Aromatic hydrocarbons in bile – the clinical significance

Nina Zaitseva 1, Tatyana Ulanova 1, Alfiya Aminova 2*, Olga Ustinova 1, Alevtina Akatova 1

1 Federal Scientific Center for Medical and Preventive Health Risk Management Technologies (Perm), Russia
2 Russian Medical Academy of Postgraduate Education, Moscow, Russia
3 First Moscow State Medical University named after I.M. Sechenov, Moscow, Russia

* Corresponding Author: Alfiya Aminova
Address: 19, str.B.Pirogovskaya, 119992, Moscow, Russia
Email: aminova62@yandex.ru | Tel: +7 (499) 248-88-41

Abstract

Background: In sanitary unhealthy areas hepatobiliary disorder occur 3-4 times more than in relatively “clean” that is probably stipulated by the impact of big quantity of industrial toxicants on the liver. It is established that the part of environmental factors makes from 14 to 36% from all the possible causes of hepatobiliary diseases.

Aim & Objectives: To evaluate the clinical significance of a method for determining the aromatic hydrocarbons in the bile in children.

Methods/Study Design: During the study 303 children were examined with “Biliary dyskinesia” (K82.8) and “sphincter of Oddi dysfunction” (83.4) according to CD-10. The study group embraced 204 children in the age from 5 to 15 with hepatobiliary disorders, living in the area of refining complex impact. 99 children living in the sanitary healthy area were chosen for the control group. We have performed chemical testing of biological media (blood and bile) in a group of children (N = 45), using the developed chromatographic method, along with clinical and anamnesis, clinical laboratory and instrumental examinations.

Results/Findings: Thus, living in the area close to the source of hydrocarbons pollution impacts on the formation and evolution of hepatobiliary disorders in children. In those children who live in the area close to the source of hydrocarbons pollution benzene, toluene, xylene were detected in biological media (blood and bile). Exposure to aromatic hydrocarbons in children, the content of these compounds in the bile, may cause such patterns of biliary dysfunction as memory impairment and irritability in children, more pronounced symptoms of dyspepsia (food belching, vomiting after eating with bile, nausea and vomiting during while riding in the car and after a fatty meal, a tendency to thin stool, sclera subicteritiousness). Aromatic hydrocarbons contribute to the hypertonia of sphincter Oddi, intrahepatic cholestasis, gallbladder hypokinesia (benzene...
0.007 + 0.001 and 0.0001 + 0.00001 and p <0.05), which is accompanied by enlargement of the right lobe of the liver, bile flow overtime.

**Conclusion:** The study results showed the adverse impact of the studied aromatic hydrocarbons on the condition of the hepatobiliary system, which induces the development of hypertonic-hypokinetik dyskinesia of the gallbladder and the development of intrahepatic cholestasis.

**Key words:** children, bile, aromatic hydrocarbons, hepatobiliary disorders.

**Introduction**

In the time when Russia survives sharp demographic crisis, the most important condition of development of any civilized society is to maintain children’s health.\(^1\) The problem of impact of environmental chemical impact on children in industrial cities is getting special importance.\(^2\) The man-made load violates adaptive capabilities that increases genetic mutations and undermines children health.\(^3\)

In sanitary unhealthy areas hepatobiliary disorder occur 3-4 times more than in relatively “clean” that is probably stipulated by the impact of big quantity of industrial toxicants on the liver.\(^4,5\)

It is established that the part of environmental factors makes from 14 to 36% from all the possible causes of hepatobiliary diseases.\(^6,7\) The high sensitivity of liver to chemical ecotoxicants is determined by the fact that liver is the main organ responsible for the metabolism of foreign compounds, and their release with bile depends on the molecular size and molecular weight.\(^8\) With increasing molecular weight of toxins the rate of their release with bile increases.\(^9,10\)

Aromatic hydrocarbons have strong hepatotoxicity.\(^11\)

It is known that these compounds have a wide action spectrum - hematopoiesis depression; violation of nucleic acids synthesis (mainly DNA) leading to inhibition of cell production, their inferiority, and violation of chromosomal structures.

