

# Risk Assessment Metabolic Disorders by Prolonged Exposure to Low Doses of Benzene

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Article History:

Submitted: 12.04.2020

Revised: 16.05.2020

Accepted: 21.06.2020

## ABSTRACT

Benzene is one of the most common environmental pollutants. However, the main attention of researchers is drawn to its carcinogenic and hematotoxic effect. The metabolic effects of this hydrocarbon have been studied to a lesser extent and are mainly related to high effects. The risks associated with the effect on the body of non-toxic doses of this pollutant are practically not studied, which was the purpose of the present study.

Experiments lasting 7 weeks were carried out on animals that received drinking water containing benzene at a concentration of 10 µg/l, which is 1 maximum permissible concentration (MPC).

It was shown that long-term consumption of water containing benzene in concentration equal to 1 MPC in animals led to changes in biochemical parameters in blood serum, characterized by a decrease in the level of total protein and albumin, a moderate increase in the activity of serum enzymes – alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Simultaneously, developed hypercholesterolemia, triacylglycerolemia and dislipoproteinemia. In addition, there was a decrease in the concentration of urea and uric acid. The detected changes are considered as changes in liver

functions under the influence of benzene. In addition, there was a decrease in the level of calcium, sodium and potassium in the blood serum, which may reflect the effect of the pollutant on the processes of reabsorption of electrolytes in the renal tubules on the one hand, as well as on the regulation of electrolyte metabolism. In addition, under the influence of low doses of benzene in animals developed insulin resistance (IR), estimated by glucose tolerance test (GTT). The possible role of oxidative stress in the development of the described disorders is discussed.

**Key words:** benzene, rats, drinking water, maximum permissible concentration, serum, total protein, uric acid, blood glucose, insulin resistance, enzymemia, dyslipoproteinemia

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DOI: [10.31838/srp.2020.6.67](https://doi.org/10.31838/srp.2020.6.67)

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## INTRODUCTION

According to the International Program on Chemical Safety (IPCS), benzene is one of the ten most hazardous substances for public health [1] and is one of the most common environmental pollutants [2]. At the same time, the vast majority of studies assess the negative impact of benzene on human health in terms of its hemotoxicity and carcinogenicity [3, 4], as there is more and more information about the data on its toxicity to various organs and systems in literature[5], rather than the carcinogenic effects of benzene. In particular, the toxicity of benzene in relation to the cardiovascular system [6], its hypertensive effect associated with a violation of the formation of nitrogen oxide [7] is shown. Administration of benzene to animals had a hepatotoxic effect, which appeared by hepatospecific hyperfermentemia [8], as well as nephrotoxic effect accompanied by depression of carbohydrate metabolism enzymes in the cortical and cerebral substance of the kidneys [9].

An important manifestation of benzene toxicity can be observed when it acts on the human and animal body insulin resistance [10, 11, 12], as well as violations of the expression of fatty acid metabolism enzymes [13].

It should be noted that, as a rule, observations related to both carcinogenic and non-carcinogenic effects of benzene relate to high doses of the toxicant, while the data available in literature indicate the need to study the effect on the body of this aromatic hydrocarbon at low exposures. However, the negative effects of benzene on the body in small doses have

not been studied enough, and the results obtained are ambiguous, which is the reason for this work [14, 15, 16].

## MATERIALS AND METHODS

The work was performed on 24 male rats of the Wistar line. The weight of the animals at the beginning of the experiment was 170 grams. The animals were kept in a vivarium, with day-night cycles of 12/12 hours. We used granulated food from Procorm (Novosibirsk, Russia) for feeding, balanced in nutrients, vitamins and microelements. The animals were given bottled water extracted from local artesian springs «Akva vita» (LLC "Zhivaya Planeta", Orenburg, Russia) for drinking. The amount of feed and water was not limited.

All experimental animals were divided into two equal groups of equal weight and size. Animals of the first group served as control group. Benzene was added to rats of the second group to a concentration of 0.01 mg / liter in drinking water, which corresponded to 1 maximum permissible concentration (MPC). The duration of the experiment was 8 weeks.

Half of the animals from each group were tested for glucose tolerance (TSH) at the 6th week of the experiment. For this, after 12 hours of starvation, but maintaining access to water, each rat was injected per os with a pipette 20% glucose solution at the rate of 2 grams of dry matter per kg of mass. Immediately before administration, and then every 30 minutes after administration of glucose, blood was collected from the tail vein of the animals, in which the glucose concentration was determined using a glucose meter and test strips "Accu-Chek performa" (Roshe, Switzerland).

All animals were removed from the experiment in compliance with ethical standards after 8 weeks. The blood was collected in test tubes containing a coagulation activator of the company "Sarshted" (Germany) after decapitation and by centrifugation at 3000 rpm, the serum was separated from the shaped elements.

