RECOMMENDATIONS FOR COLLECTION AND STORAGE OF POSTMORTEM SPECIMENS FOR FORENSIC TOXICOLOGICAL INVESTIGATIONS INCLUDING SPECIAL ASPECTS OF POST-MORTEM ANALYSIS

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1. Introduction and Definitions

The aim of forensic toxicological investigations on post-mortem material is to determine whether alcohol, legal or illegal drugs or other substances are directly or indirectly related to the cause of death or whether they have caused a person to be incapacitated or to be no longer responsible for his/her actions. To this end, collection of suitable and representative samples is an essential requirement.

Definitions

Specimen: sample material including respective container
Sampling: appropriate collection and storage of specimens

Sampling includes:

- choice of sample material suitable for analysis
- sampling at an appropriate point of time
- sufficient amount of specimen
- suitable sampling technique
- appropriate specimen container
- unique specimen identification and labelling
- appropriate specimen storage
- packaging, transport and handover of specimen(s) with request form
- confirmation of receipt in the laboratory, intermediate storage prior to analysis
- procedure and length of time of storage of remaining specimen(s)
- disposal of specimen(s)
- complete documentation of all steps involved (chain of custody)

Forensic pathologists are responsible for appropriate and correct sampling of biological material.

2. Sampling of material for forensic-toxicological investigations during autopsy

The sampling of material for post-mortem toxicological investigations depends upon the individual case and, as a rule, is much more complex and challenging than for living persons. Under certain circumstances (e.g. exsanguination, advanced putrefaction, severe burning), alternative specimens must be collected (e. g. muscle tissue instead of blood). Sampling should be performed such that the specimens can be considered representative *in toto*. All specimens taken must be recorded in a list.

2.1 Material for toxicological investigation

Specimens which may be collected *prior to* or *during* **all autopsies**, those cases **where the cause of death is uncertain** or where **unique situations arise**, are listed in Table 1.

The recommendations are based upon the minimum requirements for sampling post-mortem material for toxicological investigations as laid down in the Guidelines ‘Forensic-Medical Autopsy' of the German Society of Forensic Medicine (AWMF Nr.054/001 (1).

In cases of poisoning with an elapsed survival time period of many hours or several days prior to death, surplus volumes of body fluids from clinical laboratory investigations should be secured by the investigating authorities. Collection of drug paraphernalia, remains of drinks and tablets, containers of common household chemicals and further potential evidence may provide valuable information in cases of poisoning by unknown substances (2). In cases involving gaseous or volatile substances, collection of air samples or samples from the suspected source at the scene of the incident or crime can be useful (4).

Tab. 1. Recommendations for sampling of material prior to or during all autopsies, cases where the cause of death is uncertain or where unique situations arise (see also Tab. 2) (1-4)

All autopsies	**Additional specimens (cause of death uncertain)**	**Unique situations**
Specimens that can be collected **prior** to autopsy		
Femoral or subclavian venous blood Vomit from the scene Urine	Scalp/body hair	Vitreous humour Cerebrospinal fluid (CSF) Finger/toenails Skin/subcutaneous tissue Skin/mucosal smears
Specimens to be collected **immediately after** opening the thoracic and abdominal cavities or after organ removal		
Cardiac blood Gastric contents	Bile Liver Lungs Brain Kidneys	Muscle tissue Fatty tissue Contents of small and large intestines Pericardial fluid Fluid from thoracic cavity Bones, bone marrow Insect species

2.2. Quantities and Considerations

The selection of specimens and their quantity depends upon the circumstances, availability and background information on the cause of death. As a precautionary measure, various and numerous specimens should be collected. For toxicological investigations, specimens are preferred for which a sufficient database for the interpretation of results is available (Tab. 2).
With regard to quantities, various recommendations have been published (1,2,4). The quantities given in Table 2 are considered as established or correspond to own experience.
The quantity of specimen has to be chosen so that:
(1) all required analyses can be carried out
(2) enough material remains for complementary or repeated analyses.

Tab. 2. Specimen, recommended quantity and comments (1,2,4-6).