Main air pollution producers by aromatic hydrocarbons are refining companies and motor vehicles, the share of the latter in the total amount can be up to 85%. Living in conditions of prolonged exposure to aromatic hydrocarbons may pose a threat to public health, especially in children. However, there are not enough works in Russian and foreign scientific literature with the analysis of aromatic hydrocarbons in blood and bile, their impact on clinical and laboratory parameters and function of gall-bladder in children.
Aim & Objectives

To evaluate the clinical significance of the method for determining the aromatic hydrocarbons in the bile in children.

Methods

303 children with “Biliary dyskinesia” (K82.8) and “sphincter of Oddi dysfunction” (83.4) according to ICD-10.

The study group embraced 204 children in the age from 5 to 15 with hepatobiliary disorders, living in the area of refining complex impact. Specific for industrial enterprises chemicals are systematically recorded in residential areas at levels exceeding the hygienic standards: for toluene (up to 3.2 TLVmax) for xylenes (up to 1.2 TLVmax); calculated benzene concentration amounted to 13.14 shares of TLVmax and 3.37 shares of TLVp.day.

99 children living in the sanitary healthy area were chosen for the control group.

We have performed chemical testing of biological media (blood and bile) in a group of children (N = 45), using the developed chromatographic method, along with clinical and anamnestic, clinical laboratory and instrumental examinations - clinical and biochemical analysis of blood, fractional duodenal intubation by standard techniques using a 25% solution of magnesium sulfate as a cholangogue, ultrasound of the abdomen. During ultrasound examination multiple view scanning was used: inspection was carried out in a horizontal, vertical and side position. We made a test with use of choleretic breakfast (25 gr of chocolate). We took into account the latent phase of gallbladder volume change (increase of its volume in the first minutes after the administration of choleretic breakfast), the amount of ejected bile to food stimulus and the time of its expiration, as well as the parameter of the volume flow of the ejected bile, which is defined as the ratio of the ejected bile to the time of ejection. The important parameter characterizing the function of the biliary system is the percentage of the gallbladder volume change. According to Pykov M.I. (2008), normal gallbladder contraction occurs in the range of 20 to 50 minutes, its volume is changed to 34-67% and a mean value of the volume flow of the bile is 0.203 ± 0.058 ml / min.

Clinical and laboratory diagnosis is made by an automatic hematology "Abakus junior" (Austria), biochemical "Stat Fah-2600" (USA). Benzene, toluene, p-, m- and o-xylene are determined in accordance with the "Methodical recommendations on detection and determination of 1,2-dichloroethane and a number of aromatic hydrocarbons in biological material by gaz-liquid chromatography" approved by the URSS Ministry of Health 04/12/78 N10-8/82 (N.V. Zaitseva, 1992).
Ultrasound examination of the hepatobiliary area is made by ultrasound scanner Toshiba «APLIO XG» and Toshiba «Viamo SSA-640A» with use of linear encoders, frequency from 7.5 to 13 MHz by the standard method with the assessment of topographic and anatomical relations, the macrostructure of the liver and biliary tracts.

In developing highly sensitive methods for the determination of aromatic hydrocarbons in the bile were used gas chromatography and different techniques of sample preparation that improve the sensitivity and selectivity of the determination of the studied compounds on the background of complex biological matrices.

Development of the method is based on the following principles: study of the physical and chemical features; the establishment of the chromatographic separation of the determined compounds in the analysis conditions (separation criteria); use of a flame ionization detector; choosing optimal conditions for sample preparation to minimize the impact of complex biological media matrix; study of the completeness of extraction by method "added - found"; establishing of metrological characteristics of the measuring process.

During the study the separation of aromatic hydrocarbons in the analysis of the bile was achieved on a capillary column DB-624-30m*0.32 mm *1.8 μm at temperature: Column - 50°C -180°C; evaporator - 200°C ; detector - 250°C , carrier gas 1 flow (nitrogen ) - 20 cm³/min , carrier gas flow - 30 cm³/min. To isolate the toxic compounds from the bile was used the combined method of gas chromatographic headspace analysis.

Average completeness of extraction of the studied bile compounds using the recommended method ranged from 78.5% for p- and m-xylene to 87.5 % for o-xylene. The detection limit of the test compounds (mcg/cm³) is: benzene – 0.0035, toluene – 001, p- and m-xylene – 0.001 , o-xylene – 0.0005 . The operational margin is 8.96 – 17.5%.

