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ON DETECTING REFERENCE LEVEL OF ACROLEIN CONTENT IN CHILDREN'S BLOOD

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The article gives the results of complex chemical-analytical and clinical-laboratory research in course of which biological media of children living in Perm region were examined. To study impacts exerted by exogenous acrolein we examined 156 children in 2014–2016, aged 5–10, attending pre-school facilities and schools, and living in Perm region. As we conducted this research we detected average annual acrolein concentration in atmosphere on the examined territory; this concentration was equal to 0.000024 mg/m3, and it was 1.2 times higher than reference acrolein concentration in the air for chronic inhalation exposure. Average group acrolein concentration in children's blood was 1.2 times authentically higher ($p<0.05$) than regional background level of acrolein content in blood of children living on conditionally clean (control) territory of Perm region. Average content of malonic dialdehyde in blood plasma and IgG specific to acrolein was 1.2 and 1.4 times authentically higher than physiological standard for these parameters ($p<0.05$). Average group concentration of delta-aminolevulinic acid in urine was detected at the top limit of physiological standard. Applying odds relation criterion ($OR=e^{a0-a1x}$) we obtained authentic models for correlation between acrolein content in blood and G immunoglobulin specific to acrolein, antioxidant activity of blood plasma, crude bilirubin in blood, and delta-aminolevulinic acid in urine ($F>3.96$, $p\leq0.05$). We used increased content of delta-aminolevulinic acid in urine as a limiting marker for effects occurring at chronic inhalation exposure to acrolein. Basing on the results of the performed examination we recommend concentration equal to 0.10 mgr/dm^3 as a reference level of acrolein content in blood at chronic inhalation exposure

Key words: *acrolein, chronic exposure, response markers, blood, highly efficient liquid chromatography, delta-aminolevulinic acid, bilirubin, reference level.*

Nowadays, protection of population health is among top priorities in state policy. Atmosphere becomes more and more contaminated every year due to increased emissions of technogenic substances, and it requires greater attention and expert solutions to problems of providing safe environment for population.

As per World Health Organization data, up to 500,000 compounds are used in industry, which are potentially capable to contaminate environment. Volatile organic compounds (VOC) are the most widely spread among multi-components structure of air pollutants. Acrolein is a priority pollutant among volatile organic compounds [3].

Acrolein (acrylic aldehyde, ethylene-aldehyde, 2-propionic aldehyde) is an

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elementary unsaturated aldehyde with high reactivity; it is a colorless, tear-inducing liquid with strong smell, very volatile and with low boiling temperature (52.7 °C). It is contained in the air as vapor, vapor pressure being 0.145 MPa at 5 °C [14].

Acrolein is used in production of acrylic acid, methionine, 1,3-propandiol, pyridine, glutaralaldehyde, β-picolin, acrylonitril, medications, herbicides, flavoring agents, plasticizers, etc. [14]. Motor transport emissions, combustion activities, photooxidation of hydrocarbons which are contained in the air (propylene, 1,3-butadiene, pentadiene), households and industrial waste burial, contribute greatly into total air pollution in big cities [1, 2, 12]. In everyday life a substantial contribution is made by tobacco smoke, release out of polymeric materials, heating of butters and fats, both vegetative and animal ones, during cooking (roasting, smoking).

Average daily concentration and maximum single permissible concentration of acrolein in atmosphere amount to 0.01 and 0.03 mg/m3 correspondingly. Reference concentrations of the toxicant in the atmosphere (RfC) are extremely small and amount to 0.0001 mg/m3 for acute inhalation exposures and 0.00002 mg/m3 for chronic exposures [11]. Danger category is 2. Regional background level of acrolein content in blood of children living in Perm region amounts to 0.138 ± 0.035 mg/dm3 [9].

Acrolein enters a human body mostly via inhalation. Under chronic exposure acrolein exerts overall toxic, irritating, and allergenic effects and also has mutagenic properties [12, 16]. Research of chronic effects of acrolein on lungs functioning revealed that it made for inflammation and damage to respiratory organs tissues in adults [17]. But children to a greater extent are prone to toxicants' effects, and acrolein which is contained in tobacco smoke in concentrations equal to 1.6–3.6 μkg/m3 [21], can cause a bronchospasm and increased secretion of mucous coat cells which is characteristic for bronchial asthma. Besides, lung functioning can deteriorate and number of bronchial asthma cases can increase under chronic exposure in childhood [16].

