



SHORT COMMUNICATION

Recommendations of the working group of the Anatomische Gesellschaft on reduction of formaldehyde exposure in anatomical curricula and institutes



Jens Waschke^{a,*}, Martin Bergmann^b, Lars Bräuer^c, Erich Brenner^d, Andreas Buchhorn^e, Arlette Deutsch^f, Martin Dokter^f, Desalegn Tadesse Egu^a, Süleyman Ergün^w, Ulrich Fassnacht^g, Daniela Fietz^b, Stefanie Gundlach^h, Stephan Heermannⁱ, Bernhard Hirt^j, Daniela Kugelmann^a, Magdalena Müller-Gerbl^k, Wolfram Neiss^l, Ute Nimtschkeⁿ, Johanna Plendl^o, Michael Pretterklieber^p, Christoph Redies^q, Martin Scaal^m, Mirko H.H. Schmidt^r, Andreas Schmiedl^e, Hans-Joachim Schnittler^s, Christof Schomerus^t, Tamás Sebestény^r, Björn Spittau^u, Birte Steiniger^v, Thomas Tschernig^A, Axel Unverzagt^a, Christoph Viebahn^x, Ernst Voigt^g, Janet Weigner^o, Imke Weyers^y, Andreas Winkelmann^z, Merle Winkler^h, Friedrich Paulsen^{c,*}

^a Institute of Anatomy, Chair of Vegetative Anatomy, Medical Faculty, LMU Munich, Munich, Germany

^b Institute of Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Giessen, Germany

^c Institute for Functional and Clinical Anatomy, University of Erlangen-Nürnberg, Erlangen, Germany

^d Department of Anatomy, Histology and Embryology, Division of Clinical and Functional Anatomy, Medical University of Innsbruck, Innsbruck, Austria

^e Institute for Functional and Applied Anatomy, Medical University of Hanover, Hanover, Germany

^f Institute of Anatomy and Cell Biology, Ernst Moritz Arndt University of Greifswald, Germany

^g Institute of Anatomy and Cell Biology, University of Ulm, Ulm, Germany

^h Christian Albrecht University, Anatomical Institute, Kiel, Germany

ⁱ Institute of Anatomy and Cell Biology, Department of Molecular Embryology, University of Freiburg, Freiburg, Germany

^j Anatomical Institute, Eberhard Karl University, Tübingen, Germany

^k Anatomical Institute, University of Basel, Basel, Switzerland

^l Institute of Anatomy I, Universität zu Köln, Cologne, Germany

^m Institute of Anatomy II, Universität zu Köln, Cologne, Germany

ⁿ Institute for Anatomy of the C.G. Carus Medical Faculty of TU Dresden, Dresden, Germany

^o Institute of Veterinary Anatomy, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

^p Division of Anatomy, Centre for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Germany

^q Institute of Anatomy I, Friedrich Schiller University, Jena, Germany

^r Institute for Microscopical Anatomy and Neurobiology, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

^s Institute of Anatomy, University Clinics of Westphalian Wilhelm University of Münster, Münster, Germany

^t Dr. Senckenberg Anatomy, Johann Wolfgang Goethe University, Frankfurt, Main, Germany

^u Institute of Anatomy, Medical University of Rostock AöR, Rostock, Germany

^v Institute of Anatomy and Cell Biology, Philipps University, Marburg, Lahn, Germany

^w Institute of Anatomy and Cell Biology, University of Würzburg, Würzburg, Germany

^x Institute of Anatomy and Embryology, Center of Anatomy, University Medical Center Göttingen, Göttingen, Germany

^y Institute of Anatomy, University of Lübeck, Lübeck, Germany

^z Institute of Anatomy, Brandenburg Medical School, Neuruppin, Germany

^A Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Saarland, Homburg, Saar, Germany

ARTICLE INFO

Article history:

Received 17 October 2018

Accepted 17 October 2018

ABSTRACT

The practice of human and veterinary medicine is based on the science of anatomy and dissection courses are still irreplaceable in the teaching of anatomy. Embalming is required to preserve body donors, for which process formaldehyde (FA) is the most frequently used and well characterized biocidal substance. Since January 2016, a new occupational exposure limit (OEL) for FA of 0.37 mg/m³ issued by the European

* Corresponding authors.

E-mail addresses: jens.waschke@med.uni-muenchen.de (J. Waschke), friedrich.paulsen@fau.de (F. Paulsen).

Keywords:

Formaldehyde exposure
Body donation
Embalment

Committee on Hazardous Substances is obligatory since FA has been classified as a human 1B carcinogen. The anatomical institutes in the German-speaking region are called upon to consolidate efforts to reduce use of FA in anatomical curricula and body donations. As a result, the Anatomische Gesellschaft (AG) has formed a “Working Group for Reduction of Formaldehyde Exposure in Dissection Courses” tasked with discussion and recommendation of measures to reduce FA. Based on the assessment of the Working Group, the AG has issued an official opinion to the effect that, at this point in time, embalming of body donors without FA completely is not feasible. Therefore, a combination of approaches are to be used to reduce FA exposure, including technical and structural (architectural) adaptations, modification of protocols for fixation and preservation as well as organizational measures. One structural measure considered unavoidable is the integration of air supply and exhaust of individual dissecting tables into the ventilation system of the anatomy building. To embalm human body donors, intra-arterial perfusion fixation with up to 4% FA and a total fluid volume of 150 mL/kg body weight will suffice. For animals where body weights and biology of bodies vary widely (i.e. special needs of fixation for ruminants, large animals as horses) perfusion fixation with up to 4% FA and a quantity of fixative solution of 10–15% of the body weight may be required. Preservation of body donors in storage (immersion) can be done with 40% ethanol or in a full bath preservation containing up to 2% FA. Corpse humidification in the dissecting room is possible with 2% phenoxyethanol, in each case without FA. In veterinary anatomy, microbiological burden is often higher and therefore might lead to a need of FA in long-time storage. Compliance with the current OEL in all institutes would appear to be feasible in combination with various organizational measures.

