis, or metabolic issues, or other disruptions and predictors of somatic and reproductive 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





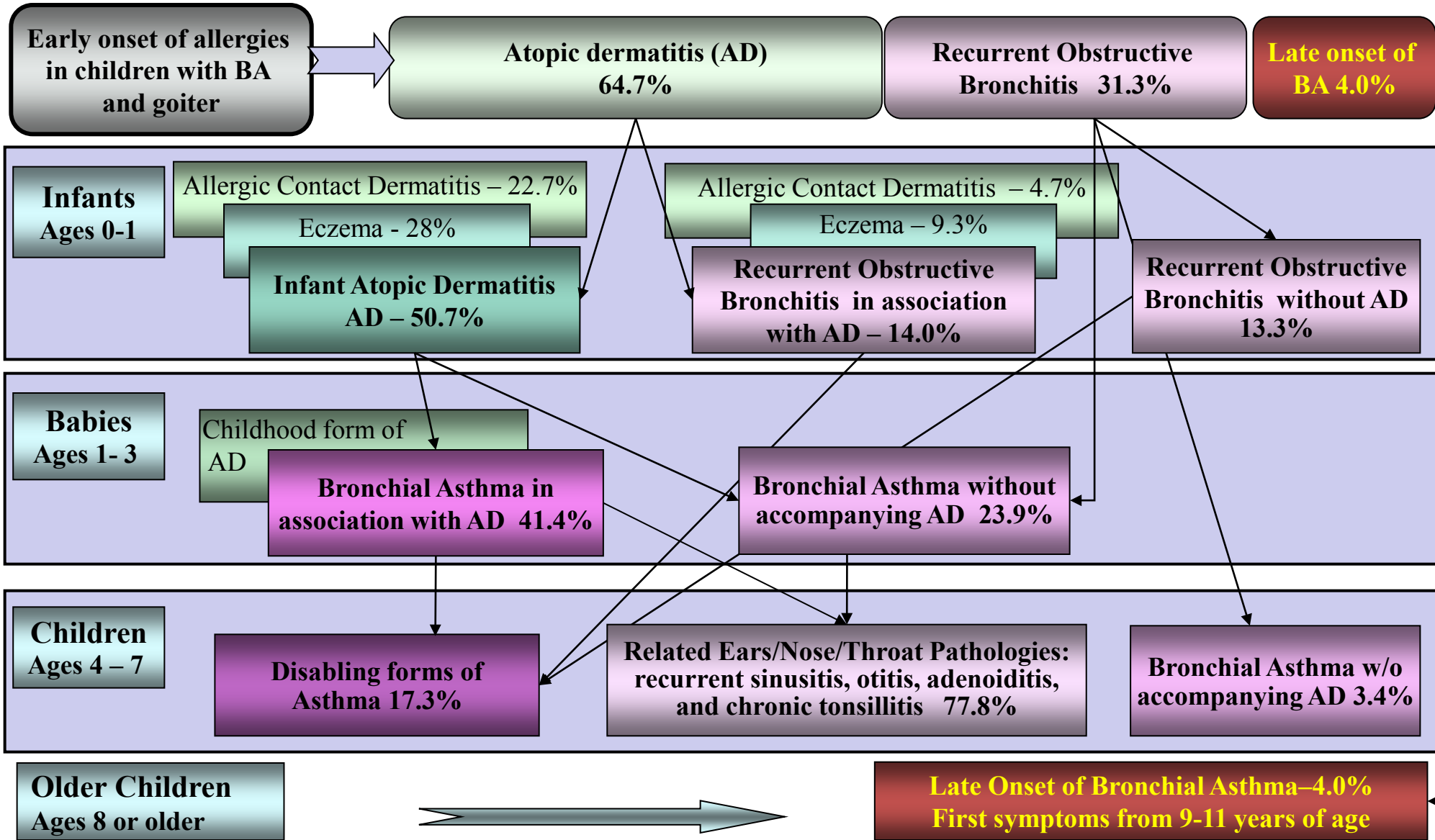
Chemical Elements, Critical Organs & Body Systems, with Referent Levels

