Measuring low formaldehyde exposure values in the dissection hall after embalming human body donors with ethanol-based fixation methods

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SUMMARY

The dissection course plays a central role in teaching anatomy and body donors are also needed in clinical-anatomical courses. In order to reduce formaldehyde exposure of students, participants and staff during these courses, we employed fixation solutions with low formaldehyde content. In this study, we present two ethanol-based protocols, and elucidate their suitability for the dissection process and compliance with occupational exposure limits for formaldehyde and other hazardous substances.

Body donors were fixed according to an ethanol-based fixation protocol for the dissection course or an ethanol-glycerin-based fixation protocol for specialist training courses. The quality of fixation was determined during the dissection process. Exposure to hazardous substances (formaldehyde, ethanol, 2-phenoxyethanol) was measured in a regular dissection course setting at different locations (room-related and person-related measurements), and exposure indices were calculated.

The quality of fixation of both methods was good and fulfilled all requirements of the student dissection course and the specialist training courses, respectively. Exposure to all hazardous substances remained well below the exposure limits. Room-related air concentration measurements were 0.073/0.058 mg/m³ (2016/2017) for formaldehyde and 65/107 mg/m³ (2016/2017) for ethanol. Person-related measurements amounted to 0.107-0.229 mg/m³ for formaldehyde and 268-388 mg/m³ for ethanol. Room-related and person-related concentrations of 2-phenoxyethanol remained below the detection limit.

The ethanol-based embalming protocols presented here offer a good alternative for the different applications. The protocols are discussed regarding current regulations and further measures to reduce formaldehyde exposure.

Key words: Ethanol – Formaldehyde – Embalming – Dissection course – Occupational exposure

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INTRODUCTION

Teaching anatomy to medical students is based on a combination of theoretical and practical lessons to which the dissection course is central (Gunderman and Wilson, 2005; Rizzolo and Stewart, 2006; Korf et al., 2008; Böckers et al., 2010; Ochs et al., 2012; Vorstenbosch et al., 2013; Schmiedl, 2017; Bogomolova et al., 2020). Learning from a body teaches the complete intricacy of reality, which has a much higher level of complexity than models, whether digital or physical (Ochs et al., 2012). So it is not surprising that the majority of final-year medical students asked at the end of their curriculum about the relevance of gross anatomy classified the dissection course with integrated clinical problems as an important prerequisite and basis for their clinical courses and are interested in additional specialized dissection courses (Pabst, 1993). Also, the relevance of gross anatomy in respect to everyday work was ranked second of all medical subjects by physicians asked some years after graduation at their specialist examination (Pabst and Rothkötter, 1997).

In addition to the classical dissection course for undergraduate medical students, dissection of specific body regions is also conducted in postgraduate education and medical specialist training (Grechenig et al., 1999; Schwarz et al., 2002; Feigl et al., 2007; Schmiedl, 2017; Wedel et al., 2019; Shichinohe et al., 2022; Suzuki et al., 2022).

The dissection course relies on donors who bequeath their body to a specific anatomical institute. This bequest must be made personally in written form during the donor's lifetime (informed consent). After death, the bodies are fixed and conserved chemically to allow storage and preparation for long periods and to preclude infection risks (Brenner, 2014). At Hannover Medical School, ethanol-based fixation techniques have been used since its foundation in 1965, and were chosen as an alternative to formalin-based fixation in order to reduce occupational health hazards for employees and students (Pabst, 1987). Since then, ethanol-based fixation techniques have evolved continuously.

Occupational exposure to formaldehyde has received renewed attention due to a reassessment of its carcinogenicity. In its 2012 review on human carcinogens, the International Agency for Research on Cancer (IARC) saw sufficient evidence that formaldehyde causes cancer of the nasopharynx and leukemia in humans, and thus graded formaldehyde as a group-1 carcinogen (IARC, 2012). Based on the United Nations' Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification, the carcinogenicity of formaldehyde was subsequently graded as category 1B by the European Union (EU), resulting in a reduction of European occupational exposure limits to air concentrations of 0.37 mg/m³ (0.3 ppm) (Commisssion Regulation (EU) 605/2014, 2014; United Nations, 2021). Likewise, legal regulations or recommendations were also adapted in other countries. Current occupational exposure limits for long-term/time-weighted average of exposure vary between countries, and range from 0.12 to 2.5 mg/m³ (0.1-2.0 ppm). Additionally, many countries have imposed short-term and/or ceiling limits. All values are listed in Table 1.