© 2018 Elsevier GmbH. All rights reserved.

1. Introduction

Anatomy is a major root of medical practice. This is the consensus of instructors of pre-clinical and clinical subjects worldwide. Whenever possible, anatomical training is based on body donors who have willed their bodies by written consent during lifetime to anatomical institutes for academic and medical research purposes. This most valuable and effective way to teach anatomy has been repeatedly defended against critics (Ochs et al., 2012; Ramsey-Stewart et al., 2010; Korf et al., 2008; Paulsen, 2007), particularly regarding criticism of cadaver-based teaching by politicians due to the relatively high costs involved (Korf et al., 2008), resulting in abandonment of dissection courses in some countries (e.g. Italy in the 70s and more recently in several American universities), measures that are now bitterly regretted and have led to student protests (Drake et al., 2009) and even re-establishment. The critics focused in particular on the virtual digital material now available, which proponents of dissection courses consider to be supplement rather than replacement (Paulsen et al., 2010; Bertelshofer et al., 2016; Kugelmann et al., 2018). A highly diverse selection of materials are indeed now available, ranging from virtual course offerings (e.g. the Virtual University in Bavaria), virtual media such as Magic Mirror or commercially purchased Anatomage® Tables, sonographic and other supplemental imaging materials to conventional supplements such as anatomical models, textbooks and colouring books. Though additional improvements in terms of learning success do appear to be feasible and students consistently welcome the use of virtual media, both students and instructors soon become aware of the limitations of these systems and are quick to confirm the assessment that the dissection course is in fact irreplaceable (Korf et al., 2008).

The preparation of donated bodies for anatomical instruction normally requires an extensive embalming process. Paulsen and Tschernig carried out a survey of body donation at German-speaking anatomical institutes based on a meeting of the prosectors of these institutes in 2003 and presented the results in a satellite symposium at the 21st Working Conference of the Anatomical Society in Würzburg in 2004. The survey revealed how varied the use of various biocidal substances and mixtures in German-speaking institutes was at the time (2003) (Tables 1 and 2). It also confirmed the position of formaldehyde (FA) as the most commonly used biocidal substance. In 2004, a working group of the International

Agency for Research on Cancer (IARC) classified FA in group 1B (containing substances likely to be carcinogenic in human) based predominantly on evidence in animal experiments, followed by the definition in 2014 by the Committee on Hazardous Substances (AGS) of a new occupational exposure limit (OEL) of 0.37 mg/m³ with an exceedance factor of 2 at peak load, with compliance to be required starting from January 2016 (Thullner et al., 2015; Thullner et al., *Gefahrenstoffe-Reinhaltung der Luft* [Clean Air – Hazardous Substances] Issue 6, 2015). It has to be taken into consideration that the layer concentration (“Schichtkonzentration”) is the relevant factor which has to be below the OEL. Even if the primary measurement was higher than 0.37 mg/m³ the layer concentration might be clearly below the OEL. In addition, it was also stated that foetal damage was not to be expected if the OEL was complied with.

Upon announcement of the new limit value, some anatomical institutes feared they would be unable to comply with it starting in 2016 as stipulated. Therefore, the Anatomical Society (AG) founded a Working Group for Reduction of Workplace Formaldehyde Exposure (RedFAEx) in September 2015 to assess and formulate measures to reduce FA exposure in both anatomy classes and dissection labs and achieve reliable compliance with the OEL. The working group was nominated during the General Assembly of the AG in the year 2015 and interested prosectors and preparators from German-speaking anatomical institutes were invited to participate. Since November 2015, the working group has convened annually in Munich and issues information to AG members once a year. The RedFAEx is in continuous contact with the accident insurance funds (Ingrid Thullner, Accident Insurance Fund, Hesse; Ludger Hohenberger, Accident Insurance Fund, North Rhine-Westphalia), the Institute for Occupational Safety and the Statutory Accident Insurance Fund (IFA; Reinhard Stockmann) as well as the Federal Office for Chemicals (BfC), and advises the Research and Consulting Institute on Hazardous Materials (FoBIG, Freiburg im Breisgau, Germany).

The aim of the article is to provide a brief overview of the current status of efforts of RedFAEx, to encourage critical engagement with the FA issue and to develop guidelines for potential FA reduction measures in anatomical teaching and in dissection practice. All information not backed by references represents results of RedFAEx discussions. Information given in this article is understood to have recommendation character, only. There is no normative aim and any of the recommendations may be adapted to the given

Table 1
Substances used for fixation of body donors (ingredients only) and storage of body donors prior to use (in 2003)^a.