The mathematical processing of evidential base of evaluating the effectiveness of the recommended method is carried out using non-parametric statistical methods with the construction and analysis of two-dimensional contingency tables, one-way ANOVA method, methods of linear and nonlinear regression analysis. To assess the reliability of the results was used F-test (assessment of the adequacy of models), Student's test (comparison of groups by variables), Pearson's chi-squared test (comparison of the structure of characters). The assessment of dependencies between characters was performed by ANOVA for qualitative characteristics and by method of correlation and regression analysis for quantitative variables. The differences of the results are considered statistically significant at p <0.05.

**Discussion**

The analysis in the study group revealed in biological media (blood) aromatic hydrocarbons (benzene, toluene, xylene) that are not identified in the control group. M-cresol concentration in
the study group exceeds reference level in 1.26 (p<0.001) and 41.4 (p<0.05) times, respectively (Table 1).

The study of the gallbladder and liver bile fractions in the study group revealed benzene, toluene, p-, m- and o-xylene (Table 2 and Table 3).

Ninety per cent of the gallbladder bile samples (batch B) in the study group were found to contain benzene, 40% of the samples contained toluene and 30% of the samples o-xylene. Ethylbenzene and p-, m-xylene were not detected. The testing of the bile samples from bile ducts (portion C) in the study group detected benzene in 43% of the samples, toluene in 36%, ethylbenzene, and p-, m-xylene in 21% and o-xylene in 21% of the samples. Chromatograms of samples are given in Figure 1.

The performed testing showed that the concentration of benzene in the samples of gallbladder bile (portion B) was 3.6 times higher than the concentration of benzene in the bile samples from bile ducts (portion C). In addition, no ethylbenzene or n-m-xylene were detected in the gallbladder bile samples (batch B) whereas the bile samples from bile ducts (portion C) were found to contain the full range of the studied aromatic hydrocarbons.

The incidence of clinical symptoms in the majority of children of study and control group had no statistically significant differences. All the children (of study and control group) complained of pain in the umbilical region and right upper quadrant after meal and after exercise, bad breath, selective appetite, belching eaten food, the changing nature of the motion (more often constipation), irritability, tearfulness. Almost every third child in both groups had abdominal pain met. Pain were sporadic (17.53 ± 2.91% and 24.44 ± 5.55%, p < 0.33, respectively) localized in the umbilical region (14.43 ± 2.11% and 22.22 ± 3.43%, p < 0.28), the right upper quadrant (14.43 ± 1.18% and 17.78 ± 3.37%, p <0.13, respectively) and in the third of cases arose after eating (17.53 ± 2.91% and 28.89 ± 6.17%, p <0.12). Half of the children in both groups complained of bad breath, selective appetite. Acid regurgitation was observed at 5 %, and constipation - in 6 % (p <0.6).

At the same time, in contrast to the control group, in the clinical performance of patients in the study asthenovegetative complaints dominated in larger percentage of cases. Night sweats were fixed 6 times more often (13.4 ± 2.34% and 2.22 ± 0.65%, p < 0.04), tearfulness - twice more often (39.18 ± 4.8% and 20 ± 4.81%, p <0.02) and night terrors (25.77 ± 3.86% and 11.11 ± 2.97%, p <0.05).

Physical examination of the study group compared to the control group detected more frequently signs of intoxication and positive Lepine’s signs: periorbital shadows (77.56 ± 2.75% and 65.69 ± 4.43%, p < 0.04), palpation pain in the umbilical region (42.48 ± 4.55% and 25.53 ± 5.58%, p <0.04) and positive (31.86 ± 4.05 % and 14.89 ± 3.72%, p <0.03). Statistical dispersive analysis
established significant causal relationship between positive Ortner-Grekov sign and elevated levels of O-xylene \((r = 0.68, p < 0.044)\) and chromium \((r = 0.74, p < 0.006)\) in the blood.