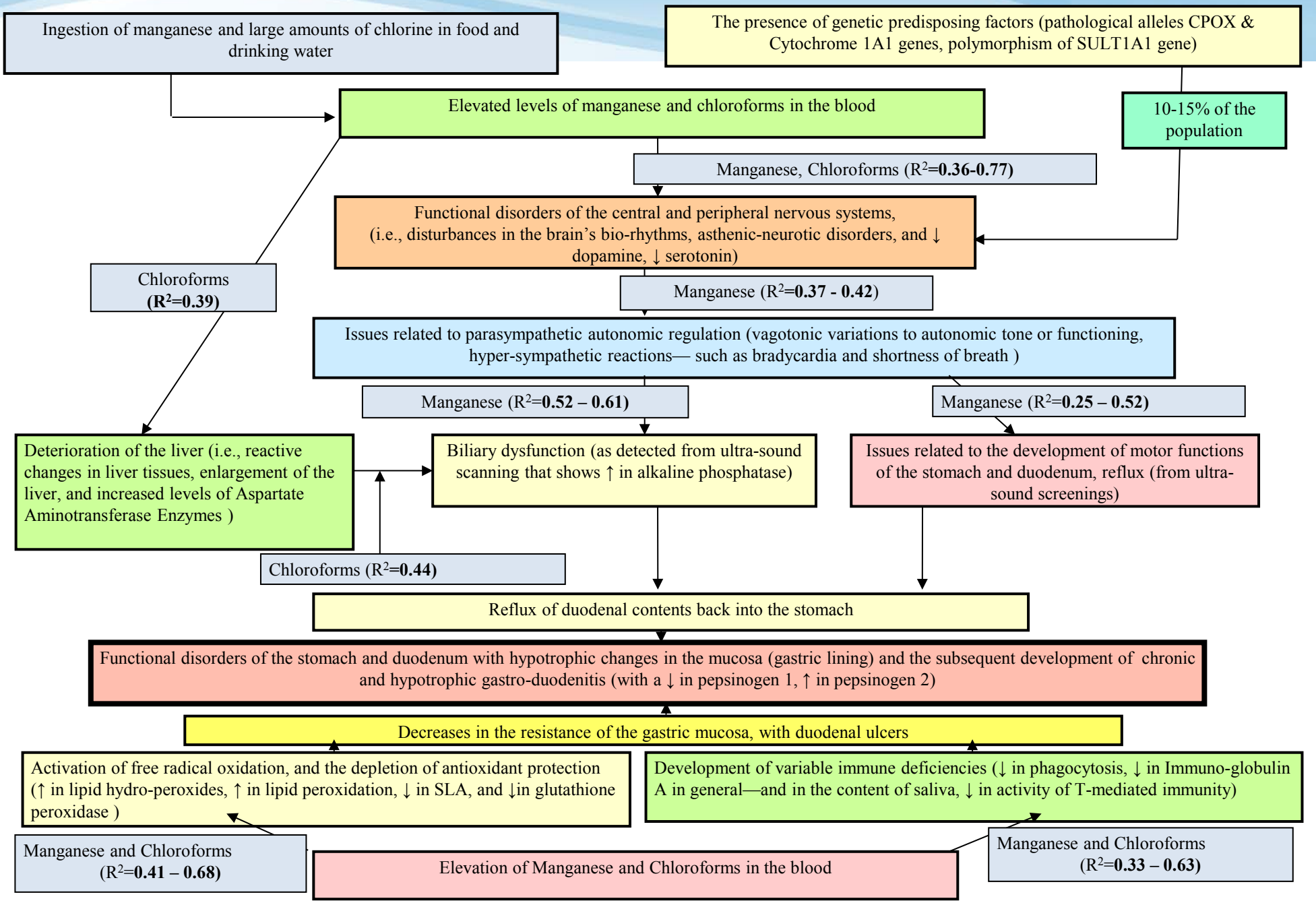
Substance	CAS	RfC,мг/м^3	Critical Organs and Systems	RFD, мг/кг	Critical Organs and Systems	Sfi
Elements, the presence of which can be used to designate an area as an Environmental Disaster Zone in Russia						
Copper (Cu)	7440-50-8	2.00E-05	Respiratory Organs	0.019	Digestive tract and liver	–
Zinc (Zn)	7440-66-6	0.0009	Respiratory organs, immune systems, blood	0.3	Blood, bio-chemicals (superoxide dismutase)	–
Arsenic (As)	7440-38-2	3.00E-05	Development (i.e., fetal development.), + nervous & cardio-vascular systems, + respiratory organs, cancer	0.0003	Skin, central nervous, immune, & cardio-vascular systems, hormones (diabetes), digestive tract	15
Lead (Pb)	7439-92-1	0.0005	Central nervous system, blood, development, reproductive and hormonal systems, kidneys	0.0035	Central nervous system, blood, bio-chemical balance, development, reproductive & hormone systems	0.042
Molybdenum (Mo)	7439-98-7	0.012	–	0.005	Kidneys	–
Tungsten (W)	7440-33-7	0.1	Respiratory organs	0.0025	–	–
Cadmium (Cd)	7440-43-9	2.00E-05	Kidneys, respiratory organs, hormone system, cancer	0.0005	Kidneys, hormonal system	6.3
Antimony (Sb)	7440-36-0	0.0004	Respiratory organs	0.0004	Biochemical balance. (glucose + cholesterol in blood), death	
Other elements that could be included in this study						
Cobalt (Co)	7440-48-4	2.00E-05	Respiratory organs	0.02	Blood	9.8
Manganese (Mn)	7439-96-5	5.00E-05	Central and overall nervous systems, respiratory organs	0.14	Central nervous system, blood	–
Mercury (Hg)	7439-97-6	0.0003	Central nervous system, hormones, kidneys	0.0003	Kidneys, reproductive, immune + central nervous systems, hormones	–
Chromium (Cr)	7440-47-3	0.0001	Respiratory organs, liver, kidneys, immune systems, digestive tract	0.005	Liver, kidneys, digestive tract, mucous	42
Nickel (Ni)	7440-02-0	5.00E-05	Respiratory organs, blood, immune and central nervous systems, cancer	0.02	Liver, cardio-vascular system, digestive tract, blood, body mass	0.84

Epidemiological Studies

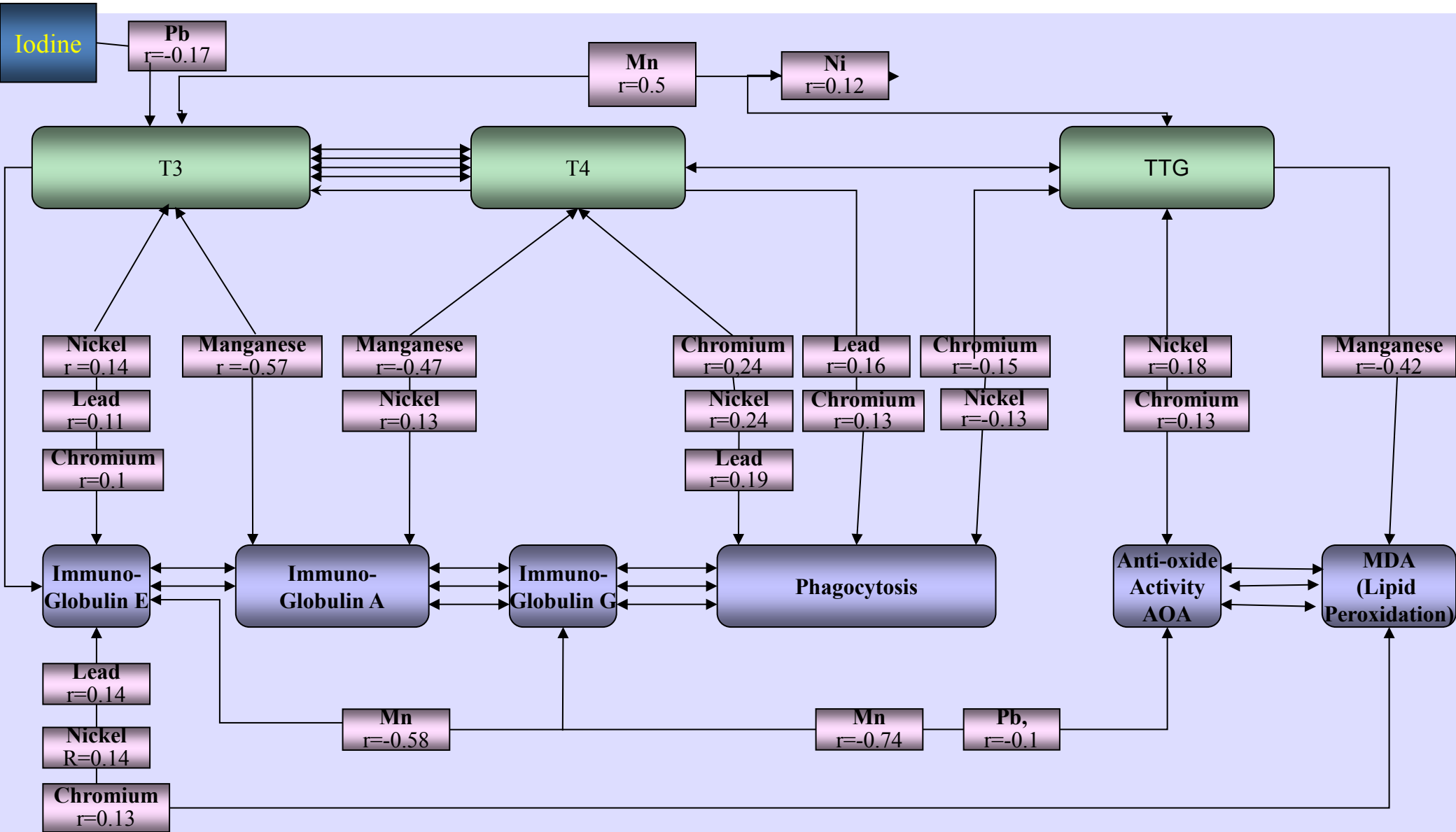
- Analysis of the dynamics, structure, and tempo in which disease grows among the residents in the area under study
- Comparative study of the dynamics and structure of specific diseases between the target area and an analogous “relatively uncontaminated” area—or with data for the entire country
- Determining 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