Acrolein induces oxidizing stress in a body. Cells death induced by acrolein and mostly having a form of necrosis, is accompanied by accumulation of active oxygen forms (AOF) in them [20, 22]. Acrolein can directly stimulate mitochondrial oxidizing stress violating the function of electrons' mitochondrial feed system [21].

But at the same time acrolein is a natural metabolite of a human body and can be found in its biological media (blood and urine) [13]. Acrolein is formed endogenously in micro-quantities as lipid peroxidation product in metabolic processes of polyamines (spermine and spermidine) [10, 23].

Our research goal was to define reference level of acrolein content in children's blood under long-term intake with atmospheric air as per results of assessing "acrolein concentration in blood - response markers" correlations.

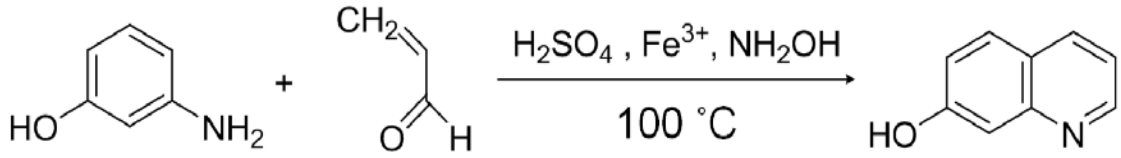
Data and methods. In accordance with the set goals, we examined a group of children (n = 156) in 2014-2016, aged 5-10 years, attending pre-school children facilities and schools and living in Perm region since their birth.

Acrolein in the atmosphere on the examined territory was detected as a derivative with the use of fluorometry in conformity with Methodical Guidelines 4.1.3356-16 "Measuring acrolein mass concentration in the atmosphere via highly efficient liquid chromatography" [8].

Biomedical research was accomplished in conformity with obligatory observation of ethical principles on medical-biological research stated in Helsinki declaration dated 1975 with supplements made in 1983 and RF State Standard 52379-2005. A legal representative of each child included in our sampling gave his or her written informed consent to voluntarily take part in biomedical research carried out by experts of Federal Scientific Center for Medical and Preventive Health Risk Management Technologies.

We determined acrolein content in blood via highly efficient liquid chromatography on

reversed phase C18 with fluorimetric detection in accordance with Methodical Guidelines 4.1.3158-14 [7]. The range of measured acrolein concentrations in blood amounted to 0.1–5.0 mg/dm3. Before the analysis, we performed a reaction of acrolein derivatization with meta-aminophenol in order to transfer the analyte from its free state into bound one and to obtain a derivative 7-hydroxyquinoline, a stable compound capable of fluorescence (figure 1).



Meta-aminophenol Acrolein 7-hydroquinoline

Figure 1. Reaction of acrolein derivatization with 3-aminophenol

We studied several biochemical parameters as response markers at acrolein inhalation intake [1, 4, 18, 19] (crude and conjugated bilirubin in blood serum, delta-aminolevulinic acid content in urine, antioxidant activity, malonic dialdehyde content in blood plasma, creatinine concentration in blood serum) with the use of unified techniques [5]; we also studied immunologic parameters (acrolein-specific IgG content) via allergosorbent testing with enzymatic mark. All the research was performed by experts of biochemical and cytogenetic diagnostics department at Federal Scientific Center for Medical and Preventive Health Risk Management Technologies.

We justified biological response markers as per odds relations calculation (OR) characterizing correlation between acrolein concentration in blood and biochemical response parameters. OR>1 condition was taken a criterion of correlation existence [13].

We detected parameters of odds relation dependence on acrolein concentration in blood via regression model making in a form of exponential function OR=ea0–a1x, where OR was odds relation parameter; x was acrolein concentration in blood, mg/dm3; a0, a1 were model parameters detected with regression analysis technique.

The validity of the obtained model was assessed via single-factor variance analysis as per Fischer criterion (F>3.63). Discrepancies in results were considered to be statistically significant at $p \leq 0.05$ [15].

Reference level was defined on the condition. We took a value corresponding to the upper 95% confidence limit of the obtained model as a reference level [6].

We accomplished processing of all the data obtained in the course of our research and model parameters assessing with the use of Statistica 6.0 applied software and other specialized software.

Results and discussion. Over 2014–2016 average annual acrolein concentration in the atmosphere amounted to 0.000024 mg/m3 on the territory where the examined group lived. It turned out to be lower than average daily concentration and maximum single permissible acrolein concentration in the atmosphere but it was 1.2 times higher than the reference acrolein concentration in the air for chronic inhalation exposure [11].