Biochemical studies of blood serum were carried out at the "Cobas-6000" station of Roche (Switzerland) and included the determination of the level of total protein, albumin, creatinine, uric acid, activity of aspartic and alanine transaminases ALAT and ASAT, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), the concentration of total cholesterol (TC) and its fractions – high-density lipoprotein cholesterol (HDL), lipoproteins very high low and low density (VLDL and LDL), triacylglycerol (TAG), ferritin, sodium, potassium, calcium, ferrum.

Statistical processing was performed by using the "Microsoft Excel" package, the reliability of differences was evaluated by the Student's test.

## RESULTS

The results of the experiments are presented in table 1.

From the data provided it follows that, in rats consumed water containing benzene at a concentration of 1 MPC, there was a decrease in the concentration of protein in the blood serum by 7%, and the albumin content was lower by 10% than in the control group. The urea content in the experimental and control groups was within the normal range, but at the same time, it was lower in animals receiving benzene by 10%. The level of serum creatinine in the groups did not differ, and the uric acid in rats treated with benzene was 1.6 times lower than in intact ones.

Table 1: Biochemical parameters of blood serum in rats with the use of water containing benzene (M ± m)

Indicators, units.	Intact	Benzene	P
	n=12	n=12	
Total protein, g/l	63,0±1,2	58,9±0,56	P <sub>1-2</sub> = 0,0055
Albumin, g/l	35,8±0,86	32,4±0,33	P <sub>1-2</sub> = 0,0014
Urea, mmol/l	5,9±0,13	5,4±0,21	P <sub>1-2</sub> = 0,055
Creatinine, mmol/l	35,3±1,61	35,8±1,57	P <sub>1-2</sub> =0,826
Uric acid, mmol/l	131,5±10,1	82,1±8,1	P <sub>1-2</sub> = 0,001
ASAT, units/l	132,5±6,9	171,0±12,0	P <sub>1-2</sub> = 0,011
ALAT, unit/l	51,7±2,5	59,03±3,1	P <sub>1-2</sub> = 0,080
ALP, units/l	150,6±3,0	166,0±6,0	P <sub>1-2</sub> = 0,032
LDH, units/l	547,0±21,0	669,0±37,0	P <sub>1-2</sub> = 0,009
TC, mol/l	1,61±0,1	1,5±0,06	P <sub>1-2</sub> = 0,356
HDL cholesterol, mol/l	1,54±0,06	1,31±0,03	P <sub>1-2</sub> = 0,003
LDL cholesterol, mol/l	0,28±0,034	0,25±0,04	P <sub>1-2</sub> = 0,574
VLDL cholesterol, mol/l	0,4±0,025	0,49±0,035	P <sub>1-2</sub> = 0,049
AI, standard unit	0,045±0,006	0,13±0,04	P <sub>1-2</sub> = 0,048
TG, mol/l	1,03±0,08	1,2±0,05	P <sub>1-2</sub> = 0,086
NEFA, mol/l	0,68±0,02	0,83±0,04	P <sub>1-2</sub> =0,003
Sodium, mmol/l	146,5±0,69	141,4±1,72	P <sub>1-2</sub> = 0,012
Potassium, mmol/l	6,09±0,25	5,26±0,17	P <sub>1-2</sub> = 0, 012
Calcium, mmol/l	2,5±0,04	2,3±0,03	P <sub>1-2</sub> = 0,001
Ferrum, µmol/l	23,5±1,29	19,12±0,39	P <sub>1-2</sub> = 0,004
Ferritin, µg/l	125±9,34	140±5,68	P <sub>1-2</sub> = 0,184

Further, the table shows that the intake of small doses of benzene into the bodies of animals led to an increase in the activity of serum enzymes, while the activity of ASAT in the experimental group of rats was 21% higher than in the control group, ALAT by 14%, ALP by 10.6%, and LDH by 22%.

Long-term intake of small doses of benzene did not lead to changes in the level of total cholesterol in the blood serum, but had a significant effect on its distribution by fractions. The table shows that the systemic exposure of HDL cholesterol in experimental animals was lower than in the control by 18%.

As a result, the Atherogenic Index (AI) in rats of the experimental series was 2.7 times higher than in intact animals.

The level of TAG and free FA in benzene-treated rats was 16 and 23%, respectively, higher than in the control group.

Further, the table shows that the content of the main serum cations-Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> - in animals receiving benzene was lower by 6, 15 and 9%, respectively, compared to rats consuming clean water.

Figure 1 shows that fasting glucose levels in intact and experimental animals did not differ.