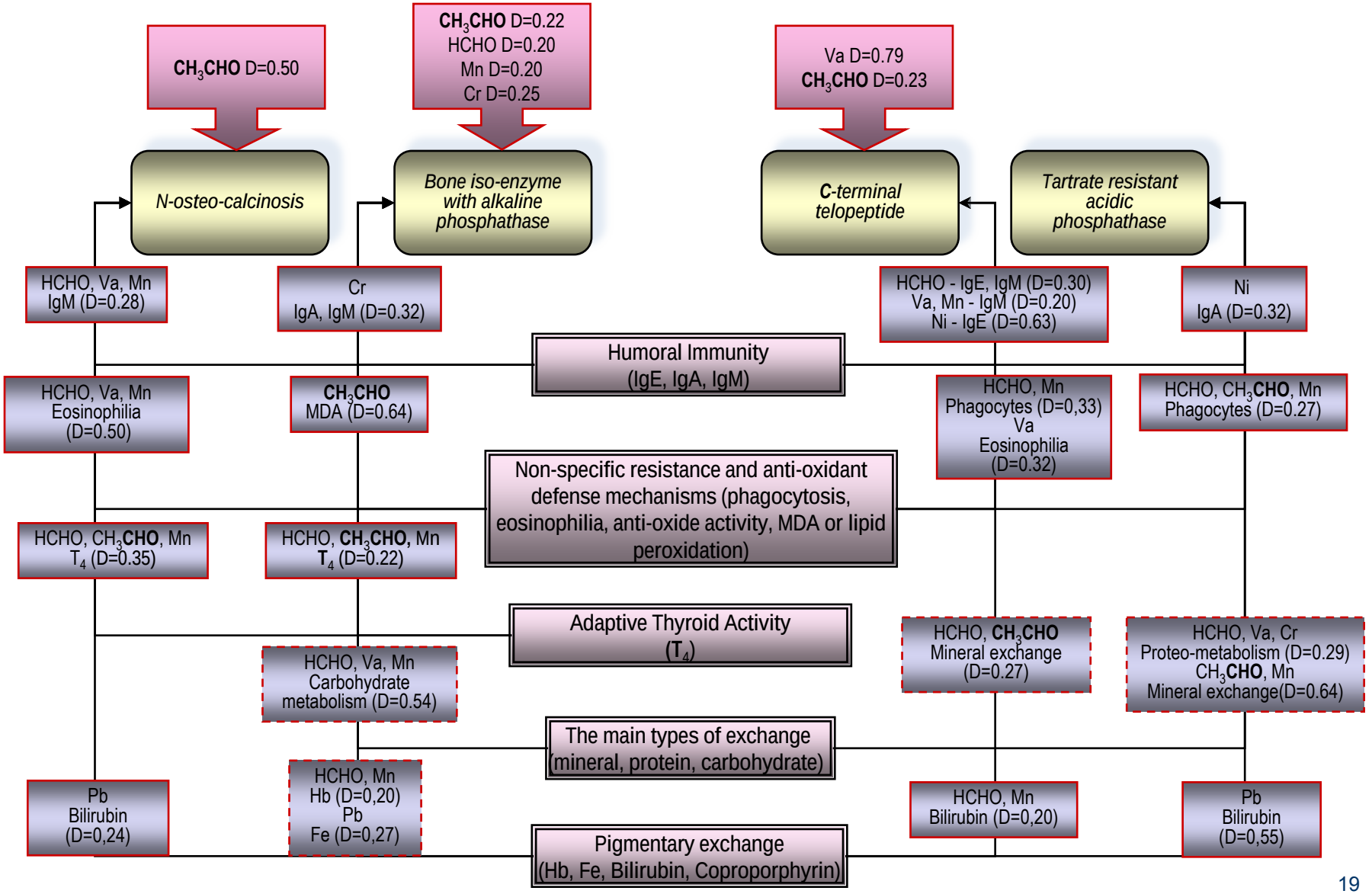



Ingestion of manganese and large amounts of chlorine in food and drinking water
The presence of genetic predisposing factors (pathological alleles CPOX & Cytochrome 1A1 genes, polymorphism of SULT1A1 gene)
Elevated levels of manganese and chloroforms in the blood
10-15% of the population
Manganese, Chloroforms (R^2=**0.36-0.77**)
Functional disorders of the central and peripheral nervous systems, (i.e., disturbances in the brain's bio-rhythms, asthenic-neurotic disorders, and ↓ dopamine, ↓ serotonin)
Chloroforms (**R^2=0.39**)
Manganese (R^2=**0.37 - 0.42**)
Issues related to parasympathetic autonomic regulation (vagotonic variations to autonomic tone or functioning, hyper-sympathetic reactions— such as bradycardia and shortness of breath)
Manganese (R^2=**0.52 – 0.61**)
Manganese (R^2=**0.25 – 0.52**)
Deterioration of the liver (i.e., reactive changes in liver tissues, enlargement of the liver, and increased levels of Aspartate Aminotransferase Enzymes)
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)
Chloroforms (R^2=**0.44**)
Reflux of duodenal contents back into the stomach
Functional disorders of the stomach and duodenum with hypotrophic changes in the mucosa (gastric lining) and the subsequent development of chronic and hypotrophic gastro-duodenitis (with a ↓ in pepsinogen 1, ↑ in pepsinogen 2)
Decreases in the resistance of the gastric mucosa, with duodenal ulcers
Activation of free radical oxidation, and the depletion of antioxidant protection (↑ in lipid hydro-peroxides, ↑ in lipid peroxidation, ↓ in SLA, and ↓in glutathione peroxidase)
Development of variable immune deficiencies (↓ in phagocytosis, ↓ in Immuno-globulin A in general—and in the content of saliva, ↓ in activity of T-mediated immunity)
Manganese and Chloroforms (R^2=**0.41 – 0.68**)
Elevation of Manganese and Chloroforms in the blood
Manganese and Chloroforms (R^2=**0.33 – 0.63**)