Acrolein concentrations detected in blood of children from the focus group varied within 0.10–2.34 mg/dm3 range. Average group concentration amounted to 0.16 ± 0.01 mg/dm3, which was authentically 1.2 times higher ($p<0.05$) than regional background level of acrolein content in blood of children living on conditionally clean territory of Perm region [9].

We studied biochemical and immunologic parameters of blood and urine taken from the examined children; the results are given in Table 1 as average group values ($M \pm m$).

Analysis of biochemical and immunologic parameters of children's blood under chronic inhalation exposure to acrolein revealed authentically 1.2 times higher (p<005) average group malonic dialdehyde content in plasma and 1.4 times higher acrolein-specific IgG content in comparison with physiological standard. Average group concentration of delta-aminolevulinic acid in urine was detected at the upper limit of physiological standard and it proves there's a trend of increase in this parameter under chronic exposure to acrolein.

We built relation models showing dependence of biochemical and immunological parameters of children's blood (effect markers) on acrolein concentration in blood (exposure marker) and it helped us to obtain authentic models (F>3.96; $p \leq 0.05$) of correlation between acrolein concentration in blood and increased crude bilirubin content in blood plasma, increased level of delta-aminolevulinic acid in urine, lower antioxidant blood plasma activity, increased acrolein-specific IgG content in blood plasma (Table 2).

Table 1

Results of analyzing biochemical and immunologic parameters of blood and urine taken from children (n = 156), 2014–2016

Parameter, unit of measure	Standard	Focus group, $M \pm m$	Validity of discrepancy, p
Antioxidant plasma activity, %	36,2–38,6	35,8 ± 1,4	>0,05
Crude bilirubin, μmol/dm^3	0–18,8	9,31 ± 1,35	>0,05
Conjugated bilirubin, μmol/dm^3	0–4,3	2,50 ± 0,17	>0,05
Delta-aminolevulinic acid in urine, μmol/dm^3	0,0012–0,013	0,013 ± 0,001	>0,05
Malonic dialdehyde in blood plasma, μmol/dm^3	1,8–2,5	3,02 ± 0,12	<0,05
Creatinine in blood plasma, μmol/dm^3	28–88	57,9 ± 1,4	>0,05
Acrolein-specific IgG, st.un.	0–0,15	0,33 ± 0,11	<0,05

Table 2

Parameters of mathematic models describing "acrolein concentration in blood - odds relation (OR = ea0–a1x) of deviations in laboratory parameter" dependence

Laboratory parameter	Model parameters		Fischer criterion, F	Validity, p	Acrolein concentration in blood, mg/dm^3
	a_0	a_1			
Antioxidant plasma activity, %	–1,27	8,09	113	<0,05	0,15
Crude bilirubin, μmol/dm^3	–5,27	37,72	74,99	<0,05	1,14
Delta-aminolevulinic acid, μmol/dm^3	–0,85	7,29	108,94	<0,05	0,10
Acrolein specific IgG	–2,50	9,04	43,21	<0,05	0,25

Crude bilirubin content in blood of children from the focus group was higher than physiological standard in 3% cases (n = 153). Dependence of odds relation for increase in bilirubin content in blood on growth in acrolein concentration in blood (F = 74.995; p<0.05) is described with the following equation $OR = e^{-5,273-37,772x}$. In the given case 95%-upper confidence limit of reference level of acrolein in blood is equal to 0.14 mg/dm^3.

Antioxidant activity of blood plasma in children from the focus group was lower than physiological standard in 52% cases (n = 121).

Authentic dependence of odd relation for decrease in antioxidant plasma activity on acrolein concentration in blood ($F = 113$; $p = 0.05$) is described with the following equation $OR = e^{-1,267-8,086x}$. In the given case 95%-upper confidence limit of reference level of acrolein in blood is equal to 0.15 mg/dm^3 (Figure 2).

Delta-aminolevulinic acid level in urine characterizing porphyrinic metabolism disorder was on average 1.5 times higher than in 44% of the examined children ($n = 98$). Dependence of odds relation for increase in delta-aminolevulinic acid in urine on acrolein concentration in blood is described with the following equation $OR = e^{-0,851-7,291x}$ ($F = 108.94$; $p = 0.05$), 95%- upper confidence limit of reference level of acrolein in blood is equal to 0.10 mg/d,^{3} (Figure 3).

