The Prophylactic and Therapeutic Effects of Cholinolytics on Perfluoroisobutylene Inhalation Induced Acute Lung Injury

Tianhong ZHANG1, Xigang ZHANG2, Zhuhua SHAO3, Rigao DING1, Shunjiang YANG3, Jinxiu RUAN1, Xiaohong SUN1, Jin XU3, Chunqian HUANG1, Zuliang Hu3 and Xianchang ZHANG1

1Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, 2307 Hospital and 3Quhua Hospital, Quhua Group Corporation, P. R. China

Abstract: The Prophylactic and Therapeutic Effects of Cholinolytics on Perfluoroisobutylene Inhalation Induced Acute Lung Injury: Tianhong ZHANG, et al. Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, P. R. China—Perfluoroisobutylene (PFIB) is a kind of fluoro-olefin that is ten times more toxic than phosgene. The mechanisms of the acute lung injury (ALI) induced by PFIB inhalation remain unclear. To find possible pharmacological interventions, mice and rats were exposed to PFIB, and the prophylactic or therapeutic effects of 3-quinuclidinyl benzilate (QNB) and anisodamine were studied and confirmed. It was observed that the wet lung/body weight and the dry lung/body weight ratios at 24 h after PFIB exposure (130 mg/m^3 for 5 min) were significantly decreased when a single dose of QNB (5 mg/kg) was administered intraperitoneally either 30 min before exposure or 10 h after exposure. Anisodamine was without any prophylactic or therapeutic effects at single doses below 30 mg/kg. The effects of QNB against PFIB inhalation induced ALI were well evidenced by the significantly decreased mice mortality at 72 h, the total protein concentration in bronchoalveolar lavage fluid at 24 h after the PFIB exposure, as well as the ultrastructural observations. The analysis of the time courses of lung sulfhydryl concentration, myeloperoxidase (MPO) activity and hemorheology assay showed that the toxicity of PFIB may be due to consumption of lung protein sulfhydryl, influx of polymorphonuclear leukocytes (PMNs) into the lung, and increased peripheral blood viscosity at a low shear rate, all of which were partially blocked by QNB intervention except for PMN influx. The results suggest that cholinolytics might have prophylactic and therapeutic roles in PFIB inhalation induced ALI.

Key words: Perfluoroisobutylene (PFIB), Acute lung injury (ALI), Cholinolytics, 3-Quinuclidinyl Benzilate (QNB), Anisodamine

Perfluoroisobutylene (PFIB), a colorless gas at normal temperature, is a kind of fluoro-olefin that is ten times more toxic than phosgene. It is usually generated as a by-product during the manufacture or the pyrolysis of polytetrafluoroethylene (PTFE), which is widely known by its trade name Teflon. Accidental inhalation can cause severe pulmonary edema, and even death because of pulmonary congestion. It is a notorious hazard to human beings in chemical industry accidents, fires and other emergencies. Although diverse mechanisms, such as hydrofluoric acid hypothesis, nucleophilic reaction and reactive intermediate species are supposed to be involved in PFIB inhalation induced acute lung injury (ALI), there is still a long way to go to fully clarify the underlying toxic mechanisms of the ALI induced by PFIB inhalation. Furthermore, there is no recognized prophylactic measures for human PFIB exposure although animal studies have indicated that increased pulmonary concentrations of free radical scavengers containing thiol groups may be of value and N-acetylcysteine has been found to be effective. Early administration of corticosteroids, as used for phosgene, seems to be the only clinical recommendation for pharmacological intervention even though proof of their beneficial effects is still lacking in general.

When comparisons are made concerning the molecular structure, the time course of ALI after the inhalation exposure and many other toxicological characteristics between PFIB and phosgene, it seems
Anisodamine administered both before and after PFIB (QNB), the most potent cholinolytic available, and ALI. Accordingly, the effects of 3-quinuclidinyl benzilate and therapeutic value against PFIB inhalation induced cholinolytics used properly might have some prophylactic pharmacological intervention, we hypothesized, that ALI and seek solutions for successful medical or endeavor to throw some light on the complex process of treatment of many diseases including ALI32, 33). In an cholinergic effects, and is widely used clinically in the lysosomal membranes30) and inhibition of the metabolism on ALI experimentally.