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

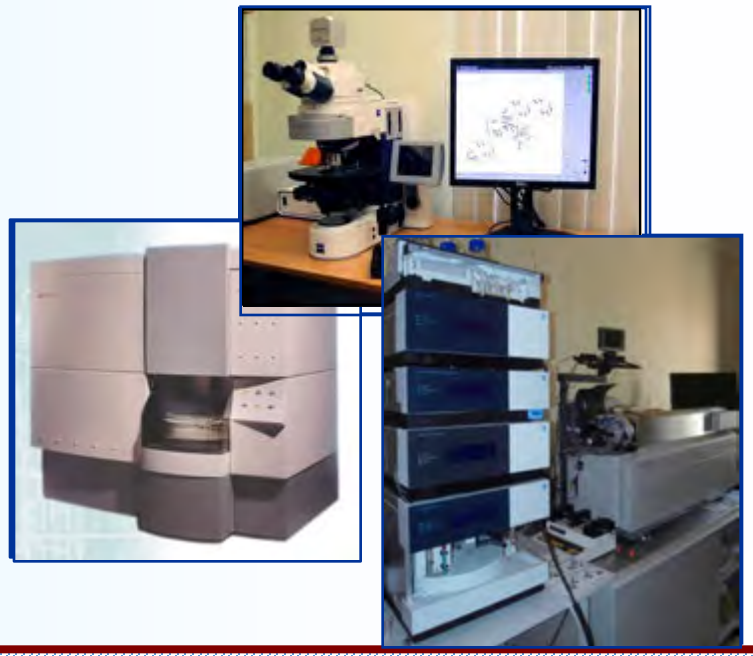


Basis for Determining Markers of Effect

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

Factors of Exposure	Non-specific
Majority of toxicants	▪ Malondealdehyde ▪ Superoxide dimutase ▪ Glutathione peroxidase ▪ Lipid hydroperoxide & catalase ▪ Antioxidant activity ▪ 8-hydroxy-2-deoxyguanosine
Hepato-toxicants — causing liver damage	▪ Enzymes and pigments ▪ Spectrum of lipids ▪ Nitrogen compounds
Haptens — causing allergic reactions	▪ Eosinophils abs. ▪ Eosinophilis lymphatic ▪ Immunoglobulin E
Hemato-toxicants — ➔ blood damage	▪ Erythrocytes—Hb, Ht ▪ Platelets, leukocytes ▪ Duration of bleeding ▪ Blood clotting time
Gastro-toxicants	▪ C-reactive proteins ▪ Immunoglobulin A
Carcinogens	▪ Carcinoembryonic antigen, C-125 ▪ Alpha-fetoprotein, Ferritin
Mutagens	▪ Karotypes ▪ Micronucleus test

Specific	*Types of exposure*
▪ Levels of Coproporphyrin ▪ Δ-amino-levulinic acids ▪ Erythrocytes with Basophil granulocytes	**Lead, Benzol**
▪ Ceruloplasmin ▪ Copper levels	**Copper**
▪ Hemiglobin ▪ Carboxyhemoglobin ▪ Heinz bodies in blood	**Aniline, carbon monoxide**
▪ Gamma-aminobutyric acid ▪ Glutamate	**Manganese**
▪ Beta-2 microglobulin	**Cadmium**
▪ Specific Immunoglobulin E	**Formaldehyde, manganese, nickel, chromium**
▪ Osteo-calcin ▪ Tartrate resistant phosphatase	**Strontium**

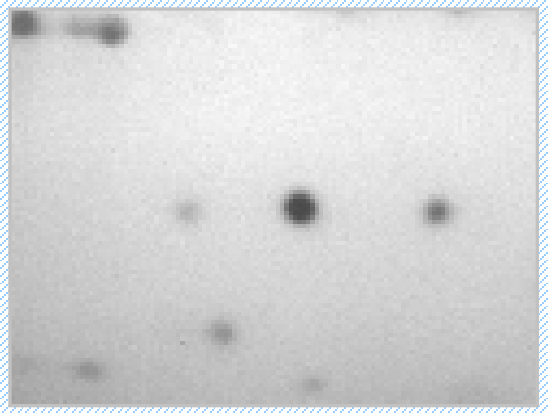
Analysis of proteomes: identifying basic new markers of effect

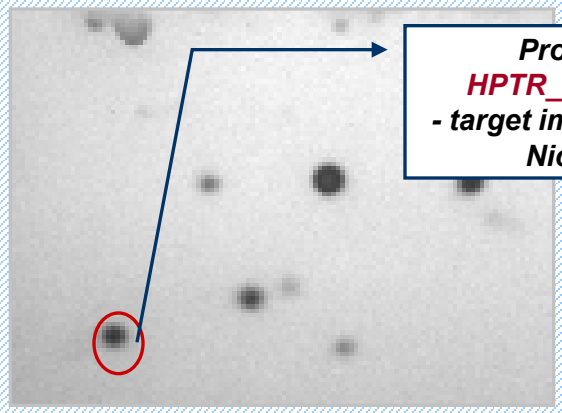
Factors of Exposure– metals (Ni)

Molecular Level

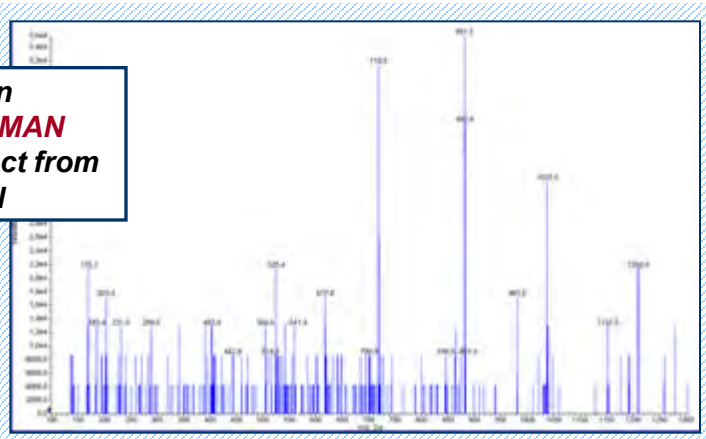
1. 2D electrophoretogram of blood plasma in children

2. Mass-spectrum identification



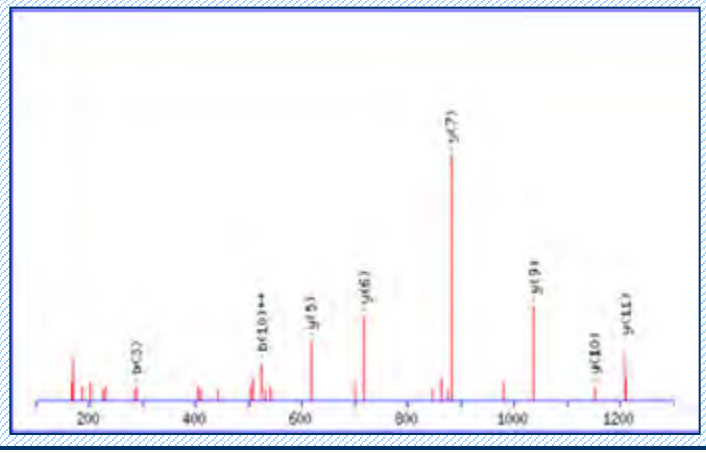


Protein HPTR_HUMAN - target impact from Nickel



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

In a 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**

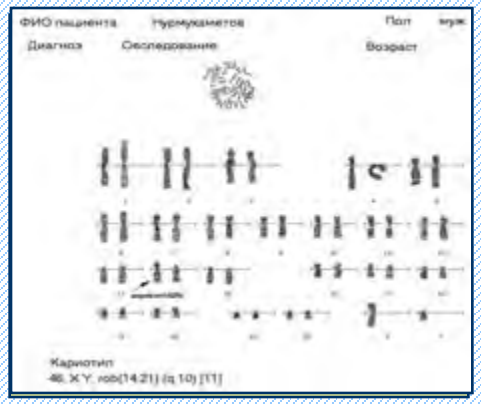
Cytogenetic analysis techniques: used for the purpose of determining markers of effect where conditions point to impacts from chemical mutagens or reproductive toxicants from the man-made environment