	Fixative solution	Storage
Aachen	Formaldehyde, ethanol	
Berlin Charité	Formaldehyde, ethanol, glycerin, water	Sprinkler system
Berlin (formerly FU)	Formaldehyde, H ₂ O, ethanol	Thalheimer system. Spraying with fixative solution free of ethanol.
Bochum	No information	No information
Bonn	Formaldehyde, glutaraldehyde, ethanol, glycerin	Thalheimer system
Dresden	Formaldehyde, ethanol, glycerine, aqua dest.	Thalheimer system
Düsseldorf	Ethyl alcohol, aqua dest., glycerin	In 8% formaldehyde
Erlangen	Formalin, ethanol, glycerin, fungicide, disinfectants, water	0.5% formaldehyde (1–1.5 years, then propanol for ½ year)
Essen	Formaldehyde in ethyl alcohol	Thalheimer system
Frankfurt	Formaldehyde	
Freiburg	Formaldehyde, Incidin Plus, Karion, thymol dissolved in ethanol, water	Thalheimer system
Giessen	Formaldehyde, phenol, thymol, water	Terralin, water
Greifswald	Formaldehyde, water, ethanol, glycerin, thymol, oil of cloves	Formaldehyde
Halle	Formaldehyde with phenol fractions	Ethanol (6 months, then cooling cell)
Hamburg	Jores I (1 day), then 96% alcohol	
Hanover	Ethanol, formalin, glycerin	Stainless steel cuvettes with ethanol (>60%)
Heidelberg	Formaldehyde, phenol, glycerin, ethanol, water	Thalheimer system
Homburg	Formaldehyde, aqua dest., Karion, Lysoformin, calcium chloride, sodium chloride, (chloral hydrate), thymol	Thalheimer system
Innsbruck	Formaldehyde 3%, phenol 3%	Immersion in phenol 4% for up to 6 month, then weld-sealed in plastic foil
Jena	Ethanol, formaldehyde, salicylic acid, thymol, glycerin	Fixative bath (70% ethanol)
Kiel	Ethanol, formaldehyde, glycerin	From 5th month: well-sealed in soaked cloths
Cologne	Ethanol, formaldehyde	Ethanol (70%), then cooling cell
Leipzig	Ethanol, formaldehyde, glycerol + Fixation acc. to Thiel	Cooling container
Lübeck	Formaldehyde, ethanol, glycerin	
Mainz	Ethanol, formaldehyde, glycerin, water	Thalheimer Wall → in containers filled with ethanol
Marburg	Ethanol, formaldehyde, fungicide	
Munich	Formaldehyde-ethanol fixation	Formaldehyde-ethanol preservation
Münster	Formaldehyde, chloral hydrate (Thiel fixation as needed)	
Regensburg	Formaldehyde, phenol, glycerin, ethanol	Tub solution 4% formalin
Rostock	Aqua dest., ethanol, glycerin, formaldehyde, thymol, salicylic acid	0.5% phenol solution in container with lid
Tübingen	Formaldehyde, glycerin, ethanol; Thiel fixation	Thalheimer Wall
Ulm	Isopropanol, lysoformin, glycerin,	Thalheimer Wall
Würzburg	Formaldehyde in saline solution with additives	Ethanol vapour atmosphere

^a From [Paulsen and Tschernig \(2004\)](#).**Table 2**
Fixatives and additives used by anatomical institutes in German-speaking countries in 2003^a.

<input type="radio"/> Formalin	<input type="radio"/> Fungicide
<input type="radio"/> Ethanol	<input type="radio"/> Disinfectants
<input type="radio"/> Glycerin	<input type="radio"/> Jores I (Jores, 1896)
<input type="radio"/> Water	<input type="radio"/> Lysoformin* (Isotridecanol, Didecyldimethylammoniumchloride, Polyhexamethylenbiguanid-HCl)
<input type="radio"/> Glutaraldehyde	<input type="radio"/> Calcium chloride
<input type="radio"/> Phenol	<input type="radio"/> Sodium chloride
<input type="radio"/> Oil of cloves	<input type="radio"/> Chloral hydrate
<input type="radio"/> Incidin Plus* (Glucoprotamine)	<input type="radio"/> Hosbeil
<input type="radio"/> Karion* (Sorbitol)	<input type="radio"/> Thiel's method (Thiel 1992)
<input type="radio"/> Thymol	<input type="radio"/> Isopropanol
<input type="radio"/> Salicylic acid	<input type="radio"/> Phenoxyethanol

^a From [Tschernig and Paulsen \(2004\)](#).

structural and organizational conditions at individual anatomical institutes.