have all shown some prophylactic or therapeutic effects into the lung27), and other pharmacological interventions28) to PFIB or phosgene exposure. In fact, the antibody of theoretically, would impede or reverse the toxicity due effectively counteract the above-mentioned factors, Any medical or pharmacological measure that can mediated by a later signal cascade network, are the two key aspects of PFIB inhalation induced ALI. This is because (1) the halogen and p-n electron conjugation in the molecular structure of PFIB make it possess a much stronger electropolytic, allowing it to attack much more easily the nucleophile groups of thiol (-SH), amino (-NH2) and hydroxy (-OH), distributed widely in the alveolar membrane (Fig. 1), which are crucial to the maintenance of the cellular structure and function; and (2) as direct injury develops, a highly complex network is initiated and established, resulting in an overshoot in inflammation5) followed by severe lung damage, fulminating pulmonary edema and even death. Multiple mediators such as TNF-α16), IL-1β17), IL-818), metabolites of arachidonic acid19), adhesion molecule 20), reactive oxygen species21), matrix metalloproteinases22), neutrophil elastase23) and many other factors24) are thought to be involved in the latter mechanism. Any medical or pharmacological measure that can effectively counteract the above-mentioned factors, theoretically, would impede or reverse the toxicity due to PFIB or phosgene exposure. In fact, the antibody of TNF-α25), the COX, inhibitor ibuprofen26), SOD induction into the lung27), and other pharmacological interventions28) have all shown some prophylactic or therapeutic effects on ALI experimentally.

Anisodamine, a typical cholinolytic extract from a Chinese herb, has been reported to possess effects of microcirculation improvement29), stabilization of lysosomal membranes30) and inhibition of the metabolism of arachidonic acid31) along with its classical anti-cholinergic effects, and is widely used clinically in the treatment of many diseases including ALI32, 33). In an endeavor to throw some light on the complex process of ALI and seek solutions for successful medical or pharmacological intervention, we hypothesized, that cholinolytics used properly might have some prophylactic and therapeutic value against PFIB inhalation induced ALI. Accordingly, the effects of 3-quinuclidinyl benzilate (QNB), the most potent cholinolytic available, and anisodamine administered both before and after PFIB exposure were studied.

Materials and Methods

1. General

1.1. Animals

Specific pathogen-free male Wistar rats (260–300 g b.w.) and specific pathogen-free male mice (18–22 g b.w.) were used in this study. All animals were obtained from the Center of Medical Experimental Animals, the Academy of Military Medical Sciences (Beijing, P. R. China). The animals were housed in quiet, humidified, clean rooms with a light-cycle of 12 h/12 h for 1 week before use. They were fed with laboratory pellet food and tap water ad libitum except when they were in the exposure chamber. All the animal experiments were performed in accordance with the Guidelines for Animal Experiments at the Chinese Academy of Medical Sciences, Beijing, P. R. China.

1.2. Doses of PFIB for animal exposure

PFIB was obtained from the Shanghai Institute of Organic Fluorine Materials at a purity of 98%. A flow-past whole-body exposure apparatus was used to expose small animals to PFIB, as described in our earlier paper30). Except for the mortality study, PFIB inhalation doses were 130 mg/m³ × 5 min and 140 mg/m³ × 8 min for mice and rats, respectively, and were chosen for the convenient observation of the pathophysiological changes without fatal consequences.

2. Experimental Design

Test animals were generally divided into four groups. In groups 1) normal/saline and 2) normal/test drug, animals experienced the same exposure procedure with only pure air circulated in the chamber, and were treated with either saline or the test drug. In groups 3) PFIB/saline and 4) PFIB/test drug, animals were exposed to PFIB in the same chamber, and administered either saline or the test drug.

In the first part of the present study, comparisons of the effects of anisodamine and QNB (i.p.) on pulmonary edema induced by PFIB inhalation were made by the weighing method; that is, the wet lung/body weight and the dry lung/body weight ratios were calculated. To obtain consistent data, the effects were expressed as relative values of both ratios, with the averages of PFIB/saline groups at the same exposure were regarded as 100%, i.e., (PFIB/test drug) / (PFIB/saline). The difference between a prophylactic and a therapeutic effect rest with the administration of the test drug 30 min before or 10 h after the PFIB exposure. The reason for the selection of the treatment time-point 10 h after PFIB exposure, was in consideration of the results from the time-course studies on the edema development pattern in our earlier study30), which showed edema occurs after a latent period (8–12 h), as well as the experience of medical doctors that most PFIB intoxicated patients usually are unconcerned in the
asymptomatic period, and only when obvious symptoms occur, such as cough, asthma and suffocation, do they come to a hospital and seek a doctor’s help.