Factors for exposure - mutagens, reproductive toxicants

Cellular/Molecular Level

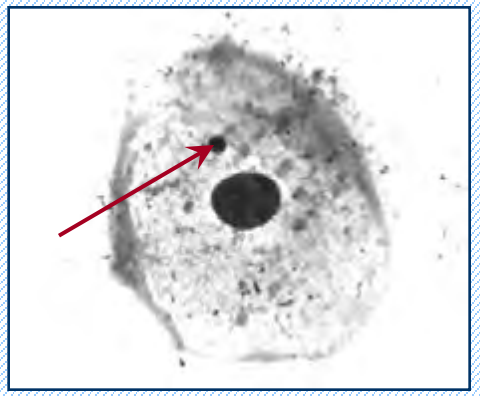
Chromosomal anomalies (chemical factors of risk for populated areas, odds ratio >7, factors of risk at production, odds ratio >10: Congenital malformations, infertility, miscarriages)

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

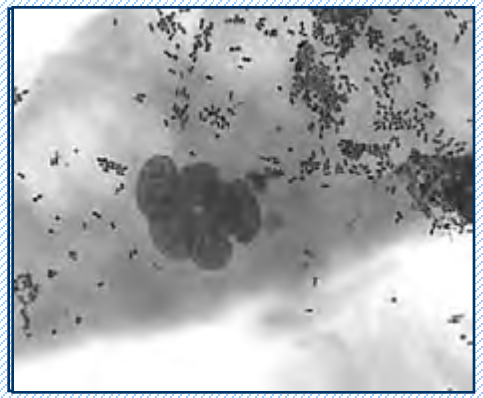


Mother – polymorphism in 14 and 21 chromosomes
Formaldehyde in blood – 10RL

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



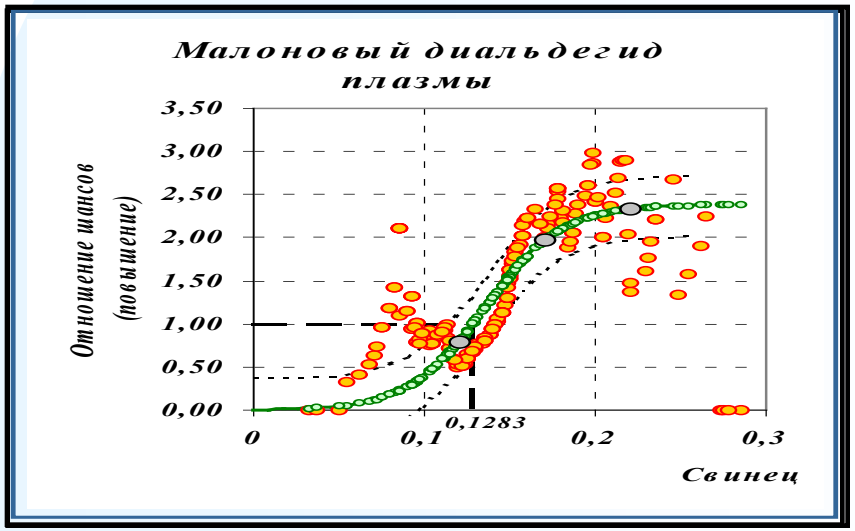
Mother – cell nucleus.
Benzol in blood– 0.03 мg/дм^3



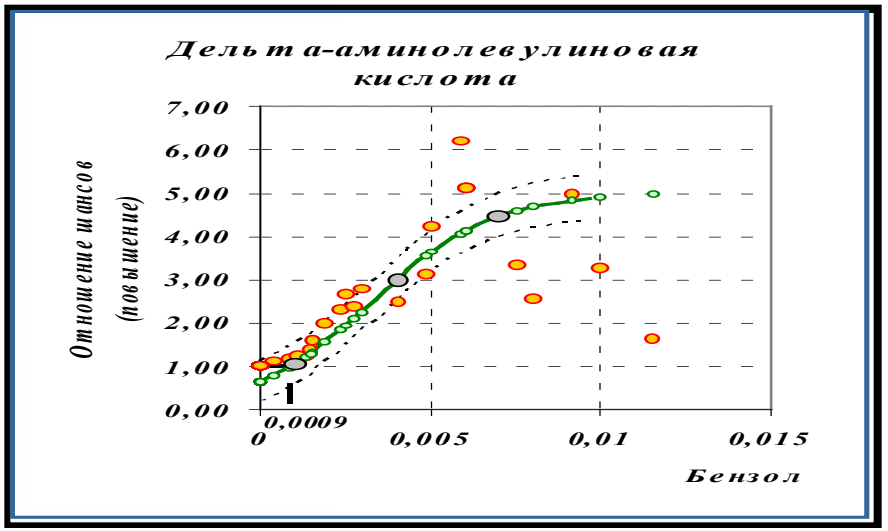
New-Born – cells with multiple nucleuses
Benzol in blood–0.02 mg/дм^3

Establishing and assessing causal relationships between "markers of exposure – markers of response"

Relationship between «lead concentrations in the blood – levels of malondialdehyde in the blood» through inhalation exposure



Relationship between «benzol concentrations in blood – levels of delta-aminolevulinic acid in the urine» through inhalation exposure

