SUMMARY
Introduction Chloroform intoxication as a result of inhalation is usually associated with occupational exposure. Fatal cases of accidental and intentional chloroform poisoning are extremely rare. The aim of this work was to report a case with fatal consequences caused by prolonged chloroform inhalation in a child.

Case outline We report a case of a three-month-old child found dead by its mother in their home. Investigation provided the data about two refrigerators in the house, which were not entirely functional, and were filled with a refrigerant based on chloroform, and about a recent house spraying with a pesticide also based on chloroform. Forensic autopsy was performed the following day. Autopsy established the existence of malnutrition, dehydration, brain and lungs edema, the presence of very scarce contents in the digestive tract, and the enlargement of the abdominal lymph nodes. The initial stage of liver degeneration was noted on histopathological examination. Chemical-toxicological analysis of organ tissue samples (brain, lungs, spleen, liver with gall bladder, kidney and bladder, stomach, small and large intestine) performed using the techniques of headspace gas chromatography with mass detector, confirmed chloroform poisoning.

Conclusion The emaciation underlying the dysfunction of organ systems provided a grim frame for a child to succumb to chloroform toxicity. The proper use and maintenance of domestic appliances, and careful application of chemical agents indoors is an imperative, especially in the vicinity of children. Low levels of chloroform inhaled from the room air may have fatal consequences in a susceptible individual.

Keywords: chloroform; intoxication; toxicology; autopsy; forensic pathology

INTRODUCTION
Chloroform (trichloromethane – CHCl₃) is a colorless, volatile liquid, whose potent anesthetic properties were recognized early after its synthesis during the 1830s [1]. However, soon after the first described applications of chloroform as an anesthetic during surgical procedures in clinical context, its acute toxicity was observed, and chloroform was determined to be the cause of death in a number of physically fit patients [2]. In spite of the fatal complications, chloroform continued to be used as a potent anesthetic agent over the next 50 years. Only in 1912, the Committee on Anesthesia of the American Medical Association proclaimed that the use of chloroform as the anesthetic for major surgery was no longer justifiable [3]. The use of chloroform as an anesthetic is abandoned because exposing the organism to high concentrations may cause hypotension and fatal cardiac arrhythmias [4, 5]. Fatal cardiac arrhythmias may be also induced by inhalation abuse of toxic substances such as chloroform. From the 1960s, deaths caused by inhalation of toxic substances have been termed “sudden sniffing death” [6, 7].

 Nowadays, chloroform has a wide application in industry, and is a subject to strict regulations as a hazardous substance. The main use of chloroform is the production of fluorocarbons used in the synthesis of tetrafluoroethylene and polytetrafluoroethylene, as a refrigerant and propellant. It is also used as an organic solvent in industry and in analytical laboratories, as an ingredient of pharmaceuticals, drugs, cosmetics, grain fumigants, dyes, and pesticides [4]. Before 1989 and the Montreal Protocol on Substances that Deplete the Ozone Layer, chloroform was also a popular refrigerant [8].

Acute chloroform poisoning, resulting in respiratory and central nervous system depression, has been described in accidental, suicidal, and homicidal cases [9, 10, 11]. Intentional fatal chloroform intoxications usually concern self-poisoning acts, while homicides by chloroform are very rare. Accidental poisoning more frequently occurs in the setting of occupational exposure, but may also be encountered in a domestic environment [11, 12]. Chronic inhalation of chloroform leads to liver damage, and causes central nervous system depression as well [3, 8].

The aim of this paper was to present a case of a child death by accidental prolonged chloroform poisoning at home, under unusual circumstances.
CASE REPORT

Circumstances of chronic exposure to chloroform inhalation

In the early morning, mother found her three-month-old child dead. She called the police, and a forensic autopsy was ordered. During the investigation, data about underprivileged, multi-member family (four adult persons and six children) who lived in a house with two rooms was obtained. There were two refrigerators in the house, which were not entirely functional, and were filled with a refrigerant based on chloroform. A short period before the relevant event, the house was sprayed with a disinsection agent, a pesticide also based on chloroform. This information was obtained from investigative authorities but the name of the compound used on that occasion was not specified. The child was found on the mattress on the floor, where he slept, in the room with the refrigerators. The event took place in the winter, and because of the poorly ventilated rooms in this period, chloroform poisoning of the infant was suspected.