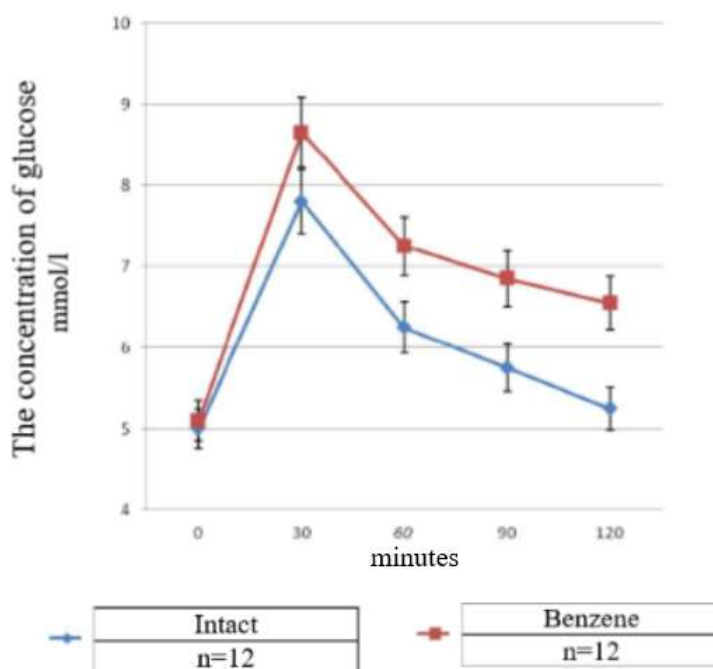


Fig. 1: The concentration of glucose in the blood of rats during the glucose tolerance test

When conducting a glucose tolerance test, changes in its concentration in the blood of intact animals were characterized by a rise to 30 minutes after oral administration, and then a decrease almost to the initial values after 2 hours (Fig. 1).

The curve showing changes in glucose concentration in animals receiving benzene was characterized, firstly, by higher values per 30 minute after oral administration, and secondly, by a slower decrease in its level in subsequent periods. At the same time, by the 180th minute of the test, the serum glucose values in the experimental group were 1.25 mmol/l higher than the control group.

## DISCUSSION

Thus, the results obtained indicate that prolonged intake of benzene in small doses with drinking water leads to noticeable changes in the metabolic status of animals.

Firstly, these changes were characterized by a decrease of the level of total protein occurring due to the albumin fraction. At the same time, the benzene-treated rats had lower levels of urea and uric acid than in the control group. These listed symptoms may indicate a slight decrease in the metabolic function of the liver. First of all, it is subject to the processes of albumin synthesis, as well as the formation of urea and catabolism of purine nucleotides.

It should be noted that these changes, which are not significant in their severity, but have a significant character, occur against the background of moderate hyperfermentemia, estimated by the activity of ASAT, ALAT, ALP and LDH, which may be evidence of increased permeability of hepatocyte membranes.

Changes in liver function can probably be associated with the observed dyslipoproteinemia, which is manifested by a decrease in the proportion of the antiatherogenic fraction of cholesterol represented by HDL and an increase in the

atherogenic index, as well as an increase in the concentration of TAG. The lower than in the control, the concentration of electrolytes in blood serum observed in rats treated with low doses of benzene, can reflect as a violation of the reabsorption processes in the renal tubules, this is more true for sodium and potassium ions, and on the other hand, a decrease in absorption in enterocytes which is possible for calcium ions. Finally, long-term consumption of water containing low concentrations of benzene led to the development of insulin resistance, assessed by the glucose tolerance test.

In other words, long-term intake of benzene in low doses led to changes in both biochemical parameters and metabolic regulation processes.

The obtained data are in accordance with studies that reflect the effects of low effects of benzene on the human body [3, 16, 17, 18].

The most likely reason for the effects of low doses of benzene described, in our opinion, is the peculiarities of its metabolism when entering the body. Thus, according to modern concepts, this aromatic hydrocarbon undergoes biotransformation in the endoplasmic reticulum of the liver, with the participation of the isoform of cytochrome P450-2E1 [19, 20].

The transformations that occur with its participation are characterized by the formation of reactive oxygen species and toxic metabolites [19] and lead to the activation of free radical oxidation of membrane lipids, both in the liver and in other organs. In other words, the metabolism of benzene entering the body is accompanied by the development of oxidative stress, which is known to cause damage to the membrane structures of cells, as well as proteins and nucleic acids, which is an important basis for mutagenesis. It should be noted that the nature of transformations does not depend on the amount of incoming benzene.

In addition, it is the phenomenon of oxidative stress that occurs when the body is exposed to benzene, is currently considered as the cause of insulin resistance and impaired insulin secretion by pancreatic islets, and may thus still be an unrecognized source of global epidemics of metabolic syndrome [21, 22].

It should be noted that the above-described biochemical shifts are a response to the effects of low doses of a pollutant, which, as shown earlier, is also accompanied by the development of oxidative stress [23] and, therefore, may be the basis for the described metabolic disorders.

**B** The results of the studies, in general, indicate that the presence of benzene in drinking water is hazardous to health, regardless of concentration, and differs only in the time of the onset of negative consequences.

This fact should be taken into account when monitoring the state of water resources [24].

### FINANCING

The study was carried out at the expense of the state building "Development and experimental testing of a method for preventing insulin resistance based on 3-b-methoxy-D-oleanene".

### CONFLICT OF INTEREST

The authors of this article report that there is no conflict of interest.

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