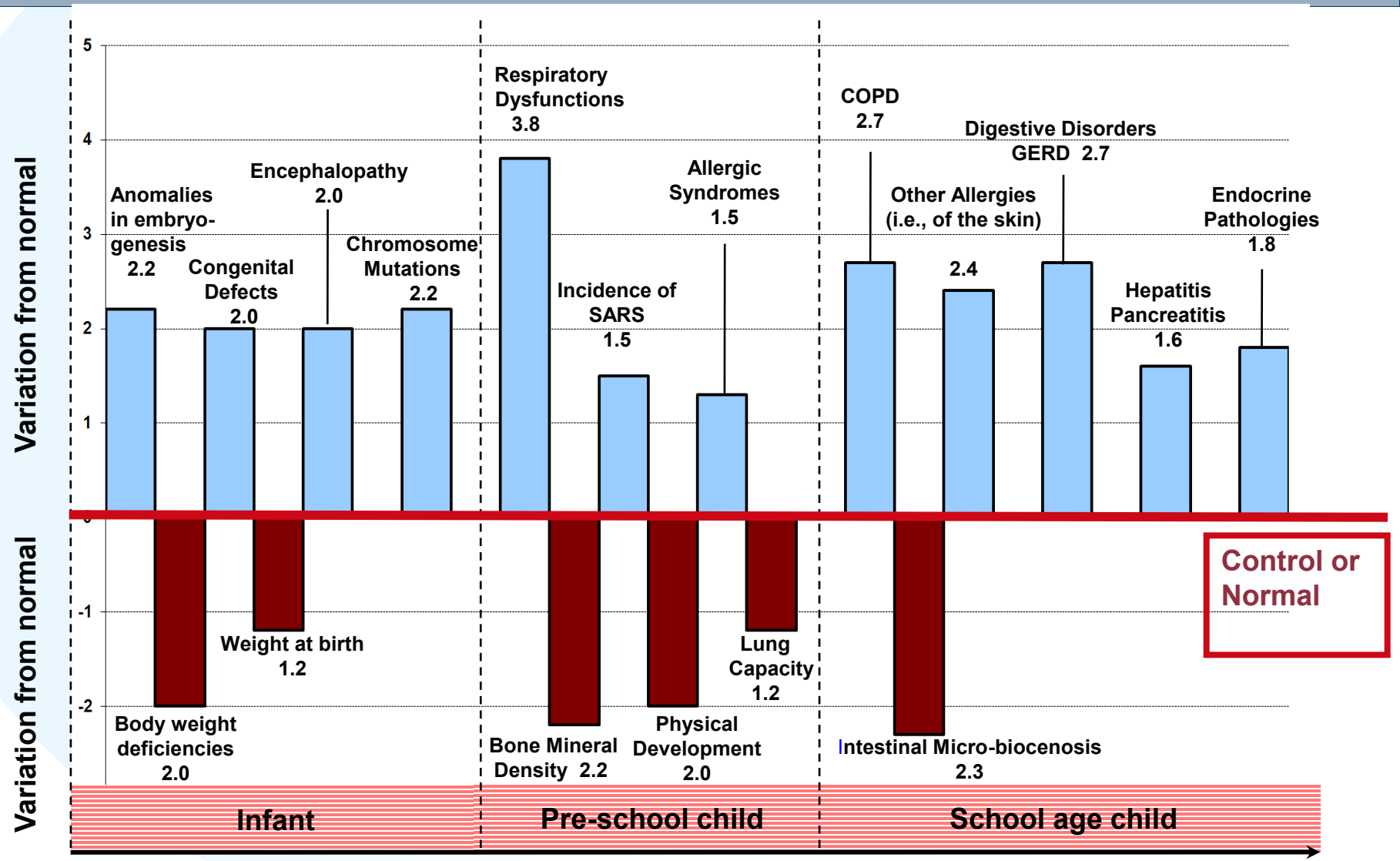


$$p_i = \frac{1}{1 + e^{-(b_0 + b_1(x-}}$$

Modeling for the dependence between «marker of exposure for manganese – the marker of response» through oral exposure

Marker of exposure	Marker of response	b0	b1	R^2	F	P
Manganese	Aminobutyric acid ↑	-0.21 ± 0.02	5.24 ± 0.001	0.55	255.6	0.001
	Glutamate ↓	0.92 ± 0.05	31.03 ± 0.50	0.69	206.8	0.000
	Ca^{-} ↓	-7.66 ± 0.01	99.12 ± 0.05	0.48	178.4	0.001

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

№/№	CRITERIA		MINIMAL CHANGE (or URINE) SYNDROME (International disease class 10: R80-R82)	GLOMULAR AND TUBULO-INTERSTITIAL KIDNEY DISEASE (International disease class: N14.3, N15.8)
1	Patient Data	Age	4-7 years old	Older than 7
2		Genetic factors	Polymorphism of homozygous and heterozygous gene variants known as: CYPOX, RCYT 450; SULTA1	Polymorphism of homozygous and heterozygous gene variants known as: CYPOX, RCYT 450; SULTA1
3		Hereditary factors	History of kidney pathologies	History of kidney pathologies
4		Possible perinatal risk factors	+/-	+
5		Abnormalities in the urinary system	+/-	+
6		Relapse rates	2-3 times per year	3 or more per year
7		Duration of relapses	Up to 1 month	Up to 1.5 or 2 months
8	Clinical manifestations	Dysfunctions in urination	Disruption of the circadian rhythm of urination (where ratio of nighttime to daytime production of urine is – 1 : 2.5)	Disruption of the circadian rhythm of urination (where ratio of nighttime to daytime production of urine is – 1 : 2.5)
		Polyuria	Absent	During flare-up periods
		Pressure in lower back region	Absent	During flare-up periods
		Reaction to temperature	Absent	Absent
		Symptoms similar to intoxication	Absent	During flare-up periods
		Arterial hypertension	Absent	Rare

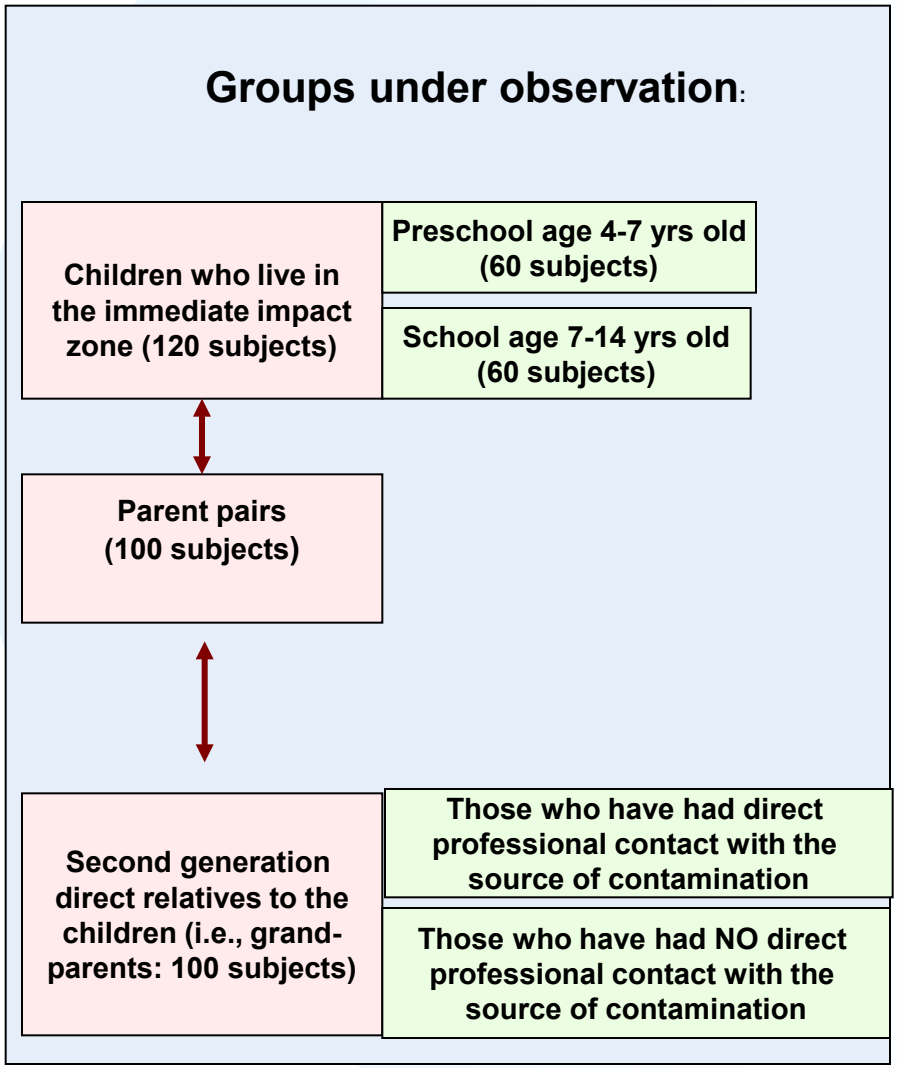
MEDICAL-BIOLOGICAL CRITERIA FOR IDENTIFYING POOLS OF CHILDREN THAT COULD UNDERGO PREVENTATIVE MEASURES FOR LOWERING THEIR CHANCES OF CHRONIC GLOMULAR + TUBULOINTERSTITIAL DISEASE OF THE KIDNEYS RELATED TO INHALATION EXPOSURE TO CADMIUM & PHENOL

