Diagnostics of fatal poisoning with 4-hydroxybutyric acid, ethanol and cannabinoids

Marek Wiergowski1,*, Jacek Sein Anand2,3, Karol Karnecki1, Ireneusz Sołtyszewski4, Zbigniew Jankowski1

Abstract: This work describes a case of acute mixed poisoning with 4-hydroxybutyric acid (GHB) and ethanol with a fatal outcome, as well as the methodology of chemical toxicological analyses of ante-mortem and post-mortem biological material in the form of blood and urine samples, as well as an exhibit: a syringe with transparent liquid.

In the course of chemical toxicological analyses of samples of biological material, toxic and fatal concentrations of the psychotropic substance GHB were detected and determined in urine. The presence of ethyl alcohol in samples of blood serum (0.60 per mille) and urine (0.74 per mille) collected from a man at 09:08 a.m. on the day of his poisoning in December 2014 indicated a possible strong combined depressive effect of GHB and ethanol on the central nervous system and the respiratory system.

The analyses additionally detected psychoactive delta-9-tetrahydrocannabinol (THC) and its inactive metabolite, 11-nor-9-carboxy-delta 9-tetrahydrocannabinol acid (THCCOOH), in concentrations indicating previous ingestion of hashish or marijuana, remaining without any significant impact on the cause of death. The transparent liquid from the syringe contained the psychotropic substance GHB, which was identical with the GHB detected in the ante-mortem biological material.

Key Words: fatal poisoning, 4-hydroxybutyric acid, ethanol, delta-9-tetrahydrocannabinol, 11-nor-9-carboxy-delta 9-tetrahydrocannabinol acid, gas chromatography, mass spectrometry.

The most often used psychoactive substances facilitating the commission of crime include: ethyl alcohol, γ-hydroxybutyric acid (GHB), benzodiazepines (such as flunitrazepam, alprazolam, and midazolam), and anaesthetics, mainly ketamine. Other, less often used substances, include 3,4-methylenedioxymethamphetamine (MDMA), marijuana, phencyclidine (PCP), opiates, hallucinogens, derivatives of barbituric acid, hypnotics such as zolpidem, antihistamines, tricyclic antidepressants, clonidine, and yohimbine.

This work describes a case of acute poisoning with GHB, ethanol and cannabinoids with a fatal outcome.

CASE DESCRIPTION

In December 2014, at about 04:10 AM, physicians from emergency medical services were providing medical assistance to a woman and two men who, as became known from an interview, ingested an unknown amount of GHB for recreational purposes. The woman and one of the men left the hospital shortly after being provided with medical assistance in the hospital emergency department. During an interview concerning their medical history, both of them reported that in contrast to their friend,
they self-induced vomiting soon after ingesting GHB, fearing its toxic effects. The man who ingested the entire dose of the substance was admitted to the intensive care unit (ICU) after two attempts at cardiopulmonary resuscitation on the street.

The hospital treatment of the person fatally poisoned with GHB, ethanol, and cannabinoids is presented in Table 1.

According to the medico-legal report drawn up after the inspection and dissection of the corpse, the man's death was caused by a diffuse, acute, ischaemic brain injury with oedema, complicated by intracranial hypertension and herniation of the cerebellar tonsils into the foramen magnum.

Chemical toxicological analyses were carried out to discover whether any intoxicating substances, including GHB, could have been the immediate cause of the man's death, and whether or not GHB was present in the exhibit submitted for analyses.

In the course of the investigation it became necessary to find answers to several important questions, including: Could the concentration of GHB in the dead man's urine have been the only cause of his death? Did the patient die due to the effect of GHB only, or as a result of an interaction of this xenobiotic with the ethyl alcohol and THC which he had ingested earlier? Can vomiting, induced immediately after the ingestion of a substance containing GHB, protect a person exposed to risk against the negative consequences of such ingestion?

**MATERIAL AND METHODS**

Samples of biological material collected and secured during an autopsy of the patient's body, including his cerebrospinal fluid, urine, and specimens of the stomach, the small intestine, liver with bile, and brain were submitted for analyses.

Samples of materials collected from the patient when he was still alive, during his hospital treatment (urine sample secured on the first day of hospitalization at 09:08 AM and a blood sample collected at the hospital – no date or time recorded) were also submitted.

A 10 ml syringe containing about 2 ml of transparent fluid, in which GHB was supposed to be present, was also submitted.

