Forensic Sci Med Pathol (2010) 6:314–325
DOI 10.1007/s12024-010-9150-4

CONTINUING MEDICAL EDUCATION REVIEW

Postmortem toxicology

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Accepted: 9 September 2009 / Published online: 4 March 2010

Abstract Results from toxicological analyses in death investigations are used to determine whether foreign substances were a cause of death, whether they contributed to death, or whether they caused impairment. Drug concentrations are likely to change during pre-terminal stages due to altered pharmacokinetics, to treatment during resuscitation or in the intensive care unit, to concomitant illness or to the presence of drug tolerance. The potential for postmortem changes must be considered in all but a few drugs. Formation of new entities as well as degradation of drugs may occur, especially in putrefied corpses; in addition, body fluids and tissues may be severely affected by autolysis and putrefaction. Specimens should be selected based on individual case history and on their availability. Analytical procedures should be performed in accordance with a proper quality assurance program for toxicological investigations. Problems are most likely to occur during the isolation and identification of a drug. Interpretation of analytical results is often limited by the inadequate information provided in a particular case.

Keywords Antemortem factors · Changes during the postmortem interval · Putrefaction · Autolysis · Drug redistribution · Selection of samples · Specimen collection · Analysis

Introduction

In many unnatural, sudden, violent or unexpected deaths the investigator often needs evidence as to whether alcohol, or illegal or prescription drugs may have caused, or are a contributing factor in the death [1, 2]. The forensic toxicologist will usually employ a 2-stage testing following selection and collection of appropriate specimens at autopsy. First, a screening test will be performed to establish whether there are any components within the sample that are not normally present. After confirmatory testing, the quantity of a foreign substance and/or its major metabolite(s) is determined, preferably from femoral venous blood and a further specimen. Quantitation of a drug is necessary to state whether its amount is sufficient to cause, prevent or be directly involved in the death [3]. Guidelines and a quality assurance program can assist in the selection and collection of specimens—as far as available—as well as in storage, transport, processing and analysis. However, the interpretation of analytical results remains the most challenging task in forensic toxicology. It is unique and fundamentally different from the situations encountered in clinical toxicology. There is evidence that substantial changes can occur in blood drug concentrations during the interval between the agonal phases of death and autopsy, mainly due to drug degradation, neo-formation or artefactual formation and postmortem redistribution [2, 4]. Interpretation may become still more difficult in decomposed or embalmed cases. It is essential to be aware that many of these changes will not be identifiable by postmortem sampling and toxicological analysis.

Antemortem factors

Basic pharmacokinetic concepts provide an estimate on the quantitative relationship between administered doses of a drug and the observed plasma or blood concentrations in a living individual. The field of pharmacokinetics is

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concerned with the liberation of a drug from its dosage form, absorption, distribution, metabolism and excretion (LADME processes). These processes, in addition to the dose, determine the concentration of drug at its active site. Pharmacokinetics assumes that a relationship exists between the concentration of a drug in an accessible site such as blood and the pharmacological or toxic response. Factors which affect the concentration-time profile of a drug are summarized in Table 1 [3, 5].

While arterio-venous differences following alcohol consumption are well documented, studies on arterio-venous differences in drug concentrations during lifetime are rare. For example, during the absorption and distribution phase, arterial plasma concentrations of amitriptyline are up to four times higher than venous concentrations. Up to 10 fold higher concentrations in arterial blood compared to venous blood were observed for diacetylmorphine and 6-acetylmorphine, whereas morphine glucuronides did not exhibit such arterio-venous differences [2].

Ethanol is commonly detected in medicolegal investigations. Many drugs interact with ethanol, commonly present in postmortem specimens, thereby altering the mechanism or effect of the alcohol and the drug involved. Ethanol is a central nervous system depressant and a similar effect is found with other hypnotic or narcotic drugs [3, 6]. Interaction may also occur through the induction of CYP2E1—a member of the cytochrome P450 family with high catalytic activity towards ethanol.

There are 2 types of drug-drug-interactions: pharmacodynamic and pharmacokinetic. Pharmacodynamic interactions take place at receptor sites and occur between drugs with similar or opposing therapeutic or adverse effects. Pharmacokinetic interactions consist of changes in the absorption, distribution or excretion, or in the quantity of drug that reaches its site of action. Most pharmacokinetic interactions occur at the metabolic level and generally result from enzyme inhibition or induction. An overview of drug-drug-interactions is available from the Drug Interaction Database [7].

Table 1 Factors affecting the concentration–time profile of xenobiotics

Dosage form, clandestine manufacturing process
Dose, route and frequency of drug administration, development of tolerance
Non-linear pharmacokinetics, e.g. acetylsalicylic acid, methylenedioxymethamphetamine
Time interval between drug administration and death and between death and collection of samples for analysis
Age, sex, ethnicity, genetic disposition
Weight, physical activity, nutritional state, general condition, disease
Nutritional ingredients, smoking, concurrent use of other drugs or alcohol

Far less is known about drug-nutrient interactions or interactions where active herbal constituents are involved. For example, grape fruit juice can inhibit the activity of CYP3A4 in the liver and the intestine and may elevate blood concentrations of drugs that are substrates of CYP3A4. A number of clinically significant interactions of St. John's wort have been identified with prescription drugs, resulting in a decrease in the concentration or effect, most probably due to induction of cytochrome P450 enzymes and the drug transporter *P*-glycoprotein [2, 3].