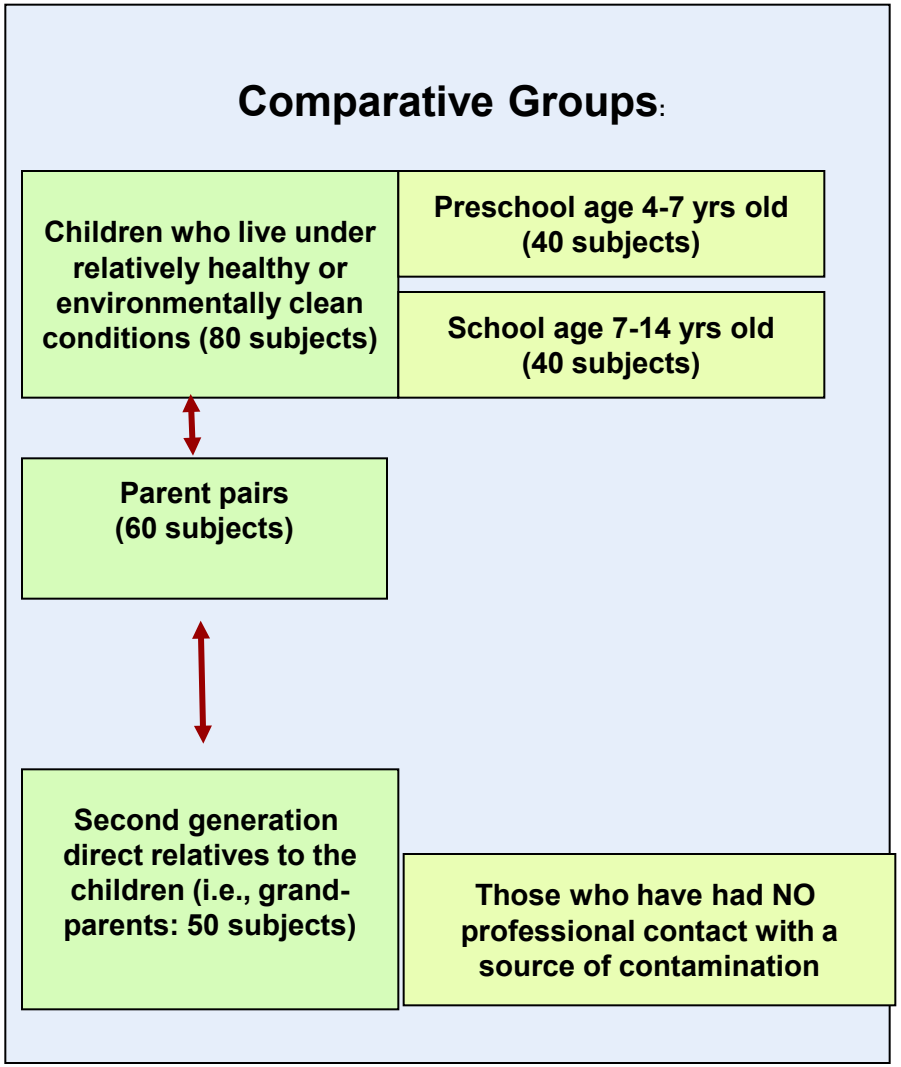
№/№	CRITERIA			MINIMAL CHANGE (or URINE) SYNDROME	GLOMULAR AND TUBULO-INTERSTITIAL KIDNEY DISEASE
9	**Characterization of Renal functions**	Renal function of Re-absorption	Decreases in the amplitude of changes in the specific weight of urine over the course of 24 hours	Up to 0.006 conventional units (CU)	Less than 0.006 CU
			Decreases in tubular reabsorption	Up to 90-95%	Less than 90%
			β2-micro-globulin in urine	Absent	Present
		Renal function of Filtration	Blood in urine (*hematuria*)	Present	Present
			Excess protein in urine (*proteinuria*)	0.033‰	0.033-0.066‰
			Abacterial leukocytes in urine	Absent	Present
			Glycosuria	Absent	+/-
			Excess excretion of uric acid	Absent	+/-
			Excess oxalates in urine	Present	Present
			Excess phosphorous in urine	Present	Present
			Excess calcium crystals in urine	Absent	+/-
10	**Diagnostic Data**	From ultrasound scanning of the kidneys		Shows up as reduction of blood flow during color-Doppler imaging of renal sub-capsular zones	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.

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

№/№	CRITERIA		MINIMAL CHANGE (or URINE) SYNDROME	GLOMULAR AND TUBULO-INTERSTITIAL KIDNEY DISEASE
11	Laboratory Data	The state of oxidation and anti-oxidation processes	Increase in the total anti-oxidant activity of the blood, and increases in the amount of superoxide dismutase, glutathione peroxidase, and lipid hydroperoxide	Fluctuations in the total antioxidant activity of the blood, as well as in the content of superoxide dismutase, glutathione peroxidase, & catalase—also increases in lipid hydroperoxides & malondialdehyde
12		The state of nonspecific resistance factors	Increased rates of phagocytic activity in blood	Decreases in phagocytic activity in blood
13		State of specific sensitization (Specific IgE to Chromium, and IgG to cadmium, lead, & phenol)	Absent	Present
14		State of mineral metabolism	Unchanged	Reduction in the concentration of sodium, potassium, chloride
15	Chemical Analyses	Concentration of chemical substances in the blood	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.	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.