In the second part of the study, in which QNB was used as a typical representative of the cholinolytics, a detailed study of QNB’s beneficial effects against PFIB induced ALI was made by the observations of the changes in mice mortality at 72 h after an over-LC50 dose of PFIB (190 mg/m³ x 5 min) exposure, the total protein concentration in bronchoalveolar lavage fluid (BALF) at 24 h after a sublethal dose of PFIB exposure, the time course of lung sulfhydryl concentration, myeloperoxidase (MPO) activity, hemorheological assay, as well as ultrastructural studies.

3. Experimental methods


At 24 h after PFIB exposure, mice were anesthetized by an intraperitoneal injection of pentobarbital (50 µg/g) and exsanguinated via abdominal aorta transection. The tracheae and lungs were then excised en bloc and cleared of all extrapulmonary tissue. Total lung wet weight was determined and the wet lung to body weight ratio was calculated. After drying at 80°C for over 24 h and weighing, the dry lung to body weight ratio was calculated.

3.2. Bronchoalveolar lavage fluid (BALF) collection and its total protein concentration assay

At 24 h after PFIB exposure, mice were anesthetized and exsanguinated, and tracheae were exposed. Each animal’s trachea was cannulated with a blunt needle secured with a silk ligature. BALF collection was performed with 0.6 ml PBS that was infused and aspirated into each lung 3 times. The lavage fluid was recovered (average fluid recovery was 0.45 ml), and centrifuged at 1,000 g, 4°C, for 10 min. The supernatants were removed and stored at –70°C until the total protein concentrations were determined by the method of Lowry et al. (1951).

3.3. Lung myeloperoxidase activity assay

At 2, 4, 8, 16 and 24 h after PFIB exposure, six mice in each group were sacrificed, and lungs isolated from the mice were rinsed with saline and blotted dry. After weighing, lung tissue was homogenized in a 1.2 ml 20 mmol/l potassium phosphate buffer, pH 7.4, and centrifuged for 30 min at 30,000 x g, at 4°C. The pellet was resuspended in 1.2 ml 50 mmol/l potassium phosphate buffer, pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide (HTAB). The resuspended pellets were frozen at –70°C until the MPO activity assay was performed. Frozen samples were thawed, sonicated, incubated in a 60°C water bath for 2 h, and centrifuged at the same condition. Supernatant, 0.1 ml, was added to 2.9 ml of 50 mmol/l potassium phosphate buffer, pH 6.0, containing 0.167 mg/ml o-dianisidine and 0.0005% hydrogen peroxide, and the absorbance of 460 nm visible light was measured for 3 min. Myeloperoxidase activity per gram wet lung was calculated as follows: MPO activity (µg lung)= ΔA × 4.05/lung weight (g). (ΔA=rate of change in absorbance at 460 nm between 1 and 3 min)

3.4. Lung sulfhydryl compounds concentrations assay

Six mice in each group were sacrificed at 2, 4, 8, 16 and 24 h after PFIB exposure, and the isolated mice lungs were frozen at –70°C. After thawing, the lung homogenates were prepared with saline in an ice bath. Sulfhydryl compounds including total sulfhydryl (TSH), nonprotein sulfhydryl (NPSH) and protein sulfhydryl (PSH) were assayed according to Sedlak (1956) using Ellman’s reagent, and a calibration curve using GSH was prepared.

3.5. Peripheral blood hemorheological studies

At 2, 8 and 16 h after PFIB exposure, six rats in each group were anesthetized. Blood was drawn from the rat carotid artery into tubes containing anticoagulant K2EDTA. Blood cells counts were performed by routine laboratory procedures. Blood viscosity was measured at 37°C with a coaxial cylinder microviscometer (BV-100, China) at a shear rate of 1 s⁻¹, followed by plasma viscosity determination after centrifugation.