In order to detect intoxicating and psychotropic substances, the ante-mortem urine sample and the post-mortem urine sample were analysed using an immunological screening method with the help of the BIO-RAD “TOX/See” tests for detecting benzodiazepines, opiates, amphetamine and methamphetamine and their analogues, cannabinoids, and cocaine and its metabolites. Further tests were carried out in order to confirm the presence of THC in ante-mortem blood and urine samples. For the above purposes, 0.5 mL blood and urine samples were collected simultaneously, and after an internal standard – isotopically labelled Δ⁹-tetrahydrocannabinol-D₉ (THC-D₉) – was added to them, they were subjected to liquid-liquid extraction. The resulting extracts were analysed by gas chromatography using an Agilent Technologies GC-7890A/MS-5975C gas chromatograph combined with a mass spectrometer (GC/MS) in the selected ion monitoring mode (SIM) using negative chemical ionisation (CI). At the same time, after adding the internal standard, a standard solution of derivatised THC was analysed. Tests were continued to confirm the presence of the main THC metabolite in the form of THC-COOH in ante-mortem blood and urine samples. In order to check the above, blood and urine samples, each of 2.0 mL, were collected simultaneously, and after an internal standard – isotopically labelled 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxy-D₉ acid (THC-COOH-D₉) – was added to them, they were subjected to liquid-liquid extraction. The obtained extracts were analysed by gas chromatography using the same GC-7890A/MS-5975C chromatograph in the selected ion monitoring mode (SIM) using electron ionisation (EI). At the same time, after the internal standard was added, a standard solution of derivatised THC COOH was analysed.

In order to detect ethyl alcohol and determine its content, an ante-mortem blood sample (no date or time of collection recorded) and an ante-mortem urine sample (collected at 09:08 AM on the first day of treatment) were also submitted.

**Table 1. Hospital treatment of a person fatally poisoned with GHB, ethanol, and cannabinoids**

<table>
<thead>
<tr>
<th>Day of treatment</th>
<th>Treatment description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>After several hours of treatment in ICU, the patient displayed muscle jerks, tremor with bending movements of his upper limbs, and tonic spasms. In view of difficulties with the effective management of his tremor, the patient was put in a medically-induced coma with the help of Thiopental. Hyperthermia was also recorded from the evening hours. Toxicological analysis carried out at 09:08 a.m. detected 0.60 per mille (g/l) of ethanol in blood serum. A sample of urine collected at 09:07 a.m. on the same day was positive for marijuana and negative for cocaine, amphetamine, methamphetamine, and opiates. The patient was still deeply unconscious and displayed signs of intravital brain-stem death. The presence of barbiturates (1.85 µg/ml, where the analytical sensitivity of the method was 0.03 µg/ml) was detected in a blood serum sample collected from the patient on the 4th day. The dosage of Thiopental was gradually reduced, and finally stopped in the morning hours on the third day of treatment.</td>
</tr>
<tr>
<td>2-4</td>
<td>At 02:45 a.m. the adjudication committee pronounced brain death, and at 02:00 p.m. the patient's organs (heart and kidneys) were recovered for transplantation.</td>
</tr>
<tr>
<td>5</td>
<td>After several hours of treatment in ICU, the patient displayed muscle jerks, tremor with bending movements of his upper limbs, and tonic spasms. In view of difficulties with the effective management of his tremor, the patient was put in a medically-induced coma with the help of Thiopental. Hyperthermia was also recorded from the evening hours. Toxicological analysis carried out at 09:08 a.m. detected 0.60 per mille (g/l) of ethanol in blood serum. A sample of urine collected at 09:07 a.m. on the same day was positive for marijuana and negative for cocaine, amphetamine, methamphetamine, and opiates. The patient was still deeply unconscious and displayed signs of intravital brain-stem death. The presence of barbiturates (1.85 µg/ml, where the analytical sensitivity of the method was 0.03 µg/ml) was detected in a blood serum sample collected from the patient on the 4th day. The dosage of Thiopental was gradually reduced, and finally stopped in the morning hours on the third day of treatment.</td>
</tr>
<tr>
<td></td>
<td>At 02:45 a.m. the adjudication committee pronounced brain death, and at 02:00 p.m. the patient's organs (heart and kidneys) were recovered for transplantation.</td>
</tr>
</tbody>
</table>
of hospitalization) were analysed by headspace gas chromatography with a flame ionization detector HS-GC/FID using a gas chromatograph "Focus GC" and ThermoFinnigan "TriPlus" headspace autosampler in compliance with an internal testing procedure, PB-01, entitled "Determination of ethanol in human biological material". At the same time, the following standards were analysed: acetone, methanol, ethanol, propanol, and butanols.