1.1. FA used as fixative in anatomical dissection courses and clinical courses

A feature shared by all embalming protocols is use of biocidal substances for effective prevention of decay and rotting and reliable killing of pathogens. Therefore, all biocidal substances are toxic by definition and can also be mutagenic and carcinogenic above a certain concentration. Probably the best characterized biocidal substance is FA, and its effect profile, including both biocidal activity and tissue stabilization, suggests that it is currently the best-suited substance. FA has been used for fixation and preservation of cadavers in anatomical applications starting about 1893 ([Blum, 1894](#); [Fox et al., 1985](#)), which made it possible for the first time to stop decay

and kill infectious germs reliably ([Brenner, 2014](#)). Since then, various protocols have been used for fixation, characterized mainly by use of 3–10% FA, so that FA can be considered the world's most commonly used biocidal substance in fixation of body donors ([Brenner, 2014](#); [Benkhadra et al., 2011](#)). In view of limitations to use in clinical courses due to pronounced changes in tissue haptics resulting from FA use, as well as tissue hardening and accompanying joint stiffening, additional protocols were developed for clinical courses, for example Thiel's embalming method ([Thiel, 1992](#)) and ethanol-glycerin embalming ([Hammer et al., 2012](#)) and the nitrite pickling salt embalming ([Janczyk et al., 2011a, 2011b](#)). In principle, these protocols are also suitable for use in anatomy classes, the method followed at some institutions. They are, however, very time-consuming and costly, which is the case with Thiel's method ([Benkhadra et al., 2011](#)) and contain other toxic substances besides FA that restrict their use ([Thiel, 1992](#)). Some mixtures, e.g. in

Table 3
Opinion and recommendation by the “Formalin” Working Group for Reduction of Formaldehyde Exposure in Dissection Courses.

Complete substitution of formaldehyde in human and veterinary medical dissection courses is not currently feasible. Nonetheless, the greatest possible reduction of formaldehyde exposure should be sought by combining structural measures with protocol amendments for fixation and storage preservation as well organizational measures.

The deadline for compliance with the new OEL for formaldehyde of 0.37 mg/m³ is 01 Jan. 2016. Proposed procedure until then:

In view of the obligations of the institutional managers responsible for cadavers regarding

- measurement of formaldehyde concentration and
- implementation of measures to reduce exposure,

the relevant state accident fund should be contacted via the Occupational Safety Staff Office to coordinate implementation of the following measures:

- 1) Measurements: The accident funds have reached an agreement on standardized procedures. Measurement methods: Active collectors (with absorber and pump), detection method: HPLC. All other measuring methods, such as test tubes or online measuring devices, are too imprecise and are suitable for orientational measuring only!
- 2) Risk Assessment: Required if limit level is exceeded.
- 3) Binding requirements: To avoid legal uncertainty, the conditions should be set out in writing under which continuation of academic courses can be approved. The accident insurance company will only then carry the liability in case of an accident. Alternatively, the supervisory authority may enforce the discontinuation of the dissecting course or other organizational measures.

glycerin-ethanol embalming solution, entail a risk of accelerated dehydration, especially when ventilation equipment is used, and require raised levels of explosion protection, e.g. ethanol. The nitrite pickling salt embalming is not expensive and is highly suitable for clinical courses as it can be used for some weeks. In addition, the anti-infection effect of the respective procedures is not entirely clarified. While natural haptics are considered advantageous by experienced operators in invasive procedures, they are impractical for inexperienced dissectors, e.g. in anatomy classes, because particularly fragile structures such as blood vessels and nerves do not lend themselves to presentation and preservation over longer periods (Benkhadra et al., 2011). These properties, and in particular the increased risk presented by infectious germs, limit the use of these protocols, at least in anatomical student tuition; on the other hand, these are the most commonly used protocols for preparation of body donations for clinical courses (Eisma and Wilkinson, 2014; Hammer et al., 2012) except when non-embalmed body donors are used after freezing and thawing.

In summary, it can be concluded that, at the present time, complete rejection of FA for preservation of body donations in curricular dissection courses is not feasible at all locations. This was confirmed in the official statement issued by the AG in 2017 based on the Red-FAEx assessment (Table 3). Abandoning FA prematurely in favour of other biocidal substances that are currently classified as non-carcinogenic, or at least less so, on the basis of less robust data, should therefore be avoided.

1.2. Measures to reduce FA exposure in anatomy

To be most effective, measures to reduce FA exposure for embalming and in dissection courses should comprise technical and structural measures, modification of fixation and preservation protocols and organizational procedures.

1.3. Technical and structural measures to reduce FA exposure

Recent years have witnessed extensive gains in knowledge of how to reduce FA exposure in dissection courses effectively by technical means. During the renovation of the Anatomical Institute in

Munich, measurements were compared between the pre-existing room extraction system and the newly installed single table extraction system. This comparison showed a reduction of measured levels by approximately one-half without modification of the overall extraction volume. Comparable results were obtained in Erlangen, where newly installed single table extraction units were connected to the old ventilation system. In the new build Anatomy at the WWU-Münster single table extraction and an top down airflow system with illumination combined with room ventilation reduced the FA exposure far below the maximal concentration of 0.3 mL/m³ or 0,3 ppm, irrespective that the body donors were perfused with 4% FA and maintained in 2,5% FA. Systematic analysis by the accident insurance funds in cooperation with the Institute for Occupational Safety and Health of the Statutory Accident Insurance (IFA) underline the effectiveness of installation of general-purpose room ventilation technology and single table extraction in particular (Thullner et al., 2015). These measures contribute decisively to reduction of FA exposure. However, the measurements also show that single table extraction units alone cannot be relied upon for OEL compliance in all facilities, demonstrating the need for revision of the embalming protocols used. The additional equipment for top-down ventilation systems further and, where the latter is not a feasible option, revision of the embalming protocols used.