Reduction or replacement of formaldehyde in fixatives requires alternative chemicals, which could also incorporate health hazards. Apart from formaldehyde, our fixatives contain ethanol, 2-phenoxyethanol and thymol, which are regulated as hazardous chemicals in the EU and other countries (Regulation (EC) 1272/2008, 2008; Commission Regulation (EU) 2018/669, 2018; Commission Delegated Regulation (EU) 2021/849, 2021). The main hazard associated with ethanol is flammability. Further hazards of the four substances include several acute hazards, which are listed in Table 2.

The aim of this study was to present two different ethanol-based fixation methods used at our institute for the dissection course or for clinical anatomy courses, and to assess occupational exposure to hazardous substances in a regular dissection course setting.

MATERIALS AND METHODS

Body donors

All body donors voluntarily bequeathed their body during lifetime and after informed consent for medical education and research to the Insti-

Table 1. International occupational exposure limits for formaldehyde, ethanol and 2-phenoxyethanol [mg/m³].

	Formaldehyde			Ethanol		2-Phenoxyethanol	
	Long- term/ TWA[1]	Short- term[2]	Ceiling[3]	Long- term/ TWA[1]	Short- term[2]	Long- term/ TWA[1]	Short- term[2]
Europe							
EU[4]	0.37 (0.15-0.37)	0.74 (0.5-0.74)	–	1900 (260-1900)	1900 (1520-9500)	– (5.7 to –)	– (5.7 to –)
Switzerland	0.37	0.74	–	960	1920	110	110
UK	2.5	2.5	–	1920	–	–	–
Asia							
Japan	0.12	0.18[5]	0.24[5]	–	–	–	–
Singapore	–	0.37	–	1880	–	–	–
South Korea	0.37	–	–	–	–	–	–
Australia/Ozeania							
Australia	1.2 (0.36[6])	2.5 (0.72[6])	–	1880	–	–	–
New Zealand	0.37	0.74	–	1880	–	–	–
America							
Canada[7]	0.9 to –	1.23 to –	1.85 to –	1880 to –	1900 to –	141 to –	–
Mexico	–	0.37	–	1900	–	–	–
USA[8]	0.93 (0.02[9])	2.47 (0.12[9])	–	1900	–	–	–

Occupational exposure limits according to national regulations and legal requirements
– A legal limit has not been imposed
[1] Long-term: General exposure limit during a working shift. It may be exceeded for short time periods. Or: TWA: Limit of time weighted average of exposures during a working shift
[2] Exposure limit for short time periods, defined by most countries as a 15 min interval
[3] Concentration should not be exceeded at any time
[4] National exposure limits can vary between countries: Listed is the limit valid in most EU countries and () range of national exposure limits
[5] Recommended by the Japan Society for Occupational Health (JSOH)
[6] Recommended by the National Industrial Chemicals Notification and Assessment Scheme (NICNAS)
[7] Exposure limits vary between Provinces
[8] Some states have different exposure limits
[9] Recommended by The National Institute for Occupational Safety and Health (NIOSH)

tute of Functional and Applied Anatomy, Hannover Medical School, where the study was performed. The authors state that every effort was made to follow all local and international ethical guidelines and laws that pertain to the use of human cadaveric donors in anatomical research (Iwanaga et al., 2022). Due to logistic reasons, most body donors come from the region of Hannover. Before conservation started, an additional second post-mortem examination by a coroner was carried out.