The obtained results correlate with the data taken from scientific research; according to these data, chronic exposure to acrolein leads to disorders in porphyrinic metabolism, lower antioxidant plasma activity and consequently to Red/Ox cells potential disorders.

Acrolein-specific IgG in children from the examined group was higher than physiological standard in 46% cases ($n = 74$). Dependence of odds relation (OR) for increased acrolein-specific IgG level on acrolein concentration in blood is described with the following equation $OR = e^{-2,503-9,044x}$ ($F = 43.213$; $p = 0.05$), 95%-upper confidence limit of reference level of acrolein in blood is equal 0.25 mg/dm^3 (Figure 4).

Basing on the calculated relation models ($p = 0,05$), we calculated levels of exposure marker content (acrolein concentration in blood, mg/dm^3), which cause immune system suppression and disorders in oxidation-reduction cells potential, porphyrinic metabolism disorders, and disorders in bilirubin metabolism.

Minimal acrolein content in blood (0.10 mg/dm3) was detected in case of increased delta-aminolevulinic acid concentration in blood which was higher than physiological standard. In relation to that, we recommend to use increased delta-aminolevulinic acid content in urine as reference level of acrolein content in blood (concentration equal to 0.10 mg/dm3) and as a limiting effect marker under chronic exposure to acrolein.

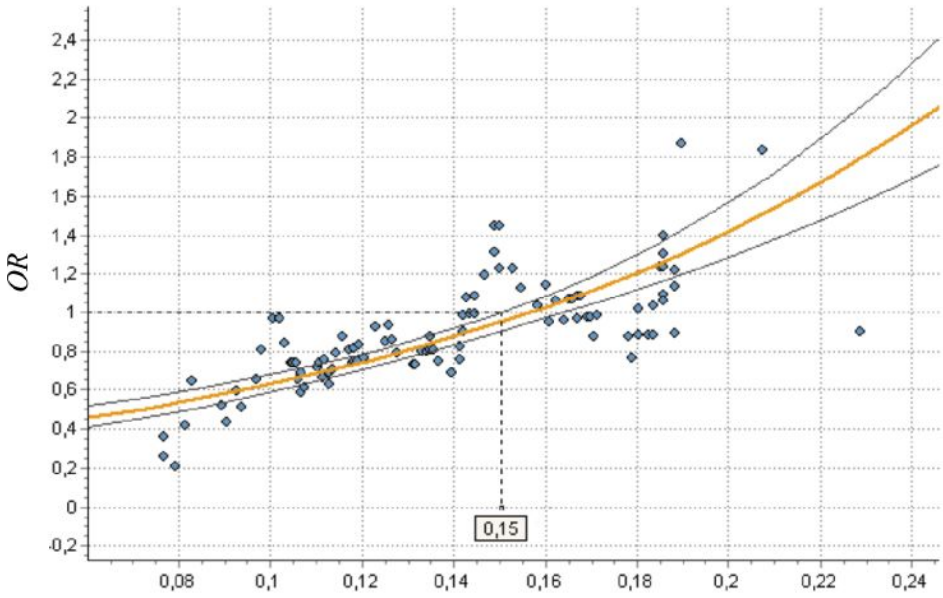


Figure 2. Dependence of odds relation (OR) for lower antioxidant activity of blood plasma on acrolein content in blood