Specimen	Quantity	Comments
Femoral/subclavian venous blood	10-20 mL	For quantitative analyses
Cardiac blood	50 mL or total*	For screening tests
Gastric contents	50 mL or total	Documentation of total amount essential. In cases of non-homogeneous contents, collect total amount.
Urine	50 mL or total	For screening tests, immunoassays. Intensive biotransformation can impede detection of parent compounds.
Organs (brain, liver, lungs, kidneys, muscle, fatty tissue)	50 g	Large database for concentrations in liver tissue, lungs and brain samples in cases of gaseous and volatile compounds; sparse data for lipophilic substances and anaesthetics in brain, kidneys and fatty tissue; specimens from kidneys, right and left ventricle in cases of cardiac glycoside poisoning.
Bile	Total	Few comparative data, high concentrations of many substances.
Scalp/body hair Finger/ toenails	Pencil-thick tuft	Retrospective information of chronic or previous use of or exposure to drugs, medication or metals. Few data available for nails or comparing the drug concentration between scalp and body hair.
Vitreous humour	Total	Detection of e.g. alcohol, cardiac glycosides, cocaine, sugar metabolism disorders. Few data available comparing concentrations in vitreous humour with those in blood.
Pericardial fluid	50 mL or total	Also for immunochemical analyses instead of urine.
CSF	Total	E. g. for diagnosis of sugar metabolism disorders.
Skin/subcutaneous tissue	ca 2 x 2 x 1 cm^3	In cases of subcutaneous injections (e.g. insulin) and percutaneous uptake of a poison, collection of a random specimen from a similar site as control.
Skin/mucosal smears		Clarification of mode of poisoning.
Intestinal contents	Fractionated where necessary	Metal, plant or mushroom poisoning; suspected rectal administration.
Thoracic cavity fluid	50 mL	Cases of putrefaction.
Bones, bone marrow	ca. 3-5 cm long, > 1 g	Cases of advanced putrefaction.
Maggots (larvae)		Cases of putrefaction or advanced corporeal decay.

* Total amount: maximum amount which can be removed or is still present

2.3 Sampling techniques

All specimens must be collected with either disposable or clean and dry utensils.
For body fluids, large diameter pipettes or syringes with needles of suitable length and gauge may be used. For viscous samples, use spoons or ladles; swabs for smears; scalpels, knives or scissors for tissues and for gaseous substances, gas-collection tubes or gas-tight syringes (2,4).

Specimen Preservation

Ideally, two parallel blood samples are collected - one into 1-5% sodium fluoride and one without. For the assay of alcohol in vitreous humour, the addition of sodium fluoride is also recommended. All other samples should be collected without additives.

Blood:

Drawn by puncture or incision from the femoral or other peripheral veins after preparation - where necessary, individual sampling from left and right vein; cardiac blood taken by puncture or incision of the cardiac chambers after opening the pericardium.

Urine:

Puncture or directly from the bladder (under direct visual control) after opening the abdominal cavity.

Bile:

Squeeze contents of the gall-bladder into a container. Needle aspiration after opening the abdominal cavity is rarely successful due to the high viscosity of contents.

CSF:

Sub-occipital puncture or - less recommended - aspiration from the ventricles after removing the cranium, or lumbar puncture.

Vitreous humour:

With a syringe and a fine needle from the anterior orbital cavity. Replace fluid with appropriate amount of physiological saline.

Gastric contents:

After opening the abdominal cavity, the stomach is ligated and removed. Contents are transferred to a container and the total amount documented.
Suspicious items such as tablet remnants or plant matter etc. should be isolated, dried (e.g. on cellulose swabs) and stored separately. If the contents are very inhomogeneous, then the whole stomach contents should be collected.

Tissues:

Place specimens in separate containers. Where gaseous or volatile substances are involved, samples of brain, lungs and blood must be collected immediately into gas-tight containers, if possible into pre-weighed and cooled glass vessels. In the presence of penetrating substances or putrefaction, remove separate liver specimens close to and at a distance from the stomach.

Smears:

Wipe suspicious parts of skin or mucosa with a cotton wool pad or other suitable absorbent. In cases of drug-related death, wipe a further clothed area of skin. If necessary, moisten the absorbent with methanol or other suitable solvent.

Hair:

Hair samples are taken preferably from the posterior vertex scalp region except from areas contaminated with blood, vomit or putrefaction fluid. A tuft of hair, approximately the thickness of a pencil, is tied firmly and cut off as close as possible to the scalp under slight tension. The stubble length remaining at the site is noted. Moist hair should be dried. Body hair is removed with a disposable razor or scalpel. Recommendations for correct hair sampling have been published (4).