The biochemical blood test in 10% of children the observation group has shown compared to the physiological norm the decrease of albumin level to \(34.35 \pm 6.99\) g/dm\(^3\) \((p <0.05)\), in 30% of children - concentration of triglycerides up to \(0.766 \pm 0.119\) mmol/dm\(^3\) \((p < 0.05)\), in 20% of children - increased ASAT activity to \(46.051 \pm 3.109\) E/dm\(^3\) \((p<0.05)\). These biochemical changes in 20% of children go with the increasing level of total sensibilization in the form of the increase of general immunoglobulin E in the blood \((to 249\ IU/ml \pm 52.67)\) in 2.0-2.4 times compared to the physiological norm \((p < 0.05)\). In contrast to the control group the metabolic maladjustment in the study group is represented by the stimulation of antioxidant activity (AOA) of the blood \((44.52 \pm 2.4\% and 35.961 \pm 1.266\%,\ respectively, p < 0.04)\) that does not compensate high levels of malondialdehyde (MDA) \((2.812 \pm 0.293\ \mu\text{mol/cm}^3\) and \(2.542 \pm 0.177\ \mu\text{mol/cm}^3, p <0.05)\). In the control group the analyzed parameters of the antioxidant system were not statistically different from the physiological norm \((p <0.05)\).

Multifractional duodenal intubation revealed that for patients in both groups hypokinetic gallbladder sphincter dysfunction is typical \((13.42 \pm 4.81\ \text{min. and 14.75} \pm 6.13\ \text{min.}, p<0.72)\), while only children in the study group had signs of hyperton of sphincter Oddi \((18.35 \pm 1.65\ \text{min. and 12.12} \pm 2.6\ \text{min} , p <0.03)\) and of sphincter Lutkens \((5.86 \pm 0.49\) and \(3.67 \pm 0.52\ \text{min} , p < 0.04)\) that gives evidence to mixed variant of biliary dyskinesia \((\text{Figs. 2 and 3})\).

The features of the ultrasonic parameters have been determined - dyscholia (loose sediment that occupies \(1/2\) of gallbladder volume) was registered in the study group 1.9 \((p <0.05)\) more often, than in the control group, concretion of gallbladder paries are registered 1.5-fold \((p <0.05)\) more often.

The test with choleretic breakfast in the study as opposed to the control group also revealed a mixed type of gallbladder dyskinesia, manifested in the increase of bile flow rate \((0.320 \pm 0.023\ \text{ml/min and 0.261} \pm 0.057\ \text{ml/min} , p< 0.001)\); increase of the maximum bile volume \((6.890 \pm 0.050\) ml and \(6.010 \pm 0.101\) ml, respectively, \(p < 0.01)\), ejected in response to choleretic breakfast and decrease of time of maximum gallbladder food stimulus \((20.115 \pm 0.804\ \text{min and 22.64} \pm 0.198\ \text{min} p < 0.04)\).

We have determined a relationship between a number of clinical and laboratory parameters and the levels of aromatic hydrocarbons in the biological media. The increased levels of benzene at concentrations higher than \(0.004 \pm 0.003\ \text{mg/dm}^3\) caused memory impairment \((p<0.002)\) and irritation in the children \((p<0.038)\), food regurgitation \((0.004 \pm 0.0001\ \text{mg/dm}^3\) and \(0.001 \pm 0.0001\ \text{mg/dm}^3, p<0.015)\), vomiting after eating \((0.006 \pm 0.0001\ \text{mg/dm}^3\) and \(0.001 \pm 0.0001\ \text{mg/dm}^3, p<0.002)\), bile \((0.007 \pm 0.001\ \text{mg/dm}^3\) and \(0.002 \pm 0.0001\ \text{mg/dm}^3, p<0.018)\). Nausea and vomiting during car or bus trip and after a greasy meal, often soft stools, sub-icteric sclera were significantly dependent on toluene levels, which exceeded the reference values \((0.005 \pm 0.001\ \text{mg/dm}^3\) and \(0.000 \pm 0.0000\ \text{mg/dm}^3, p<0.021; 0.005 \pm 0.001, p<0.046)\). Using the analysis of variance, we revealed a significant correlation between the increased levels of aromatic
hydrocarbons and the signs of hypertonic Oddi’s sphincter (toluene 0.019 ± 0.001 and 0.003 ± 0.001, p<0.001), intrahepatic cholestasis (benzene 0.006 ± 0.001 and 0.001 ± 0.0001, p<0.01; toluene 0.010 ± 0.001 and 0.0001 ± 0.0001, p<0.02), gallbladder hypokinesia (benzene 0.007 ± 0.001 and 0.0001 ± 0.00001, p<0.05).

Benzene levels were in a direct cause-and-effect relationship with the echographic dimensions of the right lobe of the liver (r=0.25, p<0.0001) and alkaline phosphatase levels (r=0.17, p<0.03).