The following technical structural parameters reduce FA exposure (Thullner et al., 2015):

- Maintenance of a low room temperature of approximately 16–18 °C during the dissection course. Lower temperatures may have undesirable effects, since the temperature difference compared to the students and instructors present – heat sources – results in air turbulence that causes FA to rise, whereas it would normally sink since it is heavier than air, thus increasing inhalative exposure.
- Dilution effects in rooms of sufficient height and volume.

If measuring results are to be reliably comparable they must be obtained according to standardized procedures. The standard sampling procedures recommended by accident insurance funds and the IFA are based on the measuring strategy of the Hessian Accident insurance Fund (IFA, 1989 IFA Workbook Hazardous Substances, 1989; ID 6045) and include time-weighted average and short-term values as well as individually worn metering units and simultaneous stationary measurements. Active accumulation systems are installed at the level of respiratory intake of the dissecting person, with stationary units at the same height, whereby the samples are then analysed by the IFA.

Based on these results, the IFA and the accident insurance funds of Hesse and North Rhine-Westphalia initiated a research project in cooperation with the company ROM (Hamburg, Germany) aimed at defining the required table extraction and ventilation specifications. To ensure practicable implementation, the project was supported by the Anatomical Institutes in Munich (Jens Waschke and Axel Unverzagt) and Marburg (Ralf Kinscherf and Jens Cordes), acting in an advisory capacity. The initial results point up the necessity of avoiding mixing ventilation due to non-laminar (turbulent) airflow between air supply and air extraction (Dahncke et al., 2016). Practicable supporting measures are:

- Air supply vents with skirting corresponding to the table size (height 20 cm) for laminar airflow. Textile vents with turbulent outflow resulted in mixing ventilation.
- Air vent exit temperatures should not exceed 17 °C.
- Dissipation of the thermal loads by means of additional chilled ceilings and additional air influx independent of the dissecting tables, the aim being to avoid air turbulence.

- Some air extraction units close to floor level and at least 50% single table extraction. A vortex hood on the air extraction slit surrounding the entire table may increase extraction efficiency (not scientifically proven).
- The extraction volume correlates with metering effectiveness and thus with measurable FA exposure. The extraction volume used by the company ROM, 2000 m³/h, was significantly more effective than the 300 m³/h applied in an existing facility. A systematic investigation of the extraction volume required for compliance with OEL was, however, not carried out and it appeared likely that high extraction volumes might result in excessive suction (and drying) effects, drafts and noise – all conditions not conducive to efficient dissection procedures.

The research project measurements were made under idealized conditions at a single dissection table using 6–12 stationary dummies (Dahncke et al., 2016). Simulations done in the research project revealed clear increases in exposure levels due to significant air turbulence caused by shifting the posture of only two dummies per table, i.e. leaning them forwards into the dissection field. The question that arises is whether such an investigation under the given conditions can be extrapolated to real dissection theatres, some of which feature historical architecture (high ceilings), and whether this is realistic under real course conditions with up to 300 persons in constant motion. It was therefore an important step when IFA tested the results in a newly constructed dissecting room at Brandenburg Medical School in Neuruppin designed in accordance with the above recommendations with two ROM dissecting tables. This involved preparation of a body donation using a standard embalming protocol with 4% FA in a setting that corresponded to the dissection course. Reliable FA level measurements were obtained of around 10% of the current OEL (Reinhard Stockmann, personal communication). The anatomists present were unable to confirm the further assumption that the high extraction volumes had resulted in disturbing drafts or noise levels. Summarizing the results of the research project, it was clearly demonstrated that single table extraction combined with coordinated ventilation and cooling technology can result in highly effective exposure reduction. When considering all of the structural and technical measures involved, it must be taken into account that financial restraints may limit implementation of these measures and that long lead times will be necessary for larger construction projects in particular.

1.4. Reduction of FA in protocols for body embalming

The embalming protocols used in anatomy departments have changed over time (Tables 1, 2 and 4) and are in many cases as old as the respective institutes, whereby adaptations have been ongoing in most cases. The individual protocols have been adapted to structural and technical requirements as well as to organizational accommodating changing of student numbers and curricula.

Until the final FA measurements were made in Munich by the IFA, the FA amount used in perfusion fixation appeared to be the main determining factor in terms of exposure (Thullner et al., 2015). However, it was then observed in Munich that reduction of FA in subsequent preservation leads to measurable differences in FA exposure. The procedure used in Munich was first to immerse all bodies for 6 months in 2% FA, followed by either further preservation for 6 months in 1% FA, or alternatively in 40% ethanol (other institutes use up to 70% ethanol for postfixation. However, such storage in cuvettes with higher alcohol concentrations requires relative expensive devices for explosion protection). Since the measured values for preservation without FA during the last 6 months were lower, the Munich group proposes considering the protocols for perfusion fixation, cadaver storage immersion and, finally, cadaver humidification in the dissection theatre separately and

Table 4

Sample embalming protocol for embalming as currently used at the Anatomical Institute, LMU Munich.

Perfusion fixation (12 L/body donor)	Storage preservation	Cadaver maintenance in dissection room
35% formaldehyde 1.2 l (3.5%). Polyethylene glycol, ethanol, phenoxyethanol, etc. according to local protocols.	40% ethanol (50L/8 body donors)	2% phenoxyethanol

reducing the amount of FA in all protocols used. Table 4 shows a sample protocol.