3.6. Ultrastructural studies

At 12 h after PFIB exposure, a mouse was sacrificed and the thorax was opened. The lung was rapidly removed and immersed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, which had been cooled to 4°C to fix overnight. Paramedian transverse slice, 3-mm thick, from the lower left lobe were removed and further diced into 1-mm cubes. Subsequent postfixation, dehydration, infiltration, polymerization and the like procedures were as described in our earlier paper (1990).

4. Statistical analysis

Data are shown as the mean ± SD unless particularly indicated. Student’s t-test or one-way analysis of variance followed by Dunnett’s test were used to detect differences between groups. Mortality data were compared by the Chi-square test.

Results

1. Comparison of anisodamine and QNB on PFIB-induced changes of wet lung/body weight and dry/body weight ratios in mice

It was observed that the wet lung/body weight and the dry lung/body weight ratios at 24 h after the PFIB exposure were significantly decreased when a single dose of QNB (1 mg/kg) was administered intraperitoneally 30 min before the PFIB (130 mg/m³ for 5 min) exposure, but no significant changes were observed when the same dosage of QNB was given 1 h after the PFIB exposure. When the QNB dosage was increased to 5 mg/kg, however, it was found that both ratios significantly decreased by administration either before or after the PFIB inhalation intoxication, and the most exceptional
result was that the above ratios decreased to the levels of the normal control group when QNB was administered twice, both before and after the PFIB inhalation (Fig. 2C, 2D). Anisodamine, the less potent cholinolytic, was without any prophylactic or therapeutic effects at single doses below 30 mg/kg (Fig. 2A, 2B).

2. Effects of QNB administration on PFIB-induced changes in mortality and BALF total protein concentration in mice

Table 1 shows the effect of QNB (5 mg/kg i.p.) on mouse mortality after 5 min exposure to PFIB (190 mg/m^3). After 3 d of observation, no mice died or showed any signs of illness in the normal control or normal/QNB groups. In contrast, mortality was dramatically elevated in the PFIB group, while QNB, whether administered 30 min before or 10 h after the PFIB exposure, significantly decreased the mortality (p<0.05).

In mice at 24 h after the inhalation of PFIB (130 mg/m^3) for 5 min, a significantly high protein concentration in BALF was observed, which was effectively reversed by QNB (5 mg/kg i.p.) either given 30 min before or 10 h...
Tianhong ZHANG, et al.: Intervention of PFIB-Induced ALI by Cholinolytics

3. Effects of QNB administration on PFIB-induced changes in lung MPO activity, sulfhydryl compounds concentrations and peripheral blood hemorheology

Inhalation of PFIB (130 mg/m³) for 5 min led to a slow increase in the lung MPO activity which peaked at 16 h after the exposure. Although there was some decrease in lung MPO activity after QNB pretreatment (5 mg/kg i.p.), it was not statistically significant. Interestingly, however, there were significant increases in the normal/QNB group at 4 h, and the PFIB/QNB group at 2 h after PFIB exposure (Table 2).

In general QNB (5 mg/kg i.p.) itself had no positive or negative effects on the lung sulfhydryl concentration. A marked depletion in PSH and TSH of lung tissue (p<0.05) was found at 8 and 16 h in the PFIB group, which was partly blocked by QNB (5 mg/kg i.p.) pretreatment. In contrast, no change in the NPSH concentration was found.

### Table 2. Effects of QNB (5 mg/kg i.p. at 30 min before exposure) on PFIB (130 mg/m³ for 5 min) inhalation-induced changes in mice lung MPO activity (µg)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/QNB</td>
<td>1.84 ± 0.52</td>
<td>2.52 ± 0.56*</td>
<td>1.86 ± 0.24</td>
<td>1.17 ± 0.60</td>
<td>1.20 ± 0.64</td>
</tr>
<tr>
<td>PFIB/saline</td>
<td>0.72 ± 0.30</td>
<td>1.28 ± 0.87</td>
<td>1.40 ± 0.21</td>
<td>6.72 ± 5.10*</td>
<td>5.31 ± 3.65*</td>
</tr>
<tr>
<td>PFIB/QNB</td>
<td>2.79 ± 1.54*</td>
<td>2.11 ± 1.37</td>
<td>1.02 ± 0.55</td>
<td>3.74 ± 1.29</td>
<td>3.38 ± 0.36</td>
</tr>
</tbody>
</table>

* p<0.05 versus normal saline group, # p<0.05 versus PFIB group at the same time, n=6.