Ethanol-based standard fixation

Our standard fixation protocol was applied to bodies to be used in our student dissection courses. Conservation started preferably within 24 h after death of the body donor by perfusion fixation via the femoral artery. On average, 15 l of standard fixative (Table 3) were infused slowly (over several hours) at a low-pressure gradient (max. 100 kPa (1 bar)). The exact volume infused varied with the size of the body donor (approx. 0.18 l/kg body weight) and also depended on the vessel quality of the corpse (visual control of arrival of fixative in all body regions). Additionally, if necessary, fixation solution was applied s.c. and i.m. in body regions like, e.g., subcutaneous tissue of the neck, which are sometimes not reached well by perfusion.

Subsequently, perfusion-fixed bodies were immersed in 70% ethanol for additional external fixation of skin and subcutaneous tissue and storage. The storage period lasted for at least 6-12 months

Table 2. Hazard statements for formaldehyde, ethanol, 2-phenoxyethanol and thymol according to CLP regulation (Commission Delegated Regulation (EU) 2021/849, 2021; Commission Regulation (EU) 2018/669, 2018; Commisssion Regulation (EU) 605/2014, 2014; Regulation (EC) 1272/2008, 2008) and Safety Data Sheets (Carl Roth GmbH 2021a, b, 2022).

Hazard statement	Formaldehyde	Ethanol	2-Phenoxy-ethanol	Thymol
H255: Flammable liquid 2		x		
H301: Acute toxicity 3 (oral)	x			
H302: Acute toxicity 4 (oral)			x	x
H311: Acute toxicity 3 (dermal)	x			
H314: Skin corrosion 1B	x			x
H317: Skin sensitisation 1	x			
H318: Eye damage 1	x		x	
H319: Eye irritation 2		x		
H331: Acute toxicity 3 (inhalation)	x			
H335: STOT SE1 3 (respiratory tract irritation)	x		x	
H341: Mutagenicity 2	x			
H350: Carcinogenicity 1B	x			
H370: STOT SE1 1	x			
H411: Aquatic chronic 2				x

[1] Specific target organ toxicity, single exposure

Table 3. Composition of fixation solution for standard fixation and soft fixation.

	Standard fixation		Soft fixation	
	Volume	Effective concentration[1]	Volume	Effective concentration
Ethanol (99%)	8750 ml	86.63%	5000 ml	43,61%
Formalin (Formaldehyde 37.5%)	750 ml	2.83%	125 ml	0,41%
2-Phenoxyethanol	250 ml	2.50%	125 ml	1,10%
Glycerin (85%)	250 ml	2.13%	4250 ml	31,83%
Thymol solution	–	–	100 ml	0,88%
Nitrite curing salt	–	–	100 g/1750 ml aqua dest.	0,88%

[1] Effective concentration: water free concentration of chemical substance

during which the ethanol concentration of the storage solution was tested regularly. During the dissection course, the bodies were sprayed regularly with 20% ethanol when uncovered to prevent drying out. After the end of the course, the corpses were covered with a cloth, which was sprayed with an ethanol/thymol solution and a plastic cover to prevent evaporation.

Ethanol-glycerin-based soft fixation

Bodies conserved by soft fixation were used in our specialist training courses. These include courses for hip surgery and hip joint injection, courses for shoulder, hip and knee joint prosthesis surgery, courses for implantation of hand and foot endoprostheses, flap surgery courses, pelvic floor courses, and courses for implantation of left ventricular assist systems. Donated bodies were

perfusion-fixed using fixation solution for soft fixation. The concentration of formaldehyde has been further reduced from 1% to 0.41% (Table 3) compared to previous years to increase the flexibility of the corpses. The perfusion process was similar to standard fixation; perfusion volume averaged 12-15 l. For subsequent storage, the bodies were immersed in 30% ethanol. Storage time was on average about 6 months. It could vary between 4 weeks and 1.5 years, depending on the time schedule and frequency of our specialist training courses.

Evaluation of conservation quality

Throughout the dissection course, feedback of students participating in the dissection courses during the last 10 years was obtained orally regarding the visibility and haptic properties of anatomical structures, dissectability and handling. For ethanol-glycerin-based soft fixation used in specialist training courses, oral feedback was obtained from participants and external instructors. For both fixation methods, visual appearance of donated bodies was documented photographically. Haptic properties were determined descriptively by palpation.