1.4.1. Arterial Perfusion

Since measurable FA exposure correlates significantly with the absolute amount of FA used in fixation (Thullner et al., 2015), this amount should be kept as low as possible. Experimental data collected by the anatomical institutes with 1,6–4% FA confirm that concentrations higher than 4% FA are unnecessary. When the 2% concentration was used, however, FA was partially replaced by other toxic biocides such as phenol or its derivatives, for example thymol. The use of phenol or thymol needs to be carefully considered since these cyclic hydrocarbons are regarded as potentially mutagenic and carcinogenic, even if this has not been clearly confirmed to date. Therefore, efforts must be made to omit or replace phenol and thymol in the fixation fluid or to reduce the concentrations used to a minimum.

In addition to the relative concentration of FA, a further main determinant of the total FA amount is the volume of fixative solution per body. The volumes used vary from 10 to 30 L per body donation, whereby 10–12 L can be considered below average and 25–30 L as above average. Data on record show that 10 L can suffice for a body weight of up to 60 kg, 12 L for greater weights (approximately 15% of body weight; fat – muscle correlation). In veterinary anatomy, volumes of FA used for embalming strongly vary due to diverse animals used for cadaver courses (4 kg cat vs. 500 kg horse) or special requirements as the rumen in ruminants but should be kept at 1–4% FA comparable to human anatomies. The prerequisite in all cases is good vascular status to ensure uniform perfusion via the arterial system, since otherwise local injections of embalming solution are required. Therefore, approximately 750 mL 37% (w/v) FA (formaline) of FA for a body of normal weight can be calculated as a useful framework for orientation. On this basis, each institution should determine the minimum total amount required to prevent any mould or rot. On the other hand, it must be taken into account that reliable assessment of any increased risk of infection, especially with resistant pathogens such as tuberculosis, is not feasible, so that a reduction to amounts below 750 mL must be considered a critical limit.

In veterinary anatomy, the enormous differences in cadaver sizes (4–500 kg) make important considerations in fixation and preservation necessary. If animal bodies are fixed in a lying position with a 2% FA solution, a quantity of fixative solution of at least 10% (small animals like cats and dogs) to a maximum of 15% (for large animals eg. cattle and horses) of the initial weight of the carcasses is necessary. For example, for a dog weighing 25 kg, 2.5 L of 2% FA solution should be used. However, if animals are fixed in a standing position, greater volumes are necessary. In order to reduce FA exposure, veterinary anatomy endeavours to use donations of smaller animals such as ponies instead of large horses or minipigs instead of regular size pig breeds. To avoid microbial contamination, animal donations are shorn, washed and disinfected after fixation before further preservation. A high level of attention is paid to ongoing cadaver maintenance such as intensive application of an FA-free

cadaver maintenance solution based on ethanol after each dissection (Janczyk et al., 2011a). Recently efforts have been made to develop alternatives to FA embalming for veterinary anatomical dissection purposes. Nitrite pickling salt–ethanol–glycol solutions appear to have no health risk to humans, are environmental friendly and cost effective, as well as producing cadavers close to their original appearance (Janczyk et al., 2011b).

1.4.2. Storage preservation and cadaver humidification in the dissecting room

To date there have been no systematic investigations of the impact of the FA content level in the preservative solution, especially since uptake of FA from the immersion solution has not been quantified. The above results, however, clearly suggest an impact of the FA quantity. It should be noted that the majority of institutes use FA for preservation purposes (Thullner et al., 2015). Some storage systems require FA spraying, since alcohol must be avoided due to the risk of explosion. However, many years of experience have also shown that post-fixation (after primary fixation with 4% FA) in 70% ethanol without FA for 6–16 weeks followed by storage in foils is possible, but requires a higher level of explosion protection. 10% ethanol with 2% Terralin^{®1} has also been used successfully. Storage for 6 months in 40% ethanol vapour in a cuvette also appears to be useful, whereby 50 L of ethanol (40%) in an 8000 L cuvette suffice for eight body donors. Increased explosion protection is not required for this preservation method, making its use in existing institutes possible in consultation with local occupational safety offices. The low ethanol volume can be replaced once a year with little expense and effort, thus avoiding a potential enrichment of FA in the preservative solution due to seepage from the donated cadavers that would otherwise alter preservation conditions over longer periods in little-known ways. Alternatively, a full bath preservation might be suitable not exceeding a FA concentration of 2%. Also encapsulation in plastic sheets is useful and has the advantage that no FA can reach the environment. At this point it can be stated that the calculated concentration of FA is often not the real concentration for different reasons. A measurement of the FA concentration is a routine procedure in laboratories using gas chromatography and yields the FA percentage of a taken fluid sample. The FA can be adjusted to the target concentration using periodic measurements.

The major advantage of preservation without FA is the elimination of FA exposure for preparators in all phases of the work so that respiratory masks can be dispensed with in these areas. Wearing of the masks would thus only be required when preparing the fixative solution and when performing perfusion fixation. Alternatively the section tables and the storage systems are equipped with appropriate ventilation and exhausting systems.

Cadaver humidification in the dissecting room can be done with 2% phenoxyethanol without FA (Table 4). Humidification of the covering cotton cloths using small watering cans makes for economical application without increased aerosol formation and prevents immersion of parts of the cadaver or microorganisms in the stock solution, which would be the case if the cloths were immersed.