### Table 3. Effects of QNB (5 mg/kg i.p. at 30 min before exposure) on PFIB (130 mg/m³ for 5 min) inhalation-induced changes in mice lung non-protein sulfhydryl, protein sulfhydryl, and total sulfhydryl concentration (µmol/g)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP-SH (normal: 0.62 ± 0.13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal/QNB</td>
<td>0.76 ± 0.14</td>
<td>0.80 ± 0.08</td>
<td>0.87 ± 0.10</td>
<td>0.90 ± 0.12</td>
<td>0.85 ± 0.11</td>
</tr>
<tr>
<td>PFIB/saline</td>
<td>0.49 ± 0.04</td>
<td>0.53 ± 0.03</td>
<td>0.60 ± 0.12</td>
<td>0.38 ± 0.17</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>PFIB/QNB</td>
<td>0.46 ± 0.07</td>
<td>0.43 ± 0.10</td>
<td>0.51 ± 0.03</td>
<td>0.63 ± 0.20</td>
<td>0.50 ± 0.05</td>
</tr>
</tbody>
</table>

| P-SH (normal: 15.17 ± 0.75) |         |         |         |         |         |
| Normal/QNB | 15.08 ± 1.28 | 15.03 ± 1.82 | 16.12 ± 0.42 | 15.53 ± 0.89 | 17.25 ± 2.75 |
| PFIB/saline | 13.64 ± 1.07 | 14.52 ± 0.33 | 12.32 ± 1.23* | 9.38 ± 1.22* | 13.00 ± 0.50 |
| PFIB/QNB   | 14.42 ± 0.33 | 13.51 ± 1.81 | 14.20 ± 1.38 | 13.15 ± 1.57* | 12.65 ± 1.11 |

| T-SH (normal: 15.79 ± 0.81) |         |         |         |         |         |
| Normal/QNB | 15.85 ± 1.17 | 15.83 ± 1.87 | 16.99 ± 0.45 | 16.43 ± 0.90 | 18.09 ± 2.80 |
| PFIB/saline | 14.13 ± 1.10 | 15.05 ± 0.33 | 12.92 ± 1.31* | 9.76 ± 1.27* | 13.53 ± 0.52 |
| PFIB/QNB   | 14.88 ± 0.33 | 13.94 ± 1.79 | 14.72 ± 1.36 | 13.78 ± 1.76* | 13.15 ± 1.14 |

* p<0.05 versus normal saline group, # p<0.05 versus PFIB group at the same time, n=6.
Compared with the normal group, the blood viscosity at shear rate of 1 s⁻¹ and erythrocyte aggregation were significantly raised in rats in parallel with the severity of pulmonary edema. The hemorheological effects of pretreatment with QNB (5 mg/kg i.p. 30 min before the PFIB exposure) were shown at 2 h post PFIB exposure. On the other hand, hematocrit and plasma viscosity were not altered significantly. Leukopenia also quickly appeared at 2 h after PFIB inhalation. The effect of QNB (5 mg/kg i.p.) pretreatment on leucocyte counts in peripheral blood was a decrease in normal animals and an increase at 16 h post PFIB inhalation (Table 4).

4. Ultrastructure study

No pathological change was found with types I and II pneumocytes and alveolar vasculature in the normal/saline and normal/QNB groups (Fig. 4A, 4B). The lungs of all mice sacrificed at 12 h after exposure to PFIB (130 mg/m³) for 5 min showed severe and generalized disruption and slough, in the form of excessive lysosomes and lamination swelling in type II epithelium cells (Fig. 4C), as well as increased vesicle vacuolation in type I epithelium cells (Fig. 4D). These pathological alterations were noticeably less after QNB (5 mg/kg i.p.) pretreatment, and was characterized by maintenance of the alveolar structure (Fig. 4E), although vacuoles and blebs in type I pneumocytes, and fluid leakage into the alveolar space could still be seen (Fig. 4F).