In order to broaden the range of the psychoactive substances sought in ante-mortem blood and urine samples, liquid-liquid extraction on diatomaceous earth was performed. After collecting 14 mL of blood and 2.4 mL of urine, 25% HCl solution was used to obtain pH of 2-3, and the samples were subjected to extraction with diethyl ether. The solution was then made alkaline with NH₄Cl/NH₃ (to reach pH of 8.5-9.5) and subjected to extraction with dichloromethane–isopropanol mixture (85+15) on Merck Extrelut columns with diatomite. After evaporation of the solvents, the solid residue was dissolved in 1 mL of methanol (blood extract) and 0.5 mL of methanol (urine extract). 0.5 mL of the transparent liquid from the syringe (the exhibit) was also collected and subjected to mechanical extraction (vortex mixer) with 0.5 mL of 1 chlorobutane. Instrumental analyses of extracts of the above biological materials and the exhibit material were conducted with the help of the following chromatography techniques:

HPLC-UV-DAD liquid chromatography using a Shimadzu “LC10Avp” liquid chromatograph. Gradient elution involved a monolithic column filled with silica gel modified with octadecyl phase, mobile phase with an aqueous solution of phosphoric acid and acetonitrile, UV detection for wavelengths 220 nm and 256 nm, and for DAD detector from 190 nm to 370 nm. The obtained results of chromatographic analysis were compared to the spectral library "UV Spectra of Toxic Compounds" containing 2,682 UV spectra of toxic and pharmacologically active substances and their metabolites, developed by Pragt et al.

GC/MS-SCAN gas chromatography using a ThermoFinnigan “DSQ Trace” GC/MS gas chromatograph with mass spectrometry, working in the following conditions: Phenomenex Zebron ZB-5MS (30 m x 0.25 mm x 0.25 μm) capillary column, sampler temperature: 250°C, programmed temperature of the chromatograph: 50°C (1 min), from 50°C to 200°C at 20°C/min, from 200°C to 240°C at 10°C/min, 240°C (1 min), carrier gas: helium, in the fragment ions scan mode (SCAN) from 35 to 380 m/z. The obtained results of chromatography analysis were compared to the mass spectral libraries NIST/EPA/NIH (2008 edition), Cayman Spectral Library (2014) and SWGDRUG (2014), containing the majority of pharmacologically active substances.

Further analyses were carried out to confirm the presence of γ-hydroxybutyric acid (GHB). 100 µL samples of biological material were collected for extraction and analysed in the GC/MS-SIM pattern using a ThermoFinnigan “DSQ Trace” gas chromatograph in compliance with the procedure developed on the basis of works by LeBeau et al. [1], and Joghansen et al. [2]. 100 µL samples of biological material and, simultaneously, blank samples and standard GHB solution (Fig. 1), were collected for analyses. 5 µL of GHB-D6 internal standard solution (0.1 mg/mL) was added to each sample and mixed with a vortex mixer. The samples were extracted with 200 µL of methanol, and they were then mixed for 0.5 min with the vortex mixer and centrifuged for 5 min (10,000 rpm). Supernatant was transferred to 5 mL glass test tubes, and the remaining content was mixed with the help of a glass stirring rod, and extracted once again. In the case of the analysis of urine samples, after the internal standard solution was added, about 1 g of anhydrous sodium sulphate (VI) was added to bind excess water, and the samples were extracted with two 400 µL batches of methanol. The entire supernatant collected was evaporated to solid residue under a gentle stream of nitrogen at 50 °C. The solid residue was derivatised by adding 50 µL of BSTFA:TMCS (99:1), and mixing energetically with the vortex mixer, after which 50 µL of acetonitrile was added and the content was mixed again. Samples were incubated at 75°C for 30 min. 2 µL of each of the extracts were batched to the GC/MS-SIM system. In the course of the instrumental analysis, the mass of ions characteristic for GHB (m/z: 233, 117, 147) and for GHB-D6 (239, 117, 147) was monitored. Extract samples were analysed using a ThermoFinnigan “DSQ Trace” GC/MS gas chromatograph with mass spectrometry working in the following conditions: Phenomenex Zebron ZB-5MS gas chromatograph with mass spectrometry detection (GC/MS-SCAN), were performed. After collecting 14 mL of blood and 2.4 mL of urine, 25% HCl solution was used to obtain pH of 8.5-9.5 and extracted with a dichloromethane–isopropanol mixture (85+15) on Merck Extrelut columns with diatomite. After evaporation of the solvents, the solid residue was dissolved in 1 mL of methanol (blood extract) and 0.5 mL of methanol (urine extract). 0.5 mL of the transparent liquid from the syringe (the exhibit) was also collected and subjected to mechanical extraction (vortex mixer) with 0.5 mL of 1 chlorobutane. Instrumental analyses of extracts of the above biological materials and the exhibit material were conducted with the help of the following chromatography techniques:

HPLC-UV-DAD liquid chromatography using a Shimadzu “LC10Avp” liquid chromatograph. Gradient elution involved a monolithic column filled with silica gel modified with octadecyl phase, mobile phase with an aqueous solution of phosphoric acid and acetonitrile, UV detection for wavelengths 220 nm and 256 nm, and for DAD detector from 190 nm to 370 nm. The obtained results of chromatographic analysis were compared to the spectral library "UV Spectra of Toxic Compounds" containing 2,682 UV spectra of toxic and pharmacologically active substances and their metabolites, developed by Pragt et al.

GC/MS-SCAN gas chromatography using a ThermoFinnigan “DSQ Trace” GC/MS gas chromatograph with mass spectrometry, working in the following conditions: Phenomenex Zebron ZB-5MS (30 m x 0.25 mm x 0.25 μm) capillary column, sampler temperature: 250°C, programmed temperature of the chromatograph: 50°C (1 min), from 50°C to 200°C at 20°C/min, from 200°C to 240°C at 10°C/min, 240°C (1 min), carrier gas: helium, in the fragment ions scan mode (SCAN) from 35 to 380 m/z. The obtained results of chromatography analysis were compared to the mass spectral libraries NIST/EPA/NIH (2008 edition), Cayman Spectral Library (2014) and SWGDRUG (2014), containing the majority of pharmacologically active substances.

Further analyses were carried out to confirm the presence of γ-hydroxybutyric acid (GHB). 100 µL samples of biological material were collected for extraction and analysed in the GC/MS-SIM pattern using a ThermoFinnigan “DSQ Trace” gas chromatograph in compliance with the procedure developed on the basis of works by LeBeau et al. [1], and Joghansen et al. [2]. 100 µL samples of biological material and, simultaneously, blank samples and standard GHB solution (Fig. 1), were collected for analyses. 5 µL of GHB-D6 internal standard solution (0.1 mg/mL) was added to each sample and mixed with a vortex mixer. The samples were extracted with 200 µL of methanol, and they were then mixed for 0.5 min with the vortex mixer and centrifuged for 5 min (10,000 rpm). Supernatant was transferred to 5 mL glass test tubes, and the remaining content was mixed with the help of a glass stirring rod, and extracted once again. In the case of the analysis of urine samples, after the internal standard solution was added, about 1 g of anhydrous sodium sulphate (VI) was added to bind excess water, and the samples were extracted with two 400 µL batches of methanol. The entire supernatant collected was evaporated to solid residue under a gentle stream of nitrogen at 50 °C. The solid residue was derivatised by adding 50 µL of BSTFA:TMCS (99:1), and mixing energetically with the vortex mixer, after which 50 µL of acetonitrile was added and the content was mixed again. Samples were incubated at 75°C for 30 min. 2 µL of each of the extracts were batched to the GC/MS-SIM system. In the course of the instrumental analysis, the mass of ions characteristic for GHB (m/z: 233, 117, 147) and for GHB-D6 (239, 117, 147) was monitored. Extract samples were analysed using a ThermoFinnigan “DSQ Trace” GC/MS gas chromatograph with mass spectrometry working in the following conditions: Phenomenex Zebron ZB-

---

**Figure 1.** Calibration curve with linear approximation prepared for the determination of GHB concentration ranging from 5 μg/mL to 100 μg/mL in relation to the internal GHB-D6 standard in blood and urine samples determined with the help of GC/MS-SIM.
RESULTS

Routine immunological tests of the ante-mortem urine sample collected from the first person revealed the presence of cannabinoids, and analysis of the post-mortem urine sample detected cannabinoids, benzodiazepines and barbiturates. Since the patient was treated with Relanium (diazepam) and Thiopental (thiopental) at the hospital, confirmation of the presence of medicines from the group of benzodiazepines and barbiturates in biological material was discontinued.