Turning to the future, the important thing will be to simplify individual embalming protocols at the various locations and, in particular, to reduce the number of biocidal substances used. This would make it possible over the medium term to agree on a framework protocol that can be applied at all institutes with slight

modifications to fit their specific situations. The technical structural and organizational measures aimed at reducing FA exposure could then be compared between all institutions and subsequently optimized. Modification of the protocols is a lengthy process, since measurements to determine effective FA reduction would not be feasible until the first dissection course following the changes. The switchover can take several years in institutions with large numbers of students requiring a correspondingly large number of body donations per course. Therefore, changes in the protocols for fixation and preservation are only suitable to a certain extent as a means of quick reduction of FA to achieve OEL compliance.

Embalming quality must also be considered in connection with particular educational aims to be achieved by dissection. It makes no sense to continue reducing the amount of FA used when advantages of dissection are lost due to poor fixation. What is needed is an assessment of the appropriate FA concentration in terms of the requirements of the specific dissection course as envisioned.

1.5. Reduction of FA exposure in the dissection course by means of organizational measures

In contrast to the previously described measures, organizational changes are in most cases readily implementable and inexpensive. Studies by the accident funds indicate that the following organizational measures can contribute to a reduction of FA exposure (Thullner et al., 2015):

- Small number of body donations and/or specimens per room. The number of body donors required can be reduced for the same number of students by having two or three groups in succession doing dissection work on each cadaver.
- Low number of students per dissection table or low overall student count in the dissection room. Since each dissecting student or instructor represents a heat source generating approximately 100 Watts, each person present increases the air turbulence that can increase the FA exposure level. This impact has been confirmed by measurements, whereby halving the number of students present results in a measurable reduction of the FA exposure.
- Cadaver presentation for the course only. This measure makes sense in small rooms without effective basic ventilation to keep the basal FA load level down.
- Preparatory measures for the dissection course. Cadavers can be rinsed with water before they are presented in the dissection room after storage in a solution containing FA.
- Cadavers can be placed already 2 up to 3 weeks before the dissection course starts.
- The body donors can be uncovered by the preparators/technical staff half an hour prior to the start of the course with the ventilation and table extraction switched on to achieve removal (evaporation) of FA that has accumulated under the cloth cover.

Further measures:

- No use of overweight body donations for the dissection course, since these require a higher total quantity of FA for fixation and take up more FA when preserved in a solution containing FA due to the subcutaneous fat tissue.
- Opening of the body cavities outside of course time. Measurement results at various locations show that FA exposure briefly increases for a short period of time when the thoracic and abdominal cavities are opened. The opening procedure could therefore precede the practice exercise, whereby this decision must be balanced against a reduced learning effect.
- Regular removal of embalming solution leaking out of body cavities and provision of drainage pails for the dissection tables,

¹ Concentrate for disinfectant cleaning of medical devices and other surfaces. 100 g solution contains the following active ingredients: 22 g Alkyl (C12–16) dimethylbenzyl ammonium chloride (ADBAC/BKC (C12–C16)), 17 g 2-Phenoxyethanol, 0.9 g Amines, N-C12–C14 (even numbered)-alkyltrimethylenedi-, reaction products with chloroacetic acid).

which, however, must be regularly disposed of to avoid additional FA sources in the room.

- Wearing of gloves during the dissection procedure to avoid absorption through the skin. Latex and vinyl are unsuitable materials and nitrile is also not consistently reliable. Therefore, appropriate gloves should be recommended by the institute. Information provided by manufacturers is not reliable, since the temperature range used in many studies is artificial and no other chemical substances are added. Thus it cannot be ruled out that FA penetrates gloves during the time course of a dissection course session, even if students use forceps for dissection at all time. Therefore, students should be instructed to change gloves during the practical exercise. It should be pointed out to students at the beginning of the course that keeping the dissection act at a certain distance from the mouth and nose in particular can effectively reduce FA exposure, since uptake of the substance is mainly by inhalation. Intake of food in the dissection room, including use of chewing gum, must also be avoided to prevent oral intake.
- Use of FA binding agents, such as Infutrace^{TM2} to prevent excessive FA from evaporation.

In summary, it should be noted that a large number of measures are available which, when combined, can effectively reduce FA exposure so that reliable compliance with the OEL would appear feasible in all anatomical institutes. In view of the high level of effectiveness for implementation of single table extraction with coordinated ventilation, these measures should be initiated as soon as possible. Also, FA exposure can be reduced ad hoc, or at least over the short term, by a large number of easily implemented organizational measures.

Acknowledgments

We thank Prof. Niels Hammer (University of Otago, NZ) for helpful discussion, Michael Beall for the translation and for manuscript editing.