Exposure measurements

Exposure measurements were taken during the regular dissection course for medical students. All students were working with body donors fixed with Hannover standard fixation. The dissection course is held in two dissection halls (area 220 m^2, air volume 840 m^3, each), which are ventilated and air conditioned (supply air: 6800 m^3/h, extract air: 6900 m^3/h, air exchange rate 8x, negative pressure ventilation; per dissection hall). Room temperature (set point: 17°C) and relative humidity were recorded during exposure measurements. Incoming air was delivered via 15 ducts located in the ceiling, and for exit air 4 suction ducts were located close to the floor. Table suction devices were not installed. Twelve bodies were placed in each dissection hall on stainless steel tables. Six bodies were dissected simultaneously while the other 6 remained covered. Covered and uncovered tables were arranged alternately, with always one covered table in between. Thus, the distance between uncovered tables amounted to 5 m and 3.5 m in longitudinal and transverse direction, respectively. Minimum diagonal distance between corners of uncovered tables was 2 m. Alternately 2 groups of 6 students worked on one body in a time-staggered manner. The students sprayed the bodies with ethanol solution (20%) regularly during the dissection course. Outside course hours, the bodies were covered and remained in the dissection hall.

Measurements of exposure to hazardous substances were conducted according to EU and German legal regulations (DIN EN 689, 1995; Regulation (EC) 1272/2008, 2008; Commisssion Regulation (EU) 605/2014, 2014; DIN EN 482, 2015; TRGS 900, 2015; TRGS 402, 2016; Chemikaliengesetz, 2021; Gefahrstoffverordnung, 2021; Arbeitsschutzgesetz, 2022) They included exposure measurements regarding formaldehyde, ethanol and 2-phenoxyethanol.

Sampling was performed during regular course hours by an accredited laboratory (TÜV Nord Umweltschutz GmbH, Hannover, Germany) on two days (18.2.2016 and 6.2.2017), and included room-related and person-related samples (TÜV NORD Umweltschutz Gmbh & Co. KG, 2016, 2017). Room-related samples were obtained in a central position in the dissection hall. Person-related samples were collected from one person (18.2.2016)/two persons (6.2.2017) directly involved in the preparation process and at inspiration height. These person-related samples were taken as long-term-exposure samples to determine mean exposure levels during the course (collection period 102-122 min). Sample collection started at the beginning of the course, including uncovering of body donors. Additionally, short-term-exposure samples were obtained from the two persons (2017) in maximum-exposure situations (collection period 15 min) (TÜV NORD Umweltschutz Gmbh & Co. KG, 2017). The sampling device (Personal Air Sampler, Type SKC 224PCEX8, ANALYT-MTC GmbH, Müllheim, Germany) comprised a pump sucking a defined air volume through substance-specific sample carriers (formaldehyde: 2,4-dinitrophenylhydrazin cartridge (Waters Sep-Pak Cartridge Type XPoSure Aldehyde Sampler); 2-phenoxyethanol and

ethanol: activated carbon tube (Type B, SKC Anasorb CSV) (TÜV NORD Umweltschutz Gmbh & Co. KG, 2016, 2017)).

The samples were analyzed by accredited laboratories. Formaldehyde analysis was performed by TÜV NORD Umweltschutz GmbH (Hamburg, Germany), using high pressure liquid chromatography (HPLC; LaChrom Ultra, VWR, Darmstadt, Germany). 2-phenoxyethanol and ethanol analysis was performed by ANECO Institut für Umweltschutz GmbH&Co. (Mönchengladbach, Germany), using capillary gas chromatography and a flame ionization detector (Capillary-GC-FID) (TÜV NORD Umweltschutz Gmbh & Co. KG, 2016, 2017).

Based on room air concentrations and occupational exposure limits according to TRGS 900, exposure indices were calculated according to TRGS 402 (TRGS, 900, 2015; TRGS 402, 2016). A substance index (I_i) was determined for formaldehyde, 2-phenoxythanol and ethanol, added to yield a total exposure index (BI) and time weighted to calculate the shift exposure index (I_M). Finally, hazards and safety precautions were evaluated according to TRGS 402 (TÜV NORD Umweltschutz Gmbh & Co. KG, 2017).