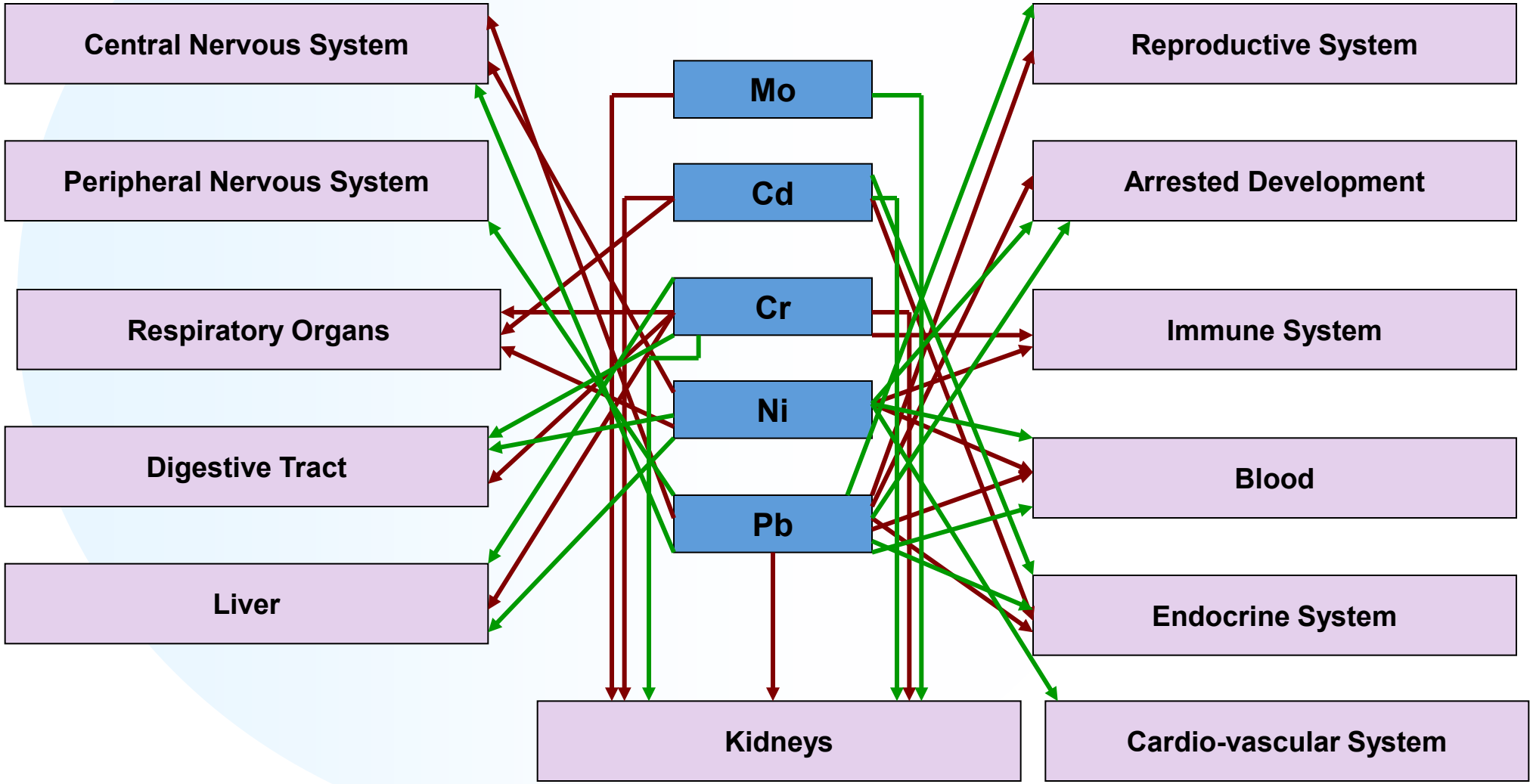
Research Design
(using the triad method)



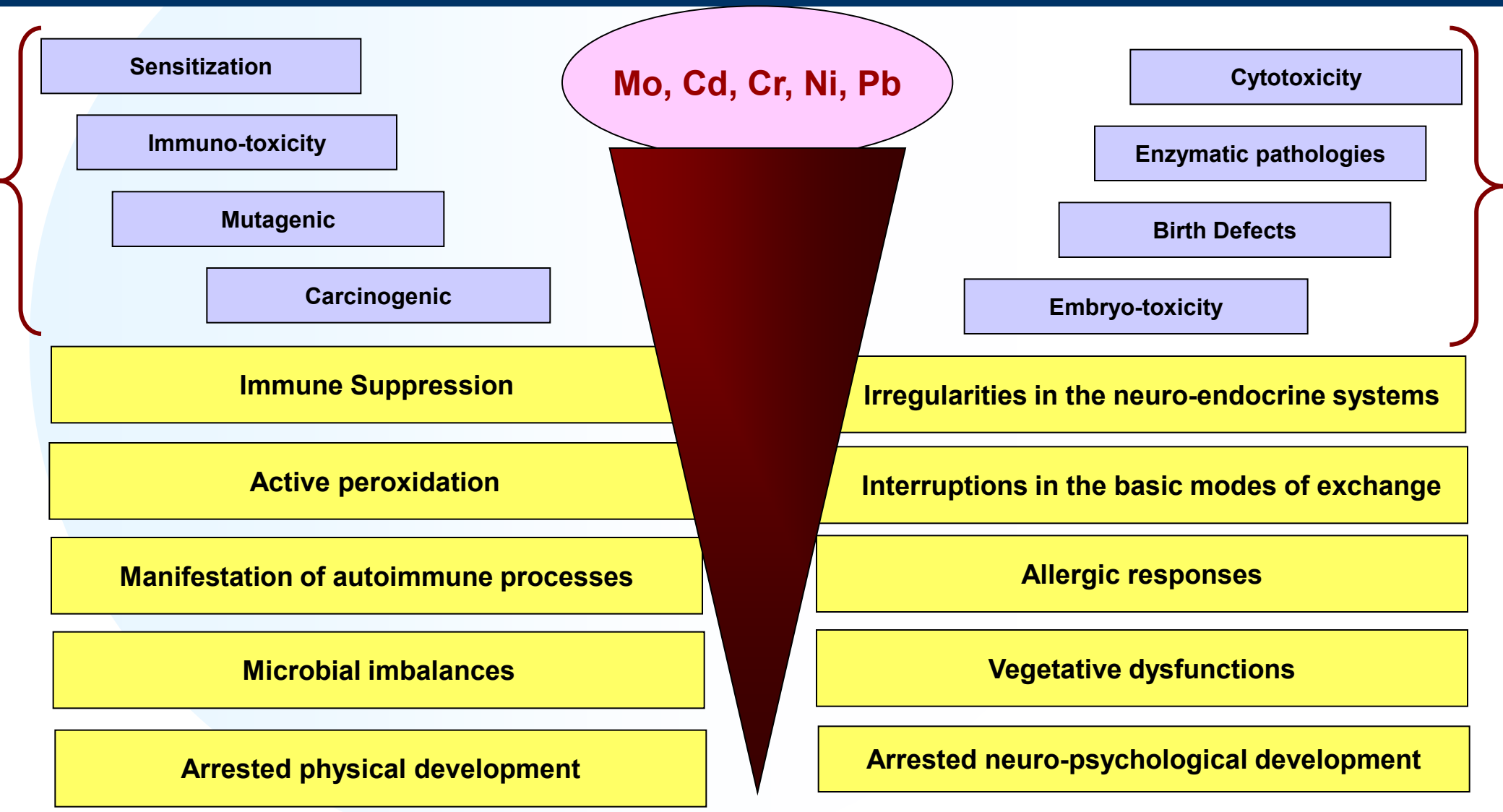


Target organs and body systems

(as associated with this list of important toxicants)



The Main Manifestations of Impact



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**
  - **EKGs**
  - **Spirography or pneumography**
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  - **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!