The study has found the dependence between the increase of aromatic hydrocarbons level in the blood and the reduction in the volume of bile (r = 0.67, p <0,01 and r = 0.87, p <0.001, respectively), reduced volume flow of bile (r = 0.33, p <0.05 and r = 0.71, p <0.04) and time extension of gall bladder contraction (r = 0.53, p <0.05 and r = 0.62, p <0.01).

Thus, living in the area close to the source of hydrocarbons pollution impacts on the formation and evolution of hepatobiliary disorders in children.

In those children who live in the area close to the source of hydrocarbons pollution benzene, toluene, xylene were detected in biological media (blood and bile).

Exposure to aromatic hydrocarbons in children, the content of these compounds in the bile, may cause such patterns of biliary dysfunction as memory impairment and irritability in children, more pronounced symptoms of dyspepsia (food belching, vomiting after eating with bile, nausea and vomiting during while riding in the car and after a fatty meal, a tendency to thin stool, scleral subicteritiousness).

Aromatic hydrocarbons contribute to the hypertonia of sphincter Oddi, intrahepatic cholestasis, gallbladder hypokinesia (benzene 0.007 ± 0.001 and 0.0001 ± 0.00001 and p <0.05), which is accompanied by enlargement of the right lobe of the liver, bile flow overtime.

**Conclusion**

The study results showed the adverse impact of the studied aromatic hydrocarbons on the condition of the hepatobiliary system, which induces the development of hypertonic-hypokinetic dyskinesia of the gallbladder and the development of intrahepatic cholestasis.

**Conflict of Interest:** None declared.

**References**


**Table 1:** The content of chemical toxicants of industrial origin in the blood of patients with hepatobiliary dysfunctions (mg/dm$^3$)

<table>
<thead>
<tr>
<th>Toxicant</th>
<th>Study group</th>
<th>Control group</th>
<th>Reference /background level</th>
<th>% of samples higher than in the control group</th>
<th>p</th>
<th>p₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene (mg/dm$^3$)</td>
<td>0.022 ±0.01</td>
<td>0.0 ±0.0</td>
<td>0</td>
<td>100</td>
<td>0.001*</td>
<td>0</td>
</tr>
<tr>
<td>M-cresol (mg/dm$^3$)</td>
<td>0.232 ±0.078</td>
<td>0.07 ±0.889</td>
<td>0.0056±0.0019</td>
<td>78.3</td>
<td>0.05*</td>
<td>0.001*</td>
</tr>
<tr>
<td>O-xylene (mg/dm$^3$)</td>
<td>0.018 ±0.008</td>
<td>0.0 ±0.0</td>
<td>0</td>
<td>18.5</td>
<td>0.001*</td>
<td>0</td>
</tr>
<tr>
<td>Toluene (mg/dm$^3$)</td>
<td>0.016 ±0.009</td>
<td>0.0 ±0.0</td>
<td>0</td>
<td>44.4</td>
<td>0.001*</td>
<td>0</td>
</tr>
</tbody>
</table>

p - statistical significance compared to the reference level
p₁ – statistical significance compared to the control group

**Table 2:** Results of the chemical analysis of bile samples (portion B)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Benzene</th>
<th>Toluene</th>
<th>P-, m-xylene</th>
<th>O-xylene</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of studied children</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Average</td>
<td>0.0114±0.0102</td>
<td>0.0022±0.0015</td>
<td>Non-detected</td>
<td>0.0007±0.0001</td>
</tr>
</tbody>
</table>

**Table 3:** Results of the chemical analysis of bile samples (portion C)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Benzene</th>
<th>Toluene</th>
<th>P-, m-xylene</th>
<th>O-xylene</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of studied children</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Average</td>
<td>0.0032±0.0027</td>
<td>0.0021±0.0003</td>
<td>0.001±0.0002</td>
<td>0.0006±0.0003</td>
</tr>
</tbody>
</table>
Figure 1

a) - standard solution of aromatic hydrocarbons in the bile
b) - sample of the bile of the inpatient

Figure represents the chromatograms of the standard solution of aromatic hydrocarbons in the bile (a) and the bile sample (b) containing the studied compounds in view of the optimal conditions of gas chromatographic separation.
Figure 2: Sphincter and gallbladder tonus of patients in the study group

Figure 3: Sphincter and gallbladder tonus of patients in the control group.