References

- Benkhadra, M., Gérard, J., Genelot, D., Trouilloud, P., Girard, C., Anderhuber, F., Feigl, G., 2011. Is Thiel's embalming method widely known? A world survey about its use. *Surg. Radiol. Anat.* 33 (4), 359–363.
- Bertelshofer, F., Brehm, O., Fitzner, P., Lammert, J., Janke, R.M., Paulsen, F., Greiner, G., 2016. Skully – an educational web application for the human skull. In: Tolxdorff, T., Deserno, T.M., Handels, H., Meinzer, H.P. (Eds.), *Bildverarbeitung für die Medizin 2016*. Springer, pp. 248–253.
- Blum, F., 1894. *Notiz über die Anwendung des Formaldehyds (Formol) als Härtungs- und Konservierungsmittel*. *Anat. Anz.* 9, 229–231.
- Brenner, E., 2014. Human body preservation-old and new techniques. *J. Anat.* 224, 316–344.
- Dahncke M, Hohenberger L, Klusmann H, Stockmann R, Thiel P, Thullner I, (2016) Formaldehyd in der vorklinischen medizinischen Ausbildung (Anatomie): Lüftungstechnischen Maßnahmen. In *Gefahrenstoffe, Reinhaltung der Luft, Ausgabe 10-2016/Band 76*, Deutsche Gesetzliche Unfallversicherung e.V. (DGUV) (Publisher).
- Drake, R.L., McBride, J.M., Lachman, N., Pawlina, W., 2009. *Medical education in the anatomical sciences: the winds of change continue to blow*. *Anat. Sci. Educ.* 2, 253–259.
- Eisma, R., Wilkinson, T., 2014. From "silent teachers" to models. *PLoS Biol.* 12 (10).
- Fox, C.H., Johnson, F.B., Whiting, J., Roller, P.P., 1985. Formaldehyde fixation. *J. Histochem. Cytochem.* 33, 845–853.
- Hammer, N., Löffler, S., Feja, C., Sandrock, M., Schmidt, W., Bechmann, I., Steinke, H., 2012. Ethanol-glycerin fixation with thymol conservation: a potential alternative to formaldehyde and phenol embalming. *Anat. Sci. Educ.* 5 (4), 225–233.
- IFA-Arbeitsmappe Messung von Gefahrenstoffen. Publisher Deutsche Gesetzliche Unfallversicherung, Berlin. Erich Schmidt, Berlin 1989-Losebl.-Ausg.
- Janczyk, P., Weigner, J., Luebke-Becker, A., Kaesmeyer, S., Plendl, J., 2011b. Nitrite pickling salt as an alternative to formaldehyde for embalming in veterinary anatomy – a study based on histo- and microbiological analyses. *Ann. Anat.* 193, 71–75.
- Janczyk, P., Weigner, J., Luebke-Becker, A., Richardson, K.C., Plendl, J., 2011a. A pilot study on ethanol-polyethylene glycol-formalin fixation of farm animal cadavers: Pilotstudie zur Fixierung von Großtieren mittels Äthanol-Polyäthylenglykol-Formalin. *Berl Munch Tierarztl Wochenschr* 124, 225–227.
- Jores, L., 1896. Die Conservirung anatomischer Präparate in Blutfarbe mittelst Formalin. *Centralbl. f. allgemeine Path. und path. Anat.* 7:134.
- Korf, H.W., Wicht, H., Snipes, R.L., Timmermans, J.P., Paulsen, F., Rune, G., Baumgart-Vogt, E., 2008. The dissection course – necessary and indispensable for teaching anatomy to medical students. *Ann. Anat.* 190, 16–22.
- Kugelmann, D., Stratmann, L., Nühlen, N., Bork, F., Hoffmann, S., Samarbarksh, G., Pfersch, A., von der Heide, A.M., Eimannsberger, A., Fallavollita, P., Navab, N., Waschke, J., 2018. An augmented reality magic mirror as additive teaching device for gross anatomy. *Ann. Anat.* 215 (January), 71–77.
- Ochs, M., Mühlfeld, C., Schmiedl, A., 2012. Präparierkurs: Grundlage ärztlichen Handelns. *Dtsch Arztebl* 109 (43), A-2126/B-1732/C-1700.
- Paulsen F. (2007) Gutes und Barmherzigkeit werden mir folgen mein Leben lang. In Ulrich J (ed) *Hallesche Universitätspredigten*. Wintersemester 2006/2007. Und siehe: Wir leben! Band IX:92-100.
- Paulsen, F., Eichhorn, M., Bräuer, L., 2010. Virtual microscopy – the future of teaching histology in the medical curriculum? *Ann. Anat.* 192, 378–382.
- Paulsen F, Tschernig T. (2004) Treffen der Prosektoren. CD mit Vorträgen zum Satellitensymposium auf der 21. Arbeitstagung der Anatomischen Gesellschaft in Würzburg am 29.09.2004. *Archiv der Anatomischen Gesellschaft*.
- Ramsey-Stewart, G., Burgess, A.W., Hill, D.A., 2010. Back to the future: teaching anatomy by whole-body dissection. *Med. J. Aust.* 193, 668–671.
- Thiel, W., 1992. Die Konservierung ganzer Leichen in natürlichen Farben. *Ann. Anat.* 174, 185–195.
- Thullner I, Stockmann R, Hohenberger L. (2015) Formaldehyd in der vorklinischen medizinischen Ausbildung (Anatomie). In *Gefahrenstoffe, Reinhaltung der Luft, Ausgabe 6-2015/Band 75*, Deutsche Gesetzliche Unfallversicherung e.V. (DGUV) (Publisher).

² InfutraceTM is a formaldehyde and phenol vapor reducing agent. It must be diluted with filtered or DI water prior to any use. Three diluted InfutraceTM concentrations are recommended for various applications: <http://www.americanbiosafety.com/files/79978104.pdf>