Bone:

A graft of cancellous bone (3-5 cm) (e.g. from a vertebral body) and a 3-5 cm piece from the femur.

Insect larvae:

As maggots and other insect larvae excrete drugs they have ingested almost immediately after removal from the nutritional source, they should be washed quickly and frozen immediately after trapping.

Collection of evidence at the scene:

Remnants of all beverages, liquids or other suspicious materials should be transferred to unbreakable, leak-proof containers and packed separately. Include all original containers. All solid matter or containers should be packed separately to avoid risk of injury. Gases or vapours can be sampled with a special gas-collection tube or in the case of immediate analysis, a gas-tight syringe. Alternatively, the gas sample can be transferred from the syringe into a headspace vial (2).

2.4 Containers, labelling and documentation

All containers should not be more than 80% full. When using headspace vials for the subsequent analysis, the vapour space over the sample should be ca. 90-95% of the total volume of the vial.
All containers should be disposable, unbreakable and leak-proof. Glass is inert and free of plasticizers, but is subject to breakage. Thus, glass tubes should be placed in a suitable storage rack and not subject to leakage during storage and shipment.

For sealing the tubes, suitable closures should be used preferably with Teflon inserts. If gaseous or volatile compounds are involved, use glass containers. For body fluids, disposable plastic tubes (Nalgene®) can be used. Many commercially available containers made of polycarbonate, polyethylene or polypropylene with very low amounts of plasticizers are suitable for collecting tissue specimens.

Specimen containers should be labelled with at least:

- Autopsy number or other identification number
- Name and surname of the deceased or other personalised identification
- Type of specimen
- Date of sampling

If a specimen involves several containers, they should all be numbered. All samples, with the exception of hair and a sample of femoral venous blood, should be bundled and packaged. A packaged unit should, with the exception of the sample materials, contain the same details as the individual specimen containers.

The document accompanying specimens should contain at least the following information (3):

- Name of post-mortem pathologist
- Name of his/her assistant
- Autopsy number, name and surname of the deceased or other personalised identification
- Date of sampling
- Type and source of sample, quantity (estimated), additives (where used)
- Particular details related to the sample (e.g. health risks due to contagious disease or dangerous substances, or details related to degree of autolysis)
- Name and signature of person responsible for checking specimens for completeness after the autopsy has been performed
- Date and time of transport or handing over of the specimens to the forensic-toxicology laboratory

2.5 Storage, transport, handover and disposal

During removal and packaging or before storage, specimens should not be left unsupervised and must be locked away for safe-keeping. Only authorised persons are entrusted with the handling and processing of specimens.
Before processing, specimens combined in one package should be stored at a minimum of -18°C. Hair samples are stored at ambient room temperature and a sample of femoral venous blood at +4°C.
Should transportation of samples be necessary, then the cold chain and chain-of-custody must be maintained according to established safety regulations and requirements.
On arrival in the laboratory, all specimens are checked for completeness, intactness and suitability for analysis. Receipt is recorded and countersigned. Annotations concerning any discrepancies are incorporated into the laboratory documentation. Every package is given a unique identification number. Until processing and completion of investigations, specimens are stored so as to avoid contamination and

any changes in the analytes of the material under investigation. If a particular request for analysis gives rise to others outside the scope of the forensic-toxicological investigations, then this must be discussed with the pathologist handling the case.

In order to precisely and economically specify the necessary analyses and provide sound interpretation of the results, the following information should be available (3):

- Request for analysis either in writing or in electronic form
- Name, address and contact numbers of the contracting authority
- Autopsy number or another unique identifier
- Name and surname of the deceased or unique identifier
- Date of birth of the deceased
- Autopsy report
- Results of the investigation
- Report of emergency physician
- Medical report or details of medication
- Source of specimens
- Date of specimen collection
- Addition of additives
- Amount of specimen
- Handling risks involved
- Correct labelling
- Complete chain-of-custody documentation
- Specified time-frame for processing specimens

In the course of the investigation, all portions of the specimens taken for analysis must be documented according to purpose and amount. In the laboratory, a traceable record of the investigations should be made in which the names of all laboratory persons involved are obvious. During the analyses, it must be ensured that the very least possible change(s) to the analyte(s) occurs. After expiry of the custody period defined in the administrative regulations or after that as arranged with the contracting authority, specimens remaining after completion of all investigations may be disposed of and their disposal recorded.