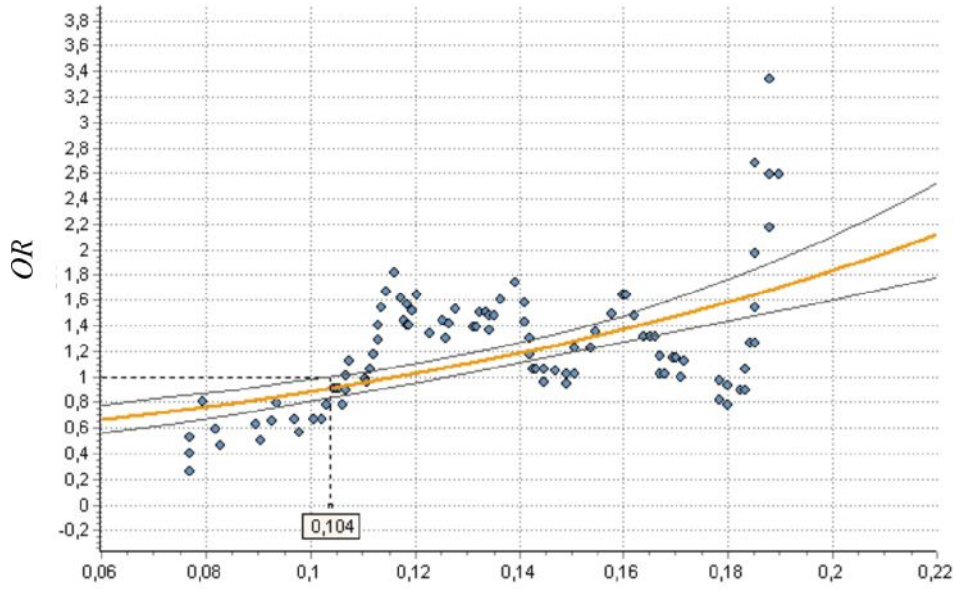


Figure 3. Dependence of odds relation (OR) for increased delta-aminolevulinic content in urine on acrolein concentration in blood concentration in blood

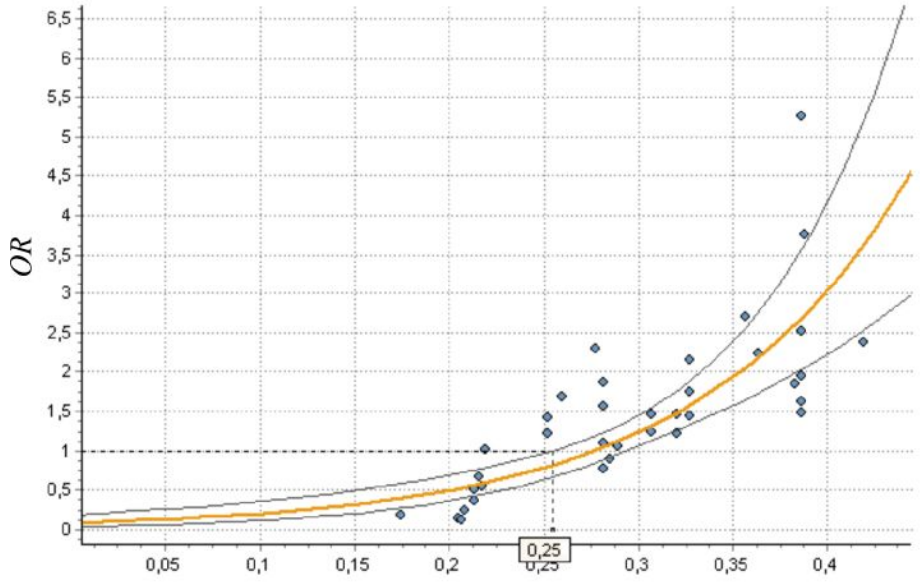


Figure 4. Dependence of odds relation (OR) for increased content of acrolein-specific IgG on acrolein content in blood

Conclusions:

1. We detected authentically higher malonic dialdehyde content in blood plasma and acrolein-specific IgG, both parameters being 1.2 and 1.4 times higher than physiological standard correspondingly ($p<0.05$), average

group concentration of delta-aminolevulinic acid in urine was detected at upper limit of physiological standard.

2. We obtained authentic models of correlation ($F>3.96$; $p\leq0.05$) between acrolein content in blood and disorders in porphyrinic metabolism as per increased delta-aminolevulinic content in urine, oxidizing stress occurrence as per antioxidant activity of blood plasma, suppressed immune response as per acrolein-specific IgG content.

3. We detected acrolein concentrations in children's blood (mg/dm3), which caused immune system suppression, decrease in antioxidant blood plasma activity, disorders in porphyrinic and bilirubin metabolism; these concentrations were equal to 0.25; 0.15; 0.14 and 0.10 mg/dm3 correspondingly.

4. We recommend acrolein concentration equal to 0.10 mg/dm3 as reference level of acrolein content in children's blood; this concentration was detected at studying dependence of odds relation (OR) for increased delta-aminolevulinic acid content in urine on acrolein concentration in blood.

5. The detected reference acrolein concentration in blood can be used as a safety parameter under long-term intake of acrolein with atmospheric air within biological monitoring frameworks, when assessing population health risks, in diagnosing ecologically-dependent changes in health state, when assessing efficiency of medical-preventive activities, as well as evidence base used in sanitary-epidemiologic studies, investigations, and examinations.

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