RESULTS

Conservation quality

Ethanol-based standard fixation was employed for donor bodies intended to be used in the dissection course. The condition of the corpses can be seen in Fig. 1. Muscles and internal organs were moderately decolored, although they retained enough color contrast for tissue differentiation. Shape and size of internal organs were not altered significantly. Tissues and organs of embalmed bodies were firm but not hard or brittle. The bodies remained in good condition during the whole dissection course (October to June). Mold infestation and tissue decompensation did not occur. Student feedback revealed that they could discern and dissect anatomical structures well, and that they were satisfied with the conservation quality.

Ethanol-glycerin-based soft fixation was performed in bodies used for specialist training courses. Figure 2 shows the conservation quality of bodies subjected to soft fixation. Organ and tissue colours were quite vivid, and exposed structures could be differentiated well. Participants and instructors of specialist training courses found it particularly helpful that extremities and trunk were flexible and most internal organs were quite soft. Thus, tissues and organs gave a realistic impression for the conduction of clinical procedures.

Exposure measurements

Ventilation and air conditioning kept the room temperature and the relative humidity at low comfortable levels during the dissection course (room temperature 18.7-20.5°C, relative humidity 30-32%, air pressure 1016 hPa).

Room related exposure measurements:

In a central position of the dissection hall a formaldehyde concentration of 0.073 and 0.058 mg/m³ was measured in 2016 and 2017, respectively. The concentration amounted to 19.7% and 15.7% of the occupational exposure limit in 2016/2017 (substance index 0.197 and 0.157; Table 4). Air concentration of ethanol was 65 and 107 mg/m³, i.e., 6.8% and 11.1% of the exposure limit in 2016 and 2017, respectively (Table 4). The exposure to 2-phenoxyethanol was below the detection threshold. The sum of all measured air concentrations resulted in a total exposure index of 0.27 in both years.

Person-related measurements:

Person-related long-term values, i.e., mean air concentrations at inspiration height directly above the corpses, were approximately twice as high as the room related values for formaldehyde and ethanol (Table 4). Air concentrations of 2-phenoxyethanol were still below the detection limit. All concentrations remained well below the exposure limit (I_i and BI < 1) (Table 4). Additionally, short-term exposure measurements were conducted for the two persons in 2017 (Table 4) when opening the abdominal cavity. This was regarded as the maximum exposure situation, not only of the course day but of the whole dissection course. From long-term and short-term exposure indices a time-weighted shift exposure index (I_M) was determined, which amounted to 0.35 for both per-