3 Principles of analytical procedure
3.1 General strategic considerations
3.1.1 Background

Each case requires an individual procedure incorporating all available information (e.g. results of police investigations including description of the scene of the crime, post-mortem report, medical reports, details of medication etc.). This information is the basis for the choice of appropriate analytical specimen(s).

3.1.2 Choice of sample material

Providing no specific requests have been made which require a complex analytical procedure, then urine and blood - if sufficiently available - are the materials of choice for a systematic toxicological analysis (STA).

For screening purposes, urine is the material of choice as it reflects a long time-window for identifying ingested substances and allows the detection of metabolites and xenobiotics often being present in high concentrations. Alternatively, extruded fluid from kidney, liver tissue, pericardial fluid, vitreous humour or other body fluids may be used for screening. Stomach contents may also be used; however, a stringent optical check must be carried out prior to analysis.

Blood drawn from peripheral veins is the material of choice for quantitative analyses. If blood samples are unavailable, then analyses may be carried out with cardiac blood, vitreous humour, CSF (particularly when identifying endogenous substances), muscle tissue (for alcohol analysis when blood samples are unavailable) and brain tissue. This must be considered in the interpretation.
Under certain circumstances (e.g. advanced corporeal decomposition or suspected poisoning), the analytical procedure must be adapted accordingly.

3.1.3. Internal standards (IS) and control material

Analytical methods and extraction procedures used for general screening must be checked with a standard test mixture. Various commercial test mixtures are available for urine to check cleavage of phase-II-metabolites, extraction and the analytical method. Alternatively, a mixture of 15 substances (THC, THC-COOH, amphetamine, benzoylecgonine-D3, cocaine, codeine, diazepam, doxepine, methadone, metoprolol, morphine, ibuprofen, paracetamol, phenobarbital, salicylic acid) as recommended by the working group 'Extraction' of the GTFCh in a suitable drug-free matrix (e.g. tissue homogenate) may be used.

Recommendations for the choice of internal standards are given in the Guidelines of the GTFCh for Quality Assurance in Forensic-Toxicological Analysis. Suitable internal standards are also required for screening tests and should always be added at the earliest stage of the analytical procedure. If the internal standard has been prepared in an organic solvent, then care must be taken to ensure that no relevant precipitation occurs when added to the sample.

3.2. Sample preparation

3.2.1. Pre-treatment

3.2.1.1 Body fluids

Body fluids which cannot be pipetted exactly must be weighed for quantitative analyses.

3.2.1.2 Urine

Phase-II metabolites must be cleaved by either enzymatic or chemical hydrolysis.

3.2.1.3 Blood

Check the homogeneity of whole-blood samples. Instead of or in addition to whole-blood samples, the supernatant of a centrifuged sample (“post mortem serum”) may be used.

3.2.1.4 Other fluids (Table 2)

Here is the sample preparation analogous to that of urine or blood. Hydrolysis is not generally required.

3.2.1.5 Tissues

Tissue samples should be homogenised. For brain and highly decomposed tissues, a suitable disintegration method may be used. Highly collagenous tissues require intensive mechanical disintegration. Ensure a uniform distribution of internal standards.

3.2.1.6 Gastric and intestinal contents, vomit

As these materials may contain large amounts of foreign substances (e.g. bodypack contents or tablets), ensure that there is no cross-contamination. If required, macroscopically visible contents (e.g. tablets) are removed prior to homogenisation.

3.2.1.7 Other materials (e.g. from the scene)

No general recommendations can be made because of the wide range of suspected substances. Attention should be given to possible dangerous substances (e.g. chemical weapons and explosives) and also to the possibility of cross-contamination. The Guidelines for Quality Assurance of Forensic-Toxicological Analysis of Narcotics and Drugs of the GTFCh are to be followed.

3.2.1.8 Other under 2.2 (Table 2) listed material

Due to the complexity of specialised sample material, relevant analytical methods must be developed and documented.

3.2.1.9 Hair samples

Hair samples are to be taken, stored and analysed according to Appendix C of the Guidelines for Quality Assurance of Forensic-Toxicological Analyses 'Requirements for the Analysis of Hair Samples'. Special attention must be given to the danger of contamination by putrefactive fluids, blood or other adherents. In special cases it may be necessary to employ more comprehensive washing stages than those used in the routine procedures. Hair samples are to be stored dry and away from light as soon as possible.