Discussion

PFIB is a most potent toxic gas that can induce pulmonary edema after inhalation, the main pathological changes of which are a destroyed lung gas-blood barrier and the leakage of protein and water including red blood cells from the circulation system into the alveolar space, as indexed by increased wet lung weight and dry lung weight, as well as high protein concentration in BALF. Cholinolytics are reported to have wide pharmacological effects besides blocking cholinergic receptors. In the current paper we hypothesized that QNB and a high dosage of anisodamine could attenuate pulmonary edema induced by PFIB inhalation and confirmed this by the observation of a significant decrease of wet lung/body weight ratio, dry lung/body weight ratio, total protein concentration in BALF and mortality.
It is speculated that the primary (direct) injury of PFIB inhalation is caused by PFIB binding to nucleophilic groups of amino acid in the alveolar membrane, the large area of alveoli providing support for this interaction. Among the nucleophilic groups of amino acid, sulfhydryl is possibly one of the most sensitive groups to active electrophilic chemicals such as PFIB and phosgene. Additionally, the maintenance of the cytoskeleton, protein molecular activity, and cell metabolism might be related to the regulation of the intracellular sulfhydryl redox state. The current results that PFIB inhalation induced consumption of protein sulfhydryl, which could be partially blocked by QNB administration, clearly supports the above speculation regarding the role of the sulfhydryl redox state, although no change of nonprotein sulfhydryl (e.g. glutathione) was observed and further studies are needed to clarify this exception.

The secondary (indirect) injury of PFIB may be caused by multiple factors including excessive inflammation and blood coagulation abnormality, both of which are involved in the pathogenesis of various kinds of ALI. The lung MPO study and leucocyte counts in peripheral...
blood showed neutrophil sequestration and accumulation in the lung may be one of the key factors in PFIB inhalation-induced ALI.

Blood viscosity is the intrinsic resistance to blood flow in the blood vessels and may a key factor involved in many pathophysiological processes; e.g., shear stress damage at the blood-endothelial interface, facilitation of plasma protein interaction with the endothelium, increased propensity for thrombosis, impaired microcirculatory flow and blood infusion to an organ. Accordingly, the current study took into account that blood viscosity at a low shear rate might affect the lung microcirculation, thus contributing to PFIB inhalation induced ALI. The results showed that PFIB inhalation did elevate the blood viscosity at a low shear rate of 1 s⁻¹ and erythrocyte aggregation with a peak at 16 h after exposure, while no changes were observed for plasma viscosity and hematocrit. Additionally, QNB pretreatment effectively attenuated the blood viscosity increase. The results, thus, suggest that an abnormal blood viscosity is an additional mechanism contributing to PFIB inhalation-induced pulmonary edema.

Evidence is accumulating that secondary injury contributing to ALI/ARDS is a complex cascade of events³⁷, including lung epithelium and endothelium apoptosis and necrosis, and thrombosis and inflammation. So it is unsurprising that QNB could not totally block PFIB toxicity. There still remains a largely unexplored area for gaining further insights into respiratory toxicology of PFIB and to discover novel prophylactic or therapeutic measures.

In conclusion, the current study confirmed that PFIB-induced ALI might be two kinds of injury, direct (e.g., sulfhydryl depletion) and indirect (e.g., PMNs accumulation and impaired hemorheology), some of which could be attenuated by QNB administration. In consideration of the high dose of anisodamine used in our experiment, and the side effects of QNB, being once as a notorious incapacitant³⁸, the significance of their clinical use in treatment of ALI induced by PFIB inhalation needs detailed investigation. However, the prophylactic and therapeutic effects of cholinolitics against PFIB-induced ALI may be of value in indicating a proper direction for the research and development of new drug with few side effects.

References
2) ID Makulova: Clinical picture of acute poisoning with perfluorobutylene. Gig Tr Prof Zabol 9, 20–23 (1965)
15) H Wang, R Ding, J Ruan, B Yuan, X Sun, X Zhang, S Yu and W Qu: Perfluoroisobutylene-Induced Acute Lung Injury and Mortality are Heralded by Neutrophil Sequestration and Accumulation. J Occup Health 43, 331–338 (2001)
19) T Nagase, N Uozumi, T Aoki-Nagase, K Terawaki, S Ishii, T Tomita, H Yamamoto, K Hashizume, Y Ouchi


30) CQ Li, ZH Shao and YL Jing: Influence of anisodamine (654–2) on plasma levels of lipid peroxides in rabbits with SAO shock. Chinese J Pathophysiol 5, 547 (1989)