The GC/MS analysis of ante-mortem blood and urine samples detected psychoactive GHB in both biological materials in concentrations below the quantitation limit for the method (<1ng/mL), and the presence of THC-COOH in concentrations of 128 ng/mL in blood and 36 ng/mL in urine.

HS-GC/FID analysis detected 0.74 per mille (g/L) of ethyl alcohol in the ante-mortem urine sample collected at 9:08 AM on the first day of hospitalisation, and was negative for ethanol (<0.20 per mille) in the ante-mortem blood sample (time and date of collection were not recorded). In the case of the ante-mortem blood sample, ethyl alcohol in a concentration equal to or greater than 0.20 per mille (g/L) was not detected, and in view of the absence of data on the time and date of the blood sample collection, we opted, for further interpretation of results, the concentration of ethyl alcohol in the blood serum collected at 9:08 AM on the first day of hospitalisation (simultaneously with the urine sample), amounting to 0.60 per mille (about 0.5 per mille of alcohol in whole blood).

Chromatographic HPLC-UV/VIS-DAD and GC/MS-SCAN analyses detected psychoactive GHB both in the ante-mortem urine sample and in the sample of transparent liquid found in the syringe secured for the purposes of the case in question. Additionally, thiopental, which was administered to the patient during his hospital treatment, was detected in the blood sample. The above GC/MS-SIM analyses detected GHB in a concentration of 5 μg/mL in the blood and 3,000 μg/mL in the urine, and confirmed the presence of the substance in the transparent liquid found in the syringe.

DISCUSSION

In the case of a suspected use of psychoactive substances to facilitate the commission of a crime, it is necessary to secure biological material for tests as soon as possible, which is particularly important for GHB, as it is quickly eliminated from the organism.
orally, GHB is absorbed very fast, producing effects as soon as after 15-30 minutes, and reaching the maximum concentration in blood after about 25-40 minutes following ingestion [6, 7]. GHB undergoes fast conversion to γ butyrolactone (GBL) and is excreted in this form. In blood serum the drug practically does not bind to proteins [8]. The half-life of GHB ranges from 20 to 50 minutes, and clearance amounts to 14 mL/min/kg, which testifies to the fast elimination of the substance from the body. Even after the ingestion of a single large dose of GHB (of up to 100 mg/kg), it can normally be detected in blood serum for just 5-8 hours, and in urine for 12 hours. Only 5-10% of the orally ingested dose is excreted with urine in an unchanged form. The remaining part is entirely metabolised to carbon dioxide and water. GHB does not create toxic metabolites. After the administration of GHB in the amount of 50 mg/kg of body weight to non-smokers, therapeutic concentrations of the substance ranging from 25 to 45 µg/mL were recorded in blood serum. The strong toxicity of GHB is recorded when the dose of 50 mg/kg of body weight is exceeded, although the interpretation of results should take into account the multiplicity of factors affecting its effects. Table 2 shows reference concentrations for GHB in biological material [9] in comparison with results obtained for ante-mortem blood and urine samples.

GHB belongs to psychoactive substances with a depressive effect on the central nervous system (CNS). This group also includes benzodiazepines, barbiturates, and ethyl alcohol. The effects of the particular psychoactive substances are normally similar, although GHB stands out from the group in terms of its properties. GHB has many features of a neurotransmitter, i.e. it is a substance which participates in the transfer of impulses between nerve cells. The substance inhibits the activity of the CNS, mainly inducing tranquillity and anaesthesia. It also inhibits the release of dopamine in the brain. It is a short-term effect, because when the drug is no longer active the entire stored dopamine is released, causing a sudden and instantaneous awakening. This drug increases the secretion of the growth hormone, stimulates protein synthesis in the body, accelerates the breakdown of lipids, and relaxes muscles [6]. What partygoers, dance clubbers, and even drivers expect most from GHB is mood improvement, relaxation, better sensitivity to music and touch, or excitement. At the same time, an increased number of cases involving the use of GHB for crime-related purposes (both robberies and rapes) have been recorded. In both cases GHB may facilitate the commission of the crime. The effects of the substance depend – as in the majority of drugs – on the ingested dose, body weight, interaction with other substances, and personal sensitivity to GHB. Studies have shown a correlation between GHB concentration in blood serum after the ingestion of an appropriate dose and the following clinical symptoms:

A single dose of GHB, which is ingested most often in amounts of 1-2 g, has a slightly euphoric effect. The person who ingested the substance is calm and feels light-hearted. He/she experiences increased sociability and a willingness to have fun and dance, and has more acute senses. A slight vertigo may also be felt. These symptoms are compared to those resulting from the drinking of a small amount of ethyl alcohol. GHB in medium doses of about 2.5g causes sleepiness, vertigo, nausea, vomiting, tremor, rapid and short-lasting spasms, bradycardia (slow heart rate), and even hallucinations. Some persons ingesting GHB report increased sexual arousal, and increased sensitivity to touch.

Doses of 3-4g of GHB result in serious loss of balance and may lead to the loss of consciousness within just a few minutes.

Persons who ingest a single dose of 4-5g of the drug or are involuntarily exposed to the effect of such a large dose of the substance may fall into a coma lasting for as long as a dozen or so hours. Such coma is reversible, although it is often difficult to wake up from it. Additionally, after waking up, the subject experiences a loss of memory concerning recent events.

These effects of GHB are most often used by persons who want to rob or sexually assault someone. The victim will not remember any persons, content of conversations, or events preceding the incident. He/she will not be able to indicate when GHB was administered to him/her, or what it was added to. Therefore, such a person will not significantly threaten the attacker from the point of view of future penal responsibility. Overdosing GHB (possible after the ingestion of as little as >2g of the drug) normally leads to acute poisoning with such symptoms as bradypnea, hypopnea, hypothermia, tremor, vomiting, and muscle spasms. In such a condition, the body does not react to external stimuli, including pain, and pupils do not react to light. A person in such a state should be immediately admitted to hospital and receive professional medical treatment. Other side effects of the use of GHB include disturbed vision, hot flashes, increased sweating, palpitation, headaches, increased salivation, constipation, and difficulty with urination. Laboratory analyses may

<table>
<thead>
<tr>
<th>Material</th>
<th>Endogenous</th>
<th>Therapeutic/ nontoxic</th>
<th>Toxic</th>
<th>Lethal</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.04</td>
<td>0.08-130</td>
<td>130-295</td>
<td>130-295</td>
<td>5</td>
</tr>
<tr>
<td>Urine</td>
<td>0-6.6</td>
<td>5-840</td>
<td>432-2,407</td>
<td>1,665-33,727</td>
<td>3000</td>
</tr>
</tbody>
</table>

Table 2. Reference concentrations of GHB in biological material in comparison with the results obtained for ante-mortem blood and urine samples
Unfortunately, the fact that the drug is also sold in liquid form, and the uncertainty as to the actual content of pure GHB in the purchased product, only add to the problem. GHB is very often mixed with alcohol [11] and/or ingested with other popular stimulants, such as ecstasy (58%), amphetamine (36%), cannabinoids (26%), ketamine (21%), LSD (3%) or cocaine (8%) [9]. In such cases, the effects of the particular substances are increased or accumulate, which may end in death.

Among cannabinoids (substances present in processed cannabis products, such as marijuana and hashish), THC is the most psychoactive substance. THC is intensely metabolised in the body. After one marijuana joint the blood concentration of the substance increases for about half an hour, to decrease in the course of several subsequent hours. At the same time, the blood and urine concentration of the main THC metabolite in the form of inactive THC-COOH acid increases. Both THC and THC-COOH are detected in the blood, but the concentration values depend on many factors, mainly the dose and time of ingestion, as well as the degree of addiction to cannabinoids and the manner of their ingestion. In relation to cannabinoids, the weakening of psychomotor functions may take place in concentrations exceeding 2 ng/ml THC in blood [12]. The concentration of the main THC-COOH metabolite exceeding 100 ng/ml in the blood indicates chronic (long-term) ingestion of cannabis preparations [13]. Fatalities following the ingestion of cannabis preparations are very rare.