3.2.1.10 Nails and skin

Special methods are required for the extraction of nails and skin. These methods must ensure that the substances to be extracted are stable.

3.2.1.11 Bone and bone marrow

Bone marrow contains a high lipid content and requires special digestion and extraction methods.

3.2.2. Dilution, precipitation, extraction

3.2.2.1 Dilution

Certain analytical methods require no further sample preparation other than a dilution step with buffer or a mobile phase for liquid chromatography.

3.2.2.2 Precipitation

Protein precipitation is a special form of dilution and is a convenient method of rapid sample preparation for both quantitative and highly specific qualitative screening

methods. Usually an organic solvent such as acetonitrile, acetone, methanol or a combination of these is used. The volume of solvent must be chosen to provide a complete precipitation (e.g. 3x the sample volume). The solvent should be cooled. For certain specialised methods, other substances such as perchloric or trichloroacetic acid or addition of zinc sulphate or barium chloride are suitable.
There is always the risk that a certain amount of the analyte will be lost during this stage. This can be assessed by analysing suitable control material.

3.2.2.3 Liquid-liquid extraction (LLE)

Liquid-liquid extraction (LLE) is a well-established method of sample preparation and is suitable for the analysis of post-mortem body fluids or tissues.
A STA requires the extraction of acidic, neutral and alkaline substances at appropriate pH levels. The pH value can be adjusted by the addition of buffer or salts. For non-targeted analyses, extraction with organic solvent mixtures such as dichloromethane/2-propanol/ethyl acetate (1:1:3, vol%) is recommended. Highly polar solvents or mixtures with a highly polar content increase the extraction yield for many substances; however, this results in extracts with a high matrix content requiring further clean-up steps (e.g. re-extraction) depending on the analytical method. It is recommended that extracts obtained at various pH values are to be kept separate and analysed individually.
LLE with 1-chlorobutane under alkaline conditions provides relatively clean extracts. Thus, in the course of an extended target screening, a large number of toxicologically relevant substances can be detected. Data on the extraction of substances with this method are available (7). The following method is recommended for blood and urine samples:
Adjust sample pH value to 8 or 9 (or other dependent on the analytes) with an equal volume of buffer solution.
Add internal standard and extract sample with 1-chlorobutane (approximately 2fold volume).
For polar analytes, addition of 2-propanol (10%) to the extraction solvent is recommended.
In general, ensure that no emulsions are formed with LLE. Suitable procedures for this include gentle shaking, addition of dry, chemically inert sorbent (supported liquid extraction) or saturated solutions of neutral salts. When the phases do not separate, high-speed centrifugation in microtubes or freezing of the aqueous phase may be used. For tissue samples, modified procedures are necessary (e.g. extraction of highly diluted homogenates with subsequent clean-up).

3.2.2.4 Solid-phase extraction (SPE)

Solid-phase extraction (SPE) methods are useful for post mortem body fluid and tissue samples particularly for highly proteinaceous samples or for automated extraction procedures. Samples should be sufficiently diluted and centrifuged to remove small particles which would impede solvent flow through the column.
Choice of solid phase should ensure a limited selectivity. Sorbents based on silica-gel or styrene polymers may be used, also mixed-phase sorbents with hydrophobic or cation-/anion-exchange capacities, thus providing a sequential separation of acidic, neutral and alkaline components. It is recommended that all extracts be analysed separately as usually the alkaline extract has less contaminating matrix components as the acidic extract.

3.2.3 Headspace techniques

Screening of volatile substances is an integral part of a STA. Together with ethanol, other volatiles such as acetone, 2-propanol, congener substances, diethyl ether, halogenated hydrocarbons, trichloro ethanol, anaesthetics, propane and butane play important roles.
Volatile substances are usually excreted unchanged via the lungs. Blood, lung and brain tissue are appropriate sample materials. In cases of suspected poisoning with volatile substances, samples must be transferred to hermetically sealed glass tubes (e.g. with PTFE-coated septa).
Analytical conditions must be chosen carefully with respect to addition of salts, incubation time and temperature, transfer of the gas-phase to the GC and also choice of GC-column.
When headspace techniques are unavailable, then the use of solid-phase micro-extraction (SPME or SPDE) provides a simple and less expensive alternative for at least qualitative analyses. Mass-spectrometric detection provides a reliable identification of substances.