Autopsy findings

The autopsy was performed on the following day. The three-month-old male child was malnourished, with signs of dehydration. The weight of the body was 3 kg, and the length was 49 cm, which, according to the table of development of infants, corresponds to the age of about a month. The cadaver had reduced muscle and bone mass, with almost complete absence of subcutaneous adipose tissue (Figures 1 and 2). On the skin of the lower part of the back, decubitus wounds were noted. No signs of injury were found on the body of the child. The examination revealed signs of brain and lungs edemas. Inside the digestive tract, only slight amounts of content were found. Abdominal lymph nodes were enlarged. The examination of the bones revealed no fractures or any other remarkable changes. In addition to pulmonary edema, the histopathological analysis demonstrated the hydropic liver degeneration. Cytoplasmic vacuolation, and swelling of the hepatocytes, with a mild architectural disarrangement of the plates, was observed in the liver tissue (Figure 3). The rest of the morphological findings were unremarkable and classified as normal.

Chemical-toxicological analysis of internal organ samples (brain, lungs, spleen, liver with gall bladder, kidney and bladder, stomach, small and large intestine) showed the presence of chloroform. We performed chemical-toxicological analysis using the techniques head space gas chromatography with flame ionization detection (HS-GC/FID) and head space gas chromatography mass spectrometry (HS-GC/MS).

The following chemicals were used in the process: chloroform and isopropanol, GC purity (J. T. Baker, Mallinckrodt, Netherlands).

The basic standard chloroform solution was prepared at a concentration of 1 mg/mL in methanol. A series of dilutions for calibration curve, in the range of 1–50 mg/L, was prepared out of the basic solution. Isopropanol was used as the internal standard at a concentration of 0.5 mg/mL in water. The identification was done by comparing mass sample spectra with mass spectra databases Willy 7 and Pfleger Maurer Weber, and the quantification was done by the internal standard method.

Conditions for gas chromatographic determination were as follows:
levels already peak within a few minutes after exposure and lasts 1 minute; the temperature is then raised to 120°C, at a rate of 50°C/minute; detector FID 260°C; carrier gas He, 30 mL/minute flow, hydrogen, 40 mL/minute flow, air 400 mL/minute flow. HS-GC/MS, Shimadzu QP 2010, Ultra, Autosampler AOC-5000; GC column: InterCap 624 (30 m × 0.25 mm i.d., film thickness 3 μm). The temperature program starts at 40°C, and lasts 1 minute; the temperature is then raised to 120°C, at a rate of 50°C/minute; detector FID 260°C; carrier gas He, 30 mL/minute flow, hydrogen, 40 mL/minute flow, air 400 mL/minute flow.

**DISCUSSION**

In the case of acute chloroform poisoning, sings as edematous swelling of the lips, focal mucositis of the facial skin and the chloroform-soaked soft covering of the airways could be very useful in determination of the cause of death [12]. In chronic poisoning, there are no previous nor indicative signs.

In case of exposure to poison in the gaseous state, blood levels already peak within a few minutes after exposure because of the extensive capillary surface area of the lungs. Because of their lipid solubility, volatiles, including chloroform, quickly cross lipid membranes and distribute to well-perfused organs such as the brain, liver, heart, and kidneys. This condition is retained if sudden death occurs, but if exposure is prolonged, toxic substance will slowly also accumulate in poorly perfused parts, such as muscles and fat tissue [13].

In fatal chloroform poisoning cases, its concentration ranges were 10–48 μg/ml in the blood, 50.4–156 μg/g in the brain, 16–27 μg/g in the kidney, 6–86.2 μg/g in the liver, and 0–60 μg/ml in urine [14]. In the case reported herein, only the concentration of chloroform in the brain (27.64 mg/kg) was below the fatal level.

Non-fatal chronic inhalation of chloroform is usually associated with features of hepatic damage that develop 2–5 days after exposure [15, 16, 17]. These pathological changes are similar to those noted in cases of the prolonged anesthesia. Signs of hepatic injury have been reported after occupational industrial exposure, up to workplace air chloroform concentrations of 205 ppm (1,005 mg/m³) [18]. Pathologic changes that may be observed in the liver tissue probably reflect the major role of hepatocytes and cytochrome P-450 enzymes in chloroform metabolism. Depending on the concentration and the quantity of oxygen, chloroform can be metabolized in the body by oxidative or reductive processes. The oxidative metabolism of chloroform, which is more commonly encountered, creates phosphene by CYP2E1 (high affinity – low capacity enzyme), while the reductive metabolism produces a dichloromethyl free radical. Both metabolic products of chloroform are highly cytotoxic and, due to their liposolubility, they rapidly penetrate into cells causing necrosis primarily of liver and kidney cells and tissue [5, 19, 20, 21]. *A priori,* it might be expected that the oxidative pathway of chloroform metabolism would predominate in *vivo*, because tissues of healthy individuals are oxygenated. Moreover, the toxic...
Fatal chloroform inhalation in a child

do not have the data on gas analysis and chloroform level in the air in the room where the child was found dead.