Pharmacokinetics during the pre-terminal phase are likely to be very different from that in study subjects or patients, due to a decrease in cardiac output and blood supply, low blood pressure, impaired ventilation, acidosis, dehydration, acute overdose and disease effects. Disease affects various organ systems and also the way drugs are absorbed, distributed, metabolized and excreted. Cardiovascular disease can substantially affect drug transportation to eliminating organs such as the kidneys and the liver. Renal diseases directly affect drug excretion, and hepatic diseases affect drug metabolism. For example, sepsis may induce a decrease in hepatic metabolism. Trauma and burns not only reduce the clearance of morphine, but also its volume of distribution. Also, volume distribution of drugs may be significantly reduced in patients with congestive heart failure [6, 8].

Disappearance of the drug may occur during lifetime in some cases. For example, paracetamol causes delayed hepatic toxicity that can be fatal, but by the time death occurs, the drug may not be detectable in the blood. Paraquat causes pulmonary fibrosis, and sometimes a slow death several weeks after ingestion [9].

Information on treatment and therapy during resuscitation or hospitalization should be provided. For example, even without restoration of the heart action during resuscitation, high concentrations of lidocaine may be present in the left side of the heart as intubation-related lidocaine may be absorbed by the trachea during cardiac massage. Interpretative problems involving alcohol and drug findings may also arise from lengthy treatment with intravenous fluids, from devices which automatically deliver medication by the parenteral route, or from transdermal patches that have been left on the body [10]. All these factors may affect drug concentrations in the body after death. However, further unique aspects of postmortem changes will be discussed below.

Changes occurring after death

Postmortem redistribution

In 1960, Curry and Sunshine reported on large differences in the amounts of barbiturates in blood obtained from

different sampling sites. Differences in drug concentrations in postmortem samples collected from different anatomical sites have been observed for numerous drugs including imipramine, diphenhydramine, codeine, methadone, doxepin, clomipramine, amitriptyline, cimetidine, cocaine, digoxin, zopiclone and methylendioxymethamphetamine. From a few studies comparing ante- and postmortem drug levels in blood it is also evident, that postmortem drug concentrations do not necessarily reflect concentrations at the time of death. For example, a 3.9 and 2.6-fold increase has been noted for amitriptyline and methadone, respectively, in postmortem blood [11, 12]. Generally, drugs with wide concentration ratios in ante- and postmortem blood also tend to have wide central/peripheral concentration ratios in postmortem specimens. Nevertheless, attempts to estimate antemortem concentrations from postmortem measurements are prone to considerable error [13].

All mechanisms and processes causing artefactual increases or decreases in drug concentration during the postmortem period can be included under the generic term of postmortem redistribution. Postmortem changes are site and time dependent. Potential factors that govern postmortem redistribution are summarized in Table 2 [2, 5, 13].

It appears that lipophilic drugs that have an apparent volume of distribution >3 L/kg and exhibit a central/peripheral blood concentration ratios >1 are candidates for postmortem redistribution (Table 3) [2, 11, 13].

Site dependent differences can arise from incomplete distribution of a drug at the time of death, release from major binding sites and/or passive diffusion through blood vessels or from the lumen of a body cavity into surrounding organs. Vascular pathways may depend on the blood remaining fluid after death. Organs such as the intestines, stomach, liver, lungs and myocardium are major sources of postmortem redistribution. Pleural and peritoneal fluids are also regarded as a route for drug exchanges between the lungs and the liver.

Redistribution from the lungs is more likely than from the stomach contents due to the large surface of the alveoli, the thin cell membranes and high vascularisation. Diffusion of ethanol from the stomach into femoral venous blood does not pose a problem. However, an investigation on postmortem diffusion from gastric residues in a human cadaver model using amitriptyline, paracetamol and lithium carbonate revealed high concentrations in the lungs as well as in the liver, whereas diffusion into gallbladder bile, cardiac and aortic blood was less pronounced. Redistribution from drug molecules sequestered in the liver may occur via hepatic vessels, or directly to adjacent organs such as the gall bladder or the stomach. Liver lobes may contain variable drug concentrations, and the left liver lobe being in close contact with the stomach will be more involved in postmortem redistribution. If a drug is also heavily concentrated in gastric contents it may be difficult to correctly determine the source of hepatic concentrations postmortem [5, 14].

Often, drug levels are higher in heart blood than in femoral venous blood. Substantial differences may be

Table 2 Major factors influencing postmortem redistribution of drugs

Physico-chemical and pharmaco-kinetic properties of the drug	Size, shape, charge, p*K*a-value, partition coefficient, apparent volume of distribution, binding to proteins, blood cells and/or tissues, residual enzyme activity during the early postmortem time period
Environmental conditions	Initial concentration, pH-value, orientation of solute flux, temperature, time, blood coagulation and hypostasis, blood movement due to fluidity changes and pressure, position of the corpse, lysosomal enzyme activities, bacterial invasion

Table 3 Drugs that are liable to postmortem redistribution

Drug	Octanol/water partition coefficient	Apparent volume of distribution (L/kg)	Concentration ratio heart/peripheral blood
Amitriptyline	4.94	ca. 15	3.1
Amphetamine	1.80	3–4	2.0
Clozapine	3.23	2–7	2.8
Cocaine	2.30	1–3	1.5–2.3
Diphenhydramine	3.30	4.5–8.0	2.3
Doxepin	2.40	20–24	5.5
Fluoxetine	4.05	ca. 27	2.9
Haloperidol	3.23	10–30	3.6
Midazolam	4.30	0.5–2.0	4.0
Trimipramine	5.43	20–50	1.6

noted for drugs such as calcium channel blockers or cardiac glycosides which are strongly bound to cardiac tissue. The lungs, the liver or gastric contents may serve as further drug sources [2, 11].