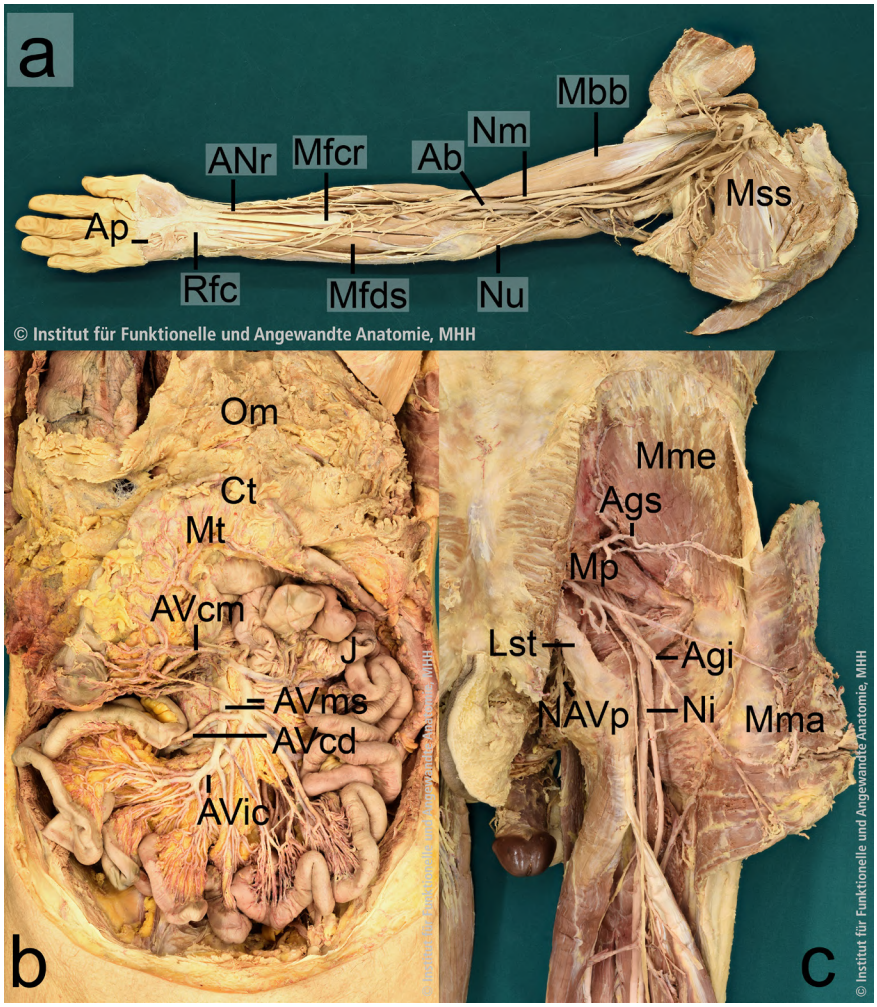

Fig. 1.- Ethanol-based standard fixation. Fixation quality was very good. Structures could be dissected easily and were well conserved. **a:** Right upper extremity. Mss Musculus (M.) subscapularis, Mbb M. biceps brachii, M. flexor carpi radialis, Mfds M. flexor digitorum superficialis, Rfc Retinaculum flexorium carpi, Ap Aponeurosis palmaris, Nm Nervus (N.) medianus, Nu N. ulnaris, Ab Arteria (a.) brachialis, ANr A. radialis and N. radialis ramus superficialis. **b:** Abdominal cavity opened and greater omentum flapped cranially. Om Omentum majus, Ct Colon transversum, Mt Mesocolon transversum, AVms A. and V. mesenterica superior, AVic A. and V. ileocolica, AVcd A. and V. colica dextra, AVcm A. and V. colica media. **c:** Right gluteal region. Mma M. gluteus maximus, Mme M. gluteus medius, Mp M. piriformis, Lst Ligamentum sacrotuberale, Ags A. glutea superior, Agi A. glutea inferior, Ni N. ischiadicus, NAVp N. pudendus, A. and A. pudenda interna.

Table 4. Air concentrations, substance indices, total exposure indices and shift exposure indices.

Location	Time interval	Formaldehyde		Ethanol		2-Phenoxyethanol		BI	I_M
		C_F [mg/m³]	I_F	C_E [mg/m³]	I_E	C_P [mg/m³]	I_P		
18.2.2016									
Room	Long- term	0.073	0.197	65	0.068	<0.238	<0.005	0.27	
Person	Long- term	0.229	0.619	268	0.279	<0.268	<0.005	0.90	0.45
6.2.2017									
Room	Long- term	0.058	0.157	107	0.111	<0.365*	<0.003	0.27	
Person 1	Long- term	0.118	0.319	278	0.290	<0.376*	<0.003	0.61	0.35
Person 1	Short- term	0.405	1.090	862	0.898	<2.760*	<0.025		
Person 2	Long- term	0.107	0.289	388	0.404	<0.336*	<0.003	0.70	0.35
Person 2	Short- term	0.135	0.365	352	0.367	<2.550*	<0.023		

Indices were determined according to TRGS 402 and TRGS 900 (TRGS 900 2015; TRGS 402 2016). According to TRGS 402, BI was not determined for short-term measurements. C Air concentration, I Substance index for formaldehyde (F), 2-phenoxyethanol (P) and ethanol (E), BI Total exposure index, IM Shift exposure index, * Detection limit with respect to sampling time and flow rate.