Ethyl alcohol (ethanol) is the most popular substance accompanying crime. It has the majority of effects characterising substances facilitating the commission of crimes. Persons under the influence of ethanol find it difficult to rationally assess the situation and defend themselves against an attacker. Additionally, the ingestion of ethyl alcohol is generally accepted, and the substance itself is legal and easily accessible. Ethanol can also cause memory loss. The impact of ethyl alcohol on the human body increases linearly with the amount of the ingested and absorbed ethanol. The same is true of its concentration. In practice, the degree of intoxication with ethanol is reliably estimated by the determination of the concentration of ethyl alcohol in the blood. Concentrations of below 1 per mille (euphoric phase) are connected with mood improvement, talkativeness, excessive self-confidence, feeling good, euphoria, poorer self-control and concentration, disturbed motor coordination, and the first signs of disturbed perception. Concentrations ranging from 1 to 2 per mille (dysphoric phase) are connected with agitation, exaggerated fear, aggression or sadness, emotional instability, erroneous beliefs, disturbed attention, and motor impairment.

Toxicokinetic and toxicodynamic analyses of interactions between ethanol and GHB show that ethanol prolongs GHB effects, and increases its sedative effect and respiratory depression, which significantly increases the risk of death [14]. The strong combined depressive effect of GHB and ethanol on both the central nervous system and the respiratory system may lead to respiratory paralysis, and in consequence to the stopping of breathing and death, as described in many works [15, 16]. On the basis of the analysis of 226 fatal poisonings following the ingestion of GHB or GBL (a substance which metabolises to GHB) in 50 US states from 1995 to 2005, it was discovered that 213 cases involved sudden cardiac arrest, and in the other cases death was caused by a fatal accident. In 35% of the deaths GHB was the only pharmacologically active substance detected, and in 41% of the deaths both GHB and ethyl alcohol were identified in biological material [17]. Similar results connected with the presence of GHB and ethyl alcohol were presented as a result of the analysis of 23 cases of death connected with GHB abuse, which were recorded in western Sweden from 2000 to 2007. In 43% of the cases, no other pharmacologically active substances apart from GHB were detected (or their impact was negligibly small), and in 30% of the cases both GHB and ethyl alcohol were identified in the biological material [18]. In both publications it was stressed that the presence of other pharmacologically active substances (including ethyl alcohol) is not necessary for death to take place. An analysis of cases of poisoning with GHB and GBL in Great Britain from 1995 to 2013 shows that fatal intoxication with these substances was recorded in 37% of the cases, and the additional presence of alcohol was identified in 14% of the cases [19]. The authors also indicated the possible increased depressive and toxic effects of GHB in the presence of depressants, and in particular ethyl alcohol and opiates.

As a physiological reflex, vomiting protects the body against the negative effect of substances which enter the gastrointestinal tract. It can be involuntary or induced. After oral ingestion, GHB reaches its maximum concentration in the blood after about 20–40 minutes, while its half-life in blood serum amounts to just 30-50 minutes. About 1–5% of GHB gets into urine unchanged, and the detection window for GHB in urine is relatively short, and ranges from 3 to 10 hours [20]. Therefore, in connection with the fast distribution of GHB in the body, vomiting the contents of the stomach within a very short time after the ingestion of GHB might have protected the man against its absorption and thus the toxic effect of poisoning.

CONCLUSION

Chemical toxicological analyses of ante-mortem samples of biological material detected the psychotropic
substance GHB, which was determined in concentrations recorded in the urine for toxic and lethal effect. Since neither the time nor the date of collection of the ante-mortem blood sample were recorded, they cannot be interpreted. The presence of ethyl alcohol in ante-mortem blood serum (0.60 per mille) and urine (0.74 per mille) samples collected from the man at 9:08 AM (i.e. after about 5 hours after the calling of the police to the place of the incident) on the date of his poisoning in December 2014 indicates the possible combined strong depressive effect of GHB and ethanol on the central nervous system and the respiratory system, which in consequence might have led to cardiac arrest and death, although the ingestion of GHB alone was sufficient to cause the lethal effect, and the ethyl alcohol detected in the victim's body was not a factor indispensable for sudden cardiac arrest and the man's death. Analyses detected psychoactive THC and its non-active metabolite THC-COOH in concentrations only indicating an earlier ingestion of cannabinoids (hashish, marijuana), without a significant impact on the cause of death. Taking into account the pharmacokinetic properties of GHB, it should be assumed that the vomiting of the ingested liquid containing GHB immediately after ingestion, eliminating the possibility of absorption of the substance from the gastrointestinal tract to the bloodstream, would make it possible to avoid the toxic consequence of the substance, including fatal outcome.

Conflict of interest. The authors declare that they have no conflict of interest concerning this article.

References