3.2.4 Dried-matrix-spot analysis (DMS)

Matrix-spots are ideal for a long-term storage of sensitive analytes. Compounds are stabilised due to the absence of water and many are stable at room temperature even months after 'spotting' on a suitable material. Spotting on chromatography paper or special materials is done immediately after receipt of the sample. The spots should be evenly homogenous with a small diameter. Volumes >20 µL should be pipetted slowly and carefully to avoid a large diameter. Tissue homogenates can also be spotted thinly.
The whole spot is used for analysis and must be punched or cut out. For quantitative analyses, the extraction yield must be verified (cf. SPE methods).
For qualitative analyses is the choice of eluent (methanol or acetonitrile) usually sufficient.

3.3. Detection methods
3.3.1 Instrumental analysis

Complementary analytical methods should be used which best cover the spectrum of those substances under investigation. Such methods include headspace techniques, immunochemical tests and gas- and liquid chromatography with individual substance identification.
Requirements for the analysis and identification of substances are given in the Guidelines for Quality Assurance in Forensic-Toxicological Analyses of the GTFCh and the literature (8,9).
In trace-metal analysis - either with atom-absorption spectroscopy (AAS), ICP-MS or X-ray fluorescence spectroscopy (TRFA) - special precautions (sample contact with metal needles or autopsy instruments, sample storage in special tubes for trace-metal analysis) must be considered. If not, then analysis of a blank sample obtained and stored under identical conditions is mandatory.
In the following, particular aspects of the analysis of post mortem material are considered. Details are given in the literature (5,10).

3.3.2 Immunochemical methods

These pre-tests alone provide no proof and all positive results must be confirmed with a clearly identifying method.
Immunoassays may be used directly with urine or other transparent fluid samples. For other matrices, protein precipitation is required or other heterogeneous methods such as ELISA can be used. ELISA methods are less susceptible to giving false-positive results because of the washing stages involved. With post mortem material however, false-positive results may occur with all tests - in particular with amphetamine and related assays.

3.3.3 Carboxyhemoglobin (CO-Hb)

The time gap between autopsy and the CO-Hb test should be reduced to a minimum. If not, a blood sample may be stabilised with fluoride.
The classical determination of CO-Hb in blood is usually based upon the measurement of the absorption of a hemolysate at different wavelengths before and after addition of dithionite. This method, however, does not allow for the possible post mortem changes resulting in methemoglobin (MetHb) or sulphemoglobin formation. In bodies exposed to fire or in heat shock victims, thermo-coagulation can lead to a significant decrease in soluble hemoglobin and an increase in methemoglobin.
A CO oximeter provides a direct measurement of various heme species. MetHb levels >10% and Hb levels <4 g/dl - as often found in post mortem samples - can lead to false CO-Hb levels.
A direct measurement of CO with chromatographic methods (GC-FID, GC-MS, GC-WLD) after acid hydrolysis requires the determination of the Hb content using a modified cyanmethemoglobin method or a normalisation either of the total CO binding capacity (sample saturation with CO) or iron content. The latter correlates well with hemoglobin but requires a separate iron assay. This method is also useful for assaying muscle tissue.
Direct CO analyses also measure that evolved during the putrefaction process but this may only be of significance when low CO-Hb levels are found.

3.3.4 Cyanide

Cyanide can be detected with Cyantesmo® (positive: blue) or Dräger® (positive: orange) test strips in blood and other specimens after acid treatment. Confirmation is achieved with either gas-chromatographic, fluorimetric or colourimetric methods.

3.3.5 Labile substances

Well-known examples of labile substances are zopiclone, olanzapine, cocaine, benzodiazepines containing a nitro moiety, organophosphorus insecticides etc. Phase II metabolites can be split and give rise to a falsely increased level of free, pharmacologically active substances (e.g. morphine glucuronides). Furthermore, advanced corporeal decay can lead to changes in concentration of almost all drugs and medication levels relative to the respective sampling site.