The hepatic damage as an effect of chloroform toxicity occurs more frequently in patients with predisposing factors, such as hypoxia, hypercapnia, dehydration, acidosis, and alcoholism [1, 3]. During the external and internal examination of the body of the child, signs of severe dehydration and malnutrition were registered, which were certainly a predisposing factor for faster appearance of toxic effects of chloroform. The emaciation underlying the dysfunction of organ systems provided a grim frame for a child to succumb to chloroform toxicity.

In the context of mental function disorders that occur during chronic chloroform poisoning, a wide range and different degrees of disturbances have been described, from a lack of concentration to the most severe ones, such as stupor and coma [1, 15]. It is possible that the child showed some signs of passivity, but the parents failed to correctly recognize them.

In recent times, chloroform intoxication has been mostly limited to professional exposure, and it has been mainly related to the period prior to the adoption of regulations on ventilation of the working area. This accidental chronic chloroform poisoning implies that the use of products containing even small amounts of chloroform in the household can have fatal consequences. The proper use and maintenance of domestic appliances, and careful application of chemical agents indoors, is imperative.

**Conflict of interest:** None declared.

## REFERENCES

11. Harada K, Ichiyama T, Ikeda H, Ishiara T, Yoshida K. An autopsy case of acute chloroform intoxication after intermittent inhalation for malnutrition [23]. In the present case, unlike the foregoing, as a result of prolonged chloroform intoxication, the microscopic examination showed the hydroptic liver degeneration with a mild architectural disarrangement of the plates. This suggests that liver changes were more likely to be caused by prolonged chloroform poisoning (Figure 3).

The circumstances recorded at the scene of death suggest that the deceased infant might have been constantly exposed to chloroform inhalation in its home. The age of the infant, the cold in the winter months when death occurred, chloroform concentrations in tissue samples, and the findings of skin decubitus on the lower part of the back point to the conclusion that the child stayed for a prolonged period of time where it was found dead. However, other family members, including the older children in the presented case, showed no signs of intoxication and did not complain of any health problems. The fact is that they went out of the house every day, which was the way of detoxification for them, thus their exposure was not constant. During the investigating procedure, the sample of the air from the family house had not been taken; therefore, we
Фатални исход изазван пролонгираним тровањем детета хлороформом

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САЖЕТАК
Увод Тровање услед удисања хлороформа је обично повезано са професионалним излагањем хлороформу. Смртни случајеви задесног и намерног тровања хлороформом су веома ретки. Циљ овог рада је био да прикаже случај тровања детета хлороформом са фаталним исходом.

Приказ случаја Тромесечно дете је мајка пронашла мртво у кући. Истрагом је утврђено да су у кући постојала два непотпуно исправна фрижидера са расхладним гасом на бази хлороформа и да је у кући недавно коришћен пестицидни спреј на бази хлороформа. Судско-медицинска обдукција је извршена наредног дана и утврђено је постојање потхранености, дехидратације, отока мозга и плућа, присуства веома оскудног садржаја у дигестивном тракту и увећања трбушних лимфних чворова. Хистопатолошким прегледом нађен је почетни степен дегенерације јетре. Хемијско-токсиколошка анализа узорака органа (мозак, плућа, слезина, јетра са жучном кесом, бубрег са бешиком, желудац, танко и дебело црево), применом технике гасне хроматографије са масеним детектором, потврдила је тровање хлороформом.

Закључак Исцрпљеност и дисфункционалност система органа утицали су на околности под којима је дете подлежео токсичном ефекту хлороформа. Неопходни су адекватна употреба и одржавање кућних апарата и пажљива употреба хемијских супстанци у затвореном простору, нарочито у близини деце. Ниске концентрације хлороформа удахнутог из ваздуха у просторији могу бити фаталне код осетљивих појединца.

Кључне речи: хлороформ; тровање; токсикологија; обдукција; форензичка патологија