Temporal changes of drug concentrations have been studied in animal models showing that the most important quantitative changes occur very rapidly during the first 24 h [2]. A few case reports have determined that there is little evidence of time dependent variability, which may be due to delayed sampling [14].

Postmortem changes of blood

Decomposition of a corpse involves the processes of autolysis and putrefaction, which also effect the composition and integrity of tissues and fluids, and thus drug analysis. As blood is the specimen of choice for detecting, quantifying and interpreting drug concentrations, a more detailed review of postmortem changes in blood will be given.

In postmortem drug analyses whole blood is used, as separation of red blood cells from serum is usually not possible. Drug concentrations provided in literature are determined from serum or plasma which is traditionally used in clinical settings. Tables of drug levels determined from plasma or serum previously reported in therapeutic or toxic conditions can serve as a reference point. But only after weighing all possible influences and—if available—referring to postmortem values established from femoral blood samples can postmortem results be reliably interpreted [5, 8, 10]. Many drugs also do not evenly distribute between the cellular and fluid constituents of blood. For example, the concentration ratio between blood and plasma varies from 0.5 for phenytoin to 2.0 for maprotiline. Blood/plasma ratios may not only vary among drugs, but may also differ between a drug and its metabolite (Table 4).

Distribution ratios are generally derived from in vitro partition experiments where plasma water, plasma proteins and red blood cells are pooled. Some caution is advisable in using these data. When spiked blood is diluted with autologous plasma, erythrocytes discharge compounds more than plasma proteins. Also, non-linear, concentration dependent variations of the blood/plasma distribution have been observed, for example for topiramate. Ratios may also differ depending on whether the sample has been collected from a living person or a corpse. Distribution ratios of cannabinoids, for example, were essentially constant over the concentration ranges in samples collected from living individuals, but scattered over a wider range of values in postmortem specimens. The mean ratio between the initial postmortem blood specimens and corresponding supernatants was 0.42, but was 0.63 in samples obtained from living subjects. It seems that the distribution ratio that exists during life due to active processes decays after death. Generally, the concentration differences observed between blood or plasma might be less important compared to other effects acting on the drug concentration prior to sampling [2, 10, 11].

Table 4 Blood/plasma concentration ratios for drugs of forensic interest

Drug	Blood/plasma ratio
Amitriptyline	1.0–1.1
Nortriptyline	1.5–1.7
Cocaine	1.00
Diazepam	0.70
Oxazepam	1.00
Ethanol	0.74–0.90
Methadone	0.75/1.00[a]
Morphine	1.02
Morphine-3- and -6-glucuronide	Dependent on the hematocrit value
Δ^9-Tetrahydrocannabinol	0.55/0.66[a]
11-Hydroxy-Δ^9-tetrahydrocannabinol	0.57/0.58[a]
11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol	0.62

[a] Depending on the particular source

Drug concentrations may also change during agonal phases. Hypoxia reduces intracellular pH thus inducing an accumulation of basic drugs into cells, whereas neutral or acidic drugs are less affected. After death had occurred, acidification up to a pH-value of 5.5 and changes in ionic strength cause damage to lysosomal membranes, and subsequently enzymatic digestion of the cell membrane and components. As the permeability of membranes increases, drugs are redistributed into the extra cellular space, and hemolysis occurs. There is a rapid progress in postmortem redistribution processes due to disintegration of physiological and anatomical barriers.

A postmortem blood specimen may differ substantially from a sample of whole blood collected from a living person. In addition to hemolysis and a fall in the pH, blood coagulates postmortem, and then becomes fluid again. The extent of these two processes will determine whether postmortem blood is clotted, fluid or partially clotted and partially fluid. Sedimentation of cellular blood components to the lower parts of the body under gravity, a phenomenon called hypostasis, is a visible manifestation of postmortem changes. There is also a wide variation in the water content of postmortem blood, ranging from 59 to 89%. All these changes may effect the original blood drug levels.

Invasion of intestinal flora into tissues and body fluids occurs after death, especially at ambient or elevated temperatures but not within a specified time frame.

Postmortem blood samples taken 6 h after death from patients who had died of causes other than infectious diseases tested positive for bacteria whereas in a study on heart blood samples collected 85 h postmortem, bacteriologic cultures gave negative results [15]. Microbial enzymes degrade lipids, carbohydrates and proteins resulting in a slow increase in the pH during the postmortem interval. Both endogenous and exogenous substrates may be utilized as nutrients or energy sources (see also section "Degradation and formation of drugs during the postmortem interval").

Degradation and formation of drugs during the postmortem interval

Degradation and formation of drugs or new entities during the postmortem interval are processes that compete with postmortem redistribution. Potential mechanisms operating on drugs postmortem are summarized in Table 5 [2, 3, 16].

A most prominent example of postmortem metabolic formation is ethanol. It appears that ethanol is not produced postmortem except by microbial action, the amount generated depending on the species of microorganisms present, the availability of substrates, the antemortem condition of the deceased and the storage conditions of the corpse prior to collection of samples for analysis. The majority of the cases attributed to neo-formation do not have significant alcohol levels ($\leq$0.07%). A few case reports, however, demonstrate that levels of up to 0.22% may be produced under the most favorable conditions. If the body is refrigerated within a few hours of death and kept in a cool place, alcohol formation could not be detected within 24 h despite positive blood cultures. Also, alcohol is not always detectable in the advanced stages of putrefaction, possibly due to its utilization by microorganisms after an initial increase in levels.

As postmortem synthesis or loss of ethanol is difficult to accurately assess, determination of further putrefactive products or markers of recent alcohol ingestion have been suggested as indicators to differentiate ethanol formed postmortem from that due to antemortem consumption. The following volatiles can be produced along with ethanol: acetaldehyde, acetone, 1- and 2-propanol, 1-butanol, isobutanol, isoamyl alcohol, acetic, propionic, butyric and isobutyric acids, and ethyl esters. Co-detection of "abnormally high concentrations" of volatiles with ethanol during ethanol analysis has been suggested as suspicious for microbial contamination. However, minimum volatile levels indicating microbial contamination, as well as the correlation of these levels with the amount of ethanol produced, have still to be established. Also, all volatiles that could be microbial products are minor ingredients of alcoholic beverages, except 1-butanol. Methanol is not likely to be formed postmortem, and levels >10 mg/L blood may be the result of heavy antemortem exposure. Aminobutyric and aminovaleric acids have been used to verify postmortem ethanol formation but are also produced at variable rates. Measurement of ethyl glucuronide is a helpful tool to determine in vivo ingestion of ethanol. If ethyl glucuronide is not detectable along with ethanol, ethanol formation might have occurred postmortem; however, alcohol synthesis can not be excluded with certainty if both ethanol and its glucuronide conjugate are present, and ethyl glucuronide may also disappear from blood due to marked putrefaction [6, 16, 17].

Although time- and temperature-dependent decreases in cyanide concentration are the usual findings from studies, increases have also been reported in certain situations, again possibly due to microbial action in poorly preserved samples.

Gamma hydroxybutyrate (GHB) is increasingly abused as a recreational drug. It is also an endogenous metabolite formed during biosynthesis and degradation of the inhibitory neurotransmitter gamma-aminobutyric acid. There is significant postmortem formation of GHB in blood and tissues and also during storage of specimens, where levels may increase up to 100 mg GHB/L blood. Further, microbial degradation of GHB has been observed [18]. A compilation of toxicological findings in deaths involving GHB has recently been published [19].

The possible role of enteric bacteria in the bioconversion of nitro benzodiazepines has been studied in detail. It is well known that higher concentrations of the 7-amino-metabolite are present in the blood in deaths involving

Table 5 Potential mechanisms operating on drugs postmortem

Mechanism	Example(s)
Chemical instability	
Hydrolysis	Diacetylmorphine, cocaine, *O*-acyl- and *N*-glucuronides
Oxidation	Sulphur containing drugs, morphine
Metabolic instability	
Esterases	Hydrolysis of ester type drugs, phase-II-metabolites such as e.g. glucuronides
	Reduction, e.g. nitro benzodiazepines
Metabolic formation	Oxidation, e.g. thioridazine
	Ethanol, volatile compounds, gamma-hydroxybutyrate, carbon monoxide, cyanide

flunitrazepam. Postmortem degradation also involves acid-labile conjugates such as the ester glucuronides of propofol or diflunisal, and to a lesser extent, ether glucuronides such as 3- and 6-morphine glucuronide [2].

Specimens stored in formaldehyde or collected after embalming

Occasionally it is necessary to perform analysis on pathologic specimens stored in formaldehyde or on samples that were collected from an embalmed body. With modern techniques of embalming, blood is drained from the veins and replaced by a fluid, usually based on formaldehyde, that is injected into one of the main arteries; cavity fluid is removed with a trocar and replaced with preservative fluid, composed of formalin mixed with alcohols and emulsifiers. The embalming procedure may therefore have diluted blood and may have partially or completely removed drugs or poisons present at the time of death from major vessels. Analysis of pathological specimens should also cover determination of the drug in fixing and storing solutions as leaching of drugs from solid specimens occurs.

Formaldehyde present in both embalming and fixing fluids is a highly reactive chemical. Most likely reaction pathways are through hydrolysis, degradation or methylation via the Eschweiler-Clarke reaction. Conversion of nortriptyline to amitriptyline may occur during formalin fixation and storage; and *N*-methylation has also been reported for amphetamine, methamphetamine, and fenfluramine. Alternatively, embalming fluid may maintain acidic conditions and contribute to a drug's stability such as e.g. succinylcholine, which will be rapidly hydrolyzed. However, some compounds such as alprazolam or midazolam decompose more rapidly under acidic conditions [2].

Autopsy findings indicating intoxication or poisoning

Clinical symptoms may provide valuable information in a case of suspected poisoning or intoxication facilitating the selection of appropriate and correct samples at autopsy. It should, however, be considered, that these symptoms may be subtle, hidden by diseases or misleading, especially in children and the elderly [20]. As the majority of drugs and chemical agents do not produce characteristic pathological findings, most drug related deaths do not leave obvious or specific signs. Often, the only finding at autopsy is pulmonary congestion and edema. In occasional cases, however, clues indicating poisoning may be observed, and in some of these specific signs may be present (Table 6) [1].

Acquisition of specimens for toxicological investigations

General considerations

The purpose of sampling is to provide a representative part of the whole that is suitable to target the analysis for likely poisons, but also to help in the interpretation of any analytical result. The term "sample" covers the fluid and tissue and its primary container, and the sampling procedure starts with the selection of appropriate samples and ends with the correct disposal of the materials (Table 7) [5, 12, 21]. It is one of the principal roles of the forensic

Table 6 Autopsy findings indicative of intoxication or poisoning

Odor	Indicative of:
Bitter almond	Cyanide, hydrogen cyanide, nitrobenzene
Fruity, aromatic	Ethanol, solvents
Like leek or garlic	Organophosphorous compounds, arsenic, phosphorous
Sweet	Chloroform or other halogenated hydrocarbons
Orifices of the body, e.g. mouth or gastrointestinal tract	
Residues of powder or colored material	Tablet or capsule remains (e.g. flunitrazepam - blue-stained gastric contents), herbicides or pesticides, intranasal drug use (e.g. cocaine)
White, corrosive staining	Hydrochloric or acetic acid
Black-brown, corrosive staining	Sulphuric acid
Glass-like, reddish necrosis	Alkaline agents, e.g. sodium hydroxide
Lividity	
Cherry red to light red	Carbon monoxide
Bright pink	Cyanide
Greyish to brownish	Nitrate, nitrite, aniline
Atrophic scarring, abscess and ulceration of the skin, puncture marks	Intravenous drug use, e.g. opiates
Perforated nasal septum	Intranasal cocaine misuse

Table 7 To maximise the reliability of toxicology results it is recommended that

The interval between death and collection of specimens at the postmortem examination is minimised
Appropriate specimens with regard to case history, legal aspects and availability are selected
Sufficient sample volumes or amounts are taken
The possibility of cross-contamination during collection is minimised
All samples should be placed in separate and clean containers capable to maintain evidential quality
Specimens are individually labelled, stored refrigerated or frozen as appropriate, and securely packaged if transported
Samples ideally be submitted in person to the laboratory
The identity and integrity of the samples is guaranteed from collection through reporting results
All information available is provided with the samples to help the laboratory in planning the analysis
The history of the sample is documented from submission through analysis, reporting results and disposal (chain of custody procedures)

pathologist to select and collect appropriate specimens. There are regulations on the minimum storage time of toxicological specimens after the toxicology report has been issued, which may be considerably longer than that for clinical samples [22, 23].

Specimens

To date, a coordinated protocol for sampling suspected poisoning or drug related deaths has not been established. Some recommendations from *Leitlinien der Deutschen Gesellschaft für Rechtsmedizin: Rechtsmedizinische Leichenöffnung* are summarized in Table 8 [2, 21].

In cases of exsanguination, burns or advanced putrefaction the availability of specimens may be limited, and alternative samples for drug identification such as skeletal muscle, pleural effusions, bone or bone marrow and entomological specimens may be collected. Insect eggs, larvae or pupae can be used not only to analyze for harmful substances and to estimate the postmortem interval, but also to indicate movement of the corpse. Postmortem samples from a patient who has died in hospital several days after a poisoning episode are likely to be negative. In these cases, specimens obtained at or soon after admission to hospital should preferably be investigated. Evidence found at the scene including spoons blackened with soot, syringes, and mugs or glasses containing drug residues, household products, solvents or pesticides, may provide additional information to assist and focus toxicological analyses [1, 3]. In cases where poisoning by volatiles or gas is suspected, a specimen should be collected directly at the scene, for example an aerosol container, or other source [4].

Quantity and use of postmortem specimens

The scope of the sampling procedure has to be case-dependent as the selection and volume or amount of specimens will vary considerably from case to case, depending on requests, legal aspects and availability. Clinical samples may be collected on several occasions, however in a death investigation there is rarely an opportunity to collect further specimens once an autopsy has been concluded. Two blood samples, including at least one

Table 8 Recommendations for sampling postmortem materials

All autopsies	Cases in which the cause of death remains uncertain	Special cases
Materials that should be collected prior to autopsy		
Peripheral blood (femoral or subclavian)	Hair sample of the scalp, alternatively: body hair or nails	Vitreous humour
Urine		Cerebrospinal fluid
Vomitus		Nails
		Skin, subcutaneous fat and control samples
		Swabs from the skin or mucous membranes and control samples
Materials that are collected during autopsy		
Heart blood	Bile bladder fluid	Deep muscle tissue
Gastric contents	Tissue samples of	Subcutaneous fat
	Liver	Hematoma (epidural, subdural)
	Lungs	Contents of the large and small intestine
	Brain	Pericardial fluid
	Kidneys	Pleural fluid
		Bone and bone marrow
		Entomological specimens

Table 9 Type, volume/amount and use of postmortem specimens

Specimen	Volume/amount	Use/comment
Blood from the femoral or subclavian veins	10–20 mL	Quantitative data, acute impairment or poisoning
Heart blood	50 mL or all available	General unknown analysis, concentration may be increased due to postmortem redistribution
Urine	50 mL or all available	Standard sample for drug screening, general unknown analysis, organophosphates, aromatic hydrocarbons (metabolites)
Gastric contents	50 mL or all available	Result should be referred to the total amount; tablets, herbal remains, etc. placed in individual containers
Tissues (brain, liver, lungs, kidneys, muscle, subcutaneous fat)	10–50 g	Body load may help to interpret postmortem blood data Lungs, brain: inhalant poisoning
Gall bladder fluid	All available	Drug screening, accumulation of drugs undergoing enterohepatic cycling
Hair sample from the scalp or the body, or nails as an alternative	Pencil-like tuft	Exposure data for weeks or months before death, tolerance
Vitreous humour	All available	Alcohol, cardiac glycosides, diabetes
Cerebrospinal fluid	All available	General unknown analysis, devoid of enzymes and proteins
Skin and subcutaneous fat	Approx $2 \times 2 \times 1$ cm^3	Skin exposure, injection marks (insulin, i.v. drug abuse), anesthetic-related incident
Contents of large and small intestine	All available and fractionated, if applicable	Suspicion of drug exposure by the rectal route, poisoning by plants or mushrooms
Pericardial fluid	50 mL or all available	In putrefied cases
Pleural fluid	50 mL	In putrefied cases
Entomological specimens species	As available	In putrefied cases, should immediately be frozen
Bone, bone marrow	Piece of 3–5 cm, >1 g	Advanced putrefaction, extensively burnt bodies
Swabs (intranasal, rectal, vaginal)	≥2 swabs	Route of administration or exposure

from a peripheral site, and urine and gastric contents should be collected as a minimum set of specimens. Collection of other appropriate fluids and tissues is recommended and even imperative in cases where the autopsy fails to determine a cause of death, or where there is an incomplete investigation. A guide to the collection of specimens is given in Table 9 [4, 5, 8, 12, 24].

Collection of samples

Separate disposable or clean devices or instruments should be used for each sample to avoid contamination. Body fluids can be taken by needle aspiration using a hypodermic syringe or by a pipette, whereas a spoon or ladle is more appropriate for viscous materials. Swabs should be taken using cotton pads, and tissue specimens can be cut out with disposable scalpels, knives or scissors. If inhalants are suspected it is important to promptly collect and seal the tissue specimen in a container as soon as possible after the body has been opened. Gas tight syringes may help to collect volatiles [4]. Collection of scalp hair, urine, femoral blood and gastric contents is shown in Figs. 1, 2, 3, 4.

Specimen preservation is not necessary except for a portion of the blood sample. Ideally, the blood sample should be divided between an unpreserved and a preserved tube containing sodium fluoride at a final concentration of 1–5%. Preservation of a vitreous humour specimen for alcohol analysis is also recommended. Generally, preservation of specimens with sodium fluoride aids in ethanol, GHB, cocaine and carbon monoxide analyses, whereas fluoride preservation must not be used when organophosphorous chemicals are involved. Instead, early acidification and storage at a temperature below −20°C are recommended to stabilize these compounds. Ascorbic acid is not widely used as an antioxidant, but may reduce losses in olanzapine during storage and also stabilize drugs such as apomorphine [2].

Although tubes containing liquids should be filled to minimize the evaporation of volatiles and oxidative losses of drugs, a small headspace of about 20% should be left if they are likely to be frozen. If analysis of volatiles is to be directly performed on a sealed container, a head space of 90–95% is recommended. Break- and leak-proof disposable tubes or containers should be used during collection and if specimens are to be stored frozen. Most types of plastic containers (polycarbonate, polyethylene or polypropylene copolymers) are suitable for the collection of body fluids and tissues in drug related fatalities. Bags that can be tightly sealed are also appropriate for the sampling of tissue specimens. Glass tubes/containers may break if

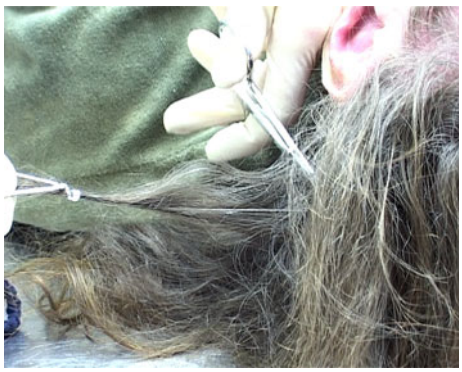

Fig. 1 Collection of a hair specimen prior to autopsy by tying with a piece of thread

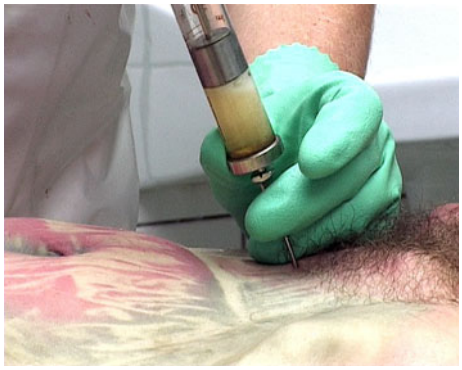

Fig. 2 Collection of a urine specimen prior to postmortem examination

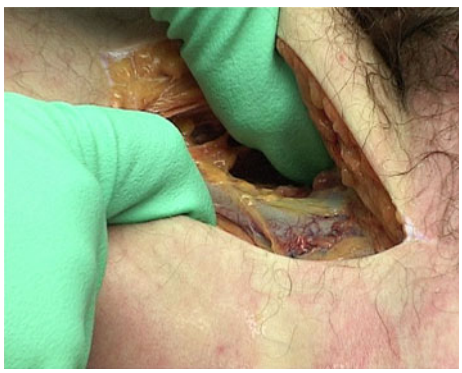

Fig. 3 Dissection of the femoral vein prior to collection of a blood sample

sent by post or upon freezing. Sampling into a glass container is, however, a must if solvent abuse or an anesthetic death is suspected. Samples should be sealed in such a way that tampering will be evident.

Specimens must be individually labelled and include the following information:

- Postmortem reference number or unique identifier
- Name or unique identifier of the deceased

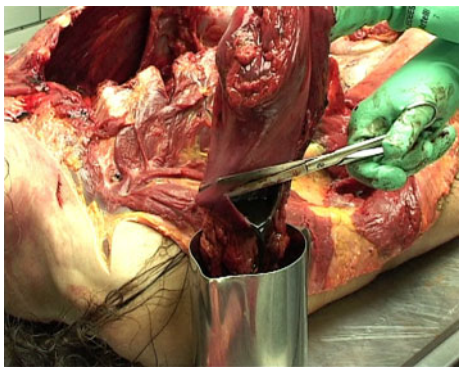

Fig. 4 Determining the total volume of gastric concents

- Type/source of specimen
- Date of specimen collection.

All samples except for the hair tuft and the portion of blood to be submitted for alcohol analysis should be combined as a batch. The specimen collection protocol should preferably include the following details (Table 10) [2, 22]:

Storage, transport, receipt and disposal of postmortem specimens

It is important to guarantee the identity and integrity of samples from collection through to the reporting of results. Safe transport within the mortuary and to the laboratory is essential. Whenever specimens are left unattended, they should be secured in a locked container, refrigerator or freezer. Handling and processing of specimens should be restricted to authorized personnel. Freezing of specimens is highly recommended except for hair and a portion of the blood sample, which should be kept at ambient temperatures or refrigerated. Also, if analyses are performed within a few days after collection, freezing may not be necessary.

Upon arrival at the laboratory, specimens should be checked for completeness and integrity. This information is essential when deciding how to approach the request of the police or coroner. Precautions should be taken to preserve the stability of the analytes present in the specimens, which often need to be kept for several months, and to avoid accidental contamination of the specimens in the laboratory, also [8].

To target the analysis to likely poisons and to simplify the interpretation of analytical results, the following information should be available when submitting samples for analysis [10]:

- Request including the name, telephone number and address of the investigating officer or coroner
- Postmortem reference number or unique identifier

Table 10 Specimen collection protocol

Name, address and telephone number of the pathologist and coroner's officer
Postmortem reference number, date of postmortem examination
Surname and first name of the deceased or unique identifier
The date of specimen collection
Type and source of specimens, amount or volume, and addition of preservatives, if applicable
Indication of any biological risk or chemical hazards associated with the specimen(s)
Abnormal appearance of the specimen or degree of autolysis/putrefaction
Specimens should be accompanied by a chain of custody form which should be signed by each individual handling the sample

- Surname/first name of the deceased or unique identifier
- Police involvement, circumstances surrounding death
- Autopsy report, at least a preliminary version
- Emergency report, recent medical history and treatment
- Specimen collection protocol (see Table 10)
- Predefined time periods to complete the toxicology investigation

The documentation of analytical results is mandatory. Once the finished report has been submitted and specimens are not required any longer, or if the predefined time for specimen storage has been reached, samples may be discarded. The date that specimens are discarded must be recorded [2, 22].

Analytical pitfalls

There is little to differentiate analytical procedures used in other branches of forensic toxicology from those used in postmortem investigations. The same quality standards as established for the analysis of specimens derived from living individuals also apply to postmortem specimens [21]. Analytical methods can be broadly classified into three types: screening procedures, confirmation procedures and specific methods. Screening for volatiles by gas chromatography (GC) is recommended, and immunoassays for common drugs of abuse are regularly included in a toxicology investigation. Interaction with putrefactive amines is commonly seen in immunoassays for amphetamine type drugs; and ambroxol was reported to show some cross reactivity in immunoassays for LSD. If death occurs within a very short time after drug administration an insufficient concentration may be present in urine to give a positive result [25].

Additionally, comprehensive chromatographic screens for basic, neutral and acidic drugs using UV- or mass spectrometric (MS) detection are required [8]. Screening methods based on MS data will provide confirmation of a suspected compound. The particular compound should also be detected in at least one other specimen. The second method may use a different detection mode or an entirely different procedure. Although GS/MS is generally accepted as unequivocal identification for many drugs, a few drawbacks have been reported:

- erroneous identification at a low analyte concentration or due to a low extraction efficiency
- misidentification due to interfering substances
- inadequate information due to similar fragmentation behaviour (e.g. barbiturates)
- production of low fragment ions (e.g. tricyclic antidepressant agents).

Several LC/MS procedures for the screening of drugs in blood have been developed in recent years using time-of-flight mass analyzers, ion traps or classical triple quadrupoles that cover a sometimes more limited range of substances compared to GC/MS [8]. At present, there is still a need for studies of matrix effects, selectivity, analyte stability and appropriate internal standards [18].

Numerous procedures exist that quantify specific drugs, especially by GC/MS or LC/MS, with LC/MS becoming a gold standard for detection of very low drug concentrations [18]. Depending on the type and quality of a sample, homogenization and modified or multiple extraction procedures have to be applied. Drug conjugates that have been co-extracted may subsequently be hydrolyzed leading to erroneously high levels of the non conjugated drug proportion. The phenomenon of conjugate instability is most common with labile conjugates such as *N*- or *O*-acyl glucuronides [2].

The extraction efficiency from a postmortem specimen may vary from case to case, or even from site to site within the same body. It may also differ from that of a blank specimen used for calibration. The use of stable isotope internal standards may provide a higher degree of accuracy in the analytical results. However, few deuterated standards are commercially available for drugs or major metabolites. If impurities present in the sample interact with the analyte to change the instrumental response, the method of standard addition may be used as an alternative; i.e. the sample can be measured before and after adding increasing amounts of the analyte. Major drawbacks are the high sample volume, an increase in analytical runs and extrapolation of the curve to "zero" concentration, e.g. beyond the valid calibration range.

It is now recognized that determination of major metabolites and degradation products along with the parent drug is essential to avoid misinterpretation of the data due to artefacts. Such a stability-indicating assay is one that can accurately and selectively differentiate the intact drug from its potential decomposition products.

Implications for practice

A toxicological death investigation is often unique and totally different from investigations in clinical toxicology. The following issues might assist in targeting requests relative to poisoning in a particular case:

- Is there adequate information on the circumstances surrounding death?
- Is there any information available on the drug, and if so, is there adequate scientific information available to define the concentration-toxicity relationship?
- Is the drug either known or suspected to undergo postmortem redistribution? Is there information on the drug stability during autolysis and putrefaction?
- Are special sample collection techniques and/or storage conditions required?
- Are there autopsy findings consistent with drug overdose?
- What kinds of specimens are available?
- When, how and where does a particular specimen have been collected?
- Were blood samples collected from a minimum of two different sites?
- Has the chain of custody be maintained?
- Is there sufficient scientific and practical experience to perform appropriate analyses on the materials provided?
- Has a request form been submitted? Have additional requests emerged from pathological or initial toxicological findings?

Drug concentration measurements cannot be interpreted without a thorough review of all the medicolegal findings and circumstances surrounding death. Success in arriving at the correct conclusion will depend on the combined efforts of all of the investigators, the pathologist and the toxicologist.

Key points

1. Many changes occurring postmortem will not be identifiable by postmortem sampling and toxicological analysis.
2. There are a lot of antemortem factors affecting the concentration-time profile of drugs.
3. All mechanisms causing artefactual changes during the postmortem period can be included under the term postmortem redistribution.
4. Degradation and formation of drugs or new entities compete with postmortem redistribution.
5. Most drug related deaths do not leave obvious or specific signs.
6. The purpose of sampling is to provide a representative part of the whole that is suitable to target the analysis for likely poisons and also help in the interpretation of the analytical results.
7. The scope of the sampling procedure has to be case-dependent.
8. The identity and integrity of the samples must be guaranteed from collection through to the reporting of results.
9. Interpretation of the analytical results may be limited by the inadequate information.

CME questionnaire

1. **No significant influences on the pharmacokinetics of a drug are to be expected from:**
   - ☐ acidosis
   - ☐ perfusion of the liver
   - ☐ cyanosis
   - ☐ dehydratation
   - ☐ hypoxia
2. **Varying drug amounts in blood samples collected from different sampling sites in a corpse are not due to:**
   - ☐ arterio-venous differences
   - ☐ development of tolerance
   - ☐ local drug reservoirs within the corpse
   - ☐ drug release from tissue binding sites
   - ☐ diffusion along a concentration gradient
3. **The phenomenon of postmortem redistribution largely depends on:**
   - ☐ a decrease of the water content of postmortem blood
   - ☐ a partial or nearly total clotting of blood
   - ☐ several processes such as a non-uniform distribution of the drug within the body, a release from its binding sites and/or passive diffusion
   - ☐ hypostasis
   - ☐ hemolysis
4. **During the postmortem interval drugs maybe degraded as a result of chemical decomposition or metabolic processes, and new entities may be formed by microbial actions. There is evidence, however, that the following compound is not likely to be formed postmortem:**
   - ☐ propanol
   - ☐ cyanide
   - ☐ ethanol
   - ☐ gamma hydroxybutyrate
   - ☐ methanol
5. **Conversion of N-desmethyl metabolites to the parent drug through reductive N-methylation has been observed in specimens stored in formaldehyde containing solutions. This reaction is named after:**
   - ☐ Eschweiler-Clarke
   - ☐ Canizzaro
   - ☐ Traub
   - ☐ Mannich
   - ☐ Grignard

6. **The following finding at postmortem examination may indicate intoxication or poisoning by the assigned agent:**
   - ☐ Garlic-like odor – arsenic compounds
   - ☐ Fruity-like odor - nitrobenzene
   - ☐ Cherry red to light red lividity – nitrate or nitrite
   - ☐ Sweet, etheral odor - cyanide
   - ☐ Glass-like, reddish necrosis – acetic acid
7. **Sampling of specimens for a toxicological investigation does not cover:**
   - ☐ Selection of the specimens
   - ☐ Packaging and transport of specimens
   - ☐ Extraction of specimens for toxicological analysis
   - ☐ Storage of samples over a fixed period of time
   - ☐ Disposal or destruction of specimens
8. **The following specimen is not suitable for the detection of:**
   - ☐ Hair sample – acute overdose
   - ☐ Urine – screening for drugs of abuse
   - ☐ Vitrous humor – cardiac glycosides
   - ☐ Skin and subcutaneous fat - injection of insulin
   - ☐ Gastric contents – identification of herbal materials
9. **Acquisition of specimens for a toxicology investigation**
   - ☐ will preferably be performed during or after dissection of a corpse.
   - ☐ should always be performed on muscle tissue.
   - ☐ will reasonably not be performed in cases of severe putrefaction
   - ☐ should be performed on skin and subcutaneous fat if solvent abuse or an anesthetic death is suspected.
   - ☐ can be done using plastic tubes if solvent abuse or an anesthetic death is suspected.
10. **The following specification is not considered an inherent part of the sample acquisition protocol:**
    - ☐ the name of the deceased
    - ☐ the name of the pathologist
    - ☐ the date of receipt for the postmortem examination of the corpse
    - ☐ information on the biological risk or chemical hazard associated with the specimen
    - ☐ date of transfer and submission to the laboratory

CME questionnaire answers

1. cyanosis
2. development of tolerance
3. several processes such as a non-uniform distribution of the drug within the body, a release from its binding sites and/or passive diffusion
4. methanol
5. Eschweiler-Clarke
6. Garlic-like—arsenic compounds
7. Extraction of specimens for toxicological analysis
8. Hair sample—acute overdose
9. should be performed on skin and subcutaneous fat if solvent abuse or an anaesthetic death is suspected
10. the date of receipt for the postmortem examination of the corpse

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