3.3.6 Alcohol

Ethanol can be produced in the course of progressive decomposition, but is rarely also degraded. A lack of ethyl glucuronide or ethyl sulphate in the presence of relevant ethanol concentrations suggests post mortem synthesis. In the case of suspected ethanol neoformation, analysis of other samples (e.g. brain, muscle being not or only slightly subjected to putrefaction, vitreous humour) in addition to blood is recommended. Ethanol may occur in large amounts in urine - particularly in that of diabetics with high urinary glucose levels - and can also increase after sampling. Simultaneous analysis of volatiles (1-propanol, 1-butanol) can provide evidence of postmortem neoformation of alcohols.

3.3.7 Gamma-hydroxybutyric acid (GHB)

GHB may also be synthesised in post mortem tissue. Therefore, it is highly recommended that, in addition to blood, other samples (e.g. urine, CSF or vitreous humour) are analysed. Analysis of urine and gastric contents (check for GBL) can prove or disclaim a suspected poisoning.

3.3.8 Trichloro compounds (Fujiwara Test)

This screening test uses a strongly alkaline pyridine solution and can be used for urine, serum and gastric contents. An intense red/purple colour indicates the presence of trichloro substances such as chloral hydrate, trichloro ethanol, chloroform, trichloroacetic acid, trichloroethane and trichloroethylene. In the presence of di- and tetra-chlorinated compounds, the colour may be different or completely absent (e. g. carbon tetrachloride).

3.3.9 Clinical chemistry parameters

If these parameters are determined in forensic-toxicology laboratories, then the respective matrices are chosen in conjunction with the respective pathologist to assess the condition of the corpse.
The determination of ketone bodies (β-hydroxybutyrate (BHB), acetoacetate, acetone) in post mortem blood provides information of a possible diabetic or alcohol-induced ketoacidosis. However, these parameters can change after death. If a ketoacidosis is the cause of death, then the concentration of BHB is usually so high that a relatively accurate diagnosis can be made, esp. in the case of a positive clinical history. Various methods are available to assay BHB - direct assay with gas-chromatography/mass spectrometry after derivatisation or indirect after enzymatic hydrolysis and detection of acetone. Determination of glucose, lactate, acetone and 2-propanol in vitreous humour, CSF and urine together with glycosylated hemoglobin (HbA-1c) in whole blood can be used to confirm whether a sugar metabolism disorder was present prior to death.

3.3.10 Identification and quantitation

Mass spectrometry is used to identify most toxicologically relevant substances.
Other unspecific detection methods may also be used to quantitate.
Identification criteria are given in the GFTCh Guidelines for Quality Assurance in Forensic Toxicology.

When using mass-selective methods, isotopically labelled standards should be used when available. The concentration of these standards should correlate with the levels to be expected.

3.3.11 Standard addition

When no isotopically labelled or suitable internal standard is available, then the standard addition method may be applied provided sufficient sample is available. It must be confirmed that a representative aliquot is used, a precise amount is weighed in, the linearity is correct throughout the range of measurement and that the method is reliable. It is recommended that for a standard addition to the original sample (level 1), three other levels (levels 2-4) are used. Based upon the estimated concentration x, then level 2 is 0.5x added followed by level 3 (1x) and level 4 (1.5x).

4 Interpretation of post-mortem results

The following points illustrate the multifactorial challenges encountered when interpreting results from samples obtained post mortem. Analysis and interpretation are solely the responsibility of an experienced forensic toxicologist. The final report can only be formulated after careful consideration of all aspects of the case including discussions with the respective forensic pathologist (11). Aspects to be considered in certain cases:

4.1. Aspects unrelated to the result

- Aim of the investigations, question(s) to be answered
- Information on the circumstances of death
- Scene
- Duration of the post mortem interval, storage of the corpse
- Medical history (pre-existing diseases, medication, addiction)

4.2. Aspects related to the result

- Significance of a result in a particular matrix
- Mode of administration of the xenobiotic, interpretation of results in different compartments
- Validity check of results (various matrices, metabolites, various analytical methods)
- Stability of analytes
- Availability of reference ranges
- Post mortem redistribution

4.3. Pharmacological aspects

- Age, organ function
- Pharmacodynamic and pharmacokinetic interactions
- Polymorphisms (pharmacodynamic, pharmacokinetic)
- Metabolite ratios, metabolite saturation

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6. Implementation

These recommendations in their present form were ratified by the executive committee of the GTFCh on 30th. November 2016 and are effective as of the date of publication in Toxichem Krimtech.

Only the original German version of these guidelines is applicable for forensic purposes.

The current version is available on the GFTCh homepage (www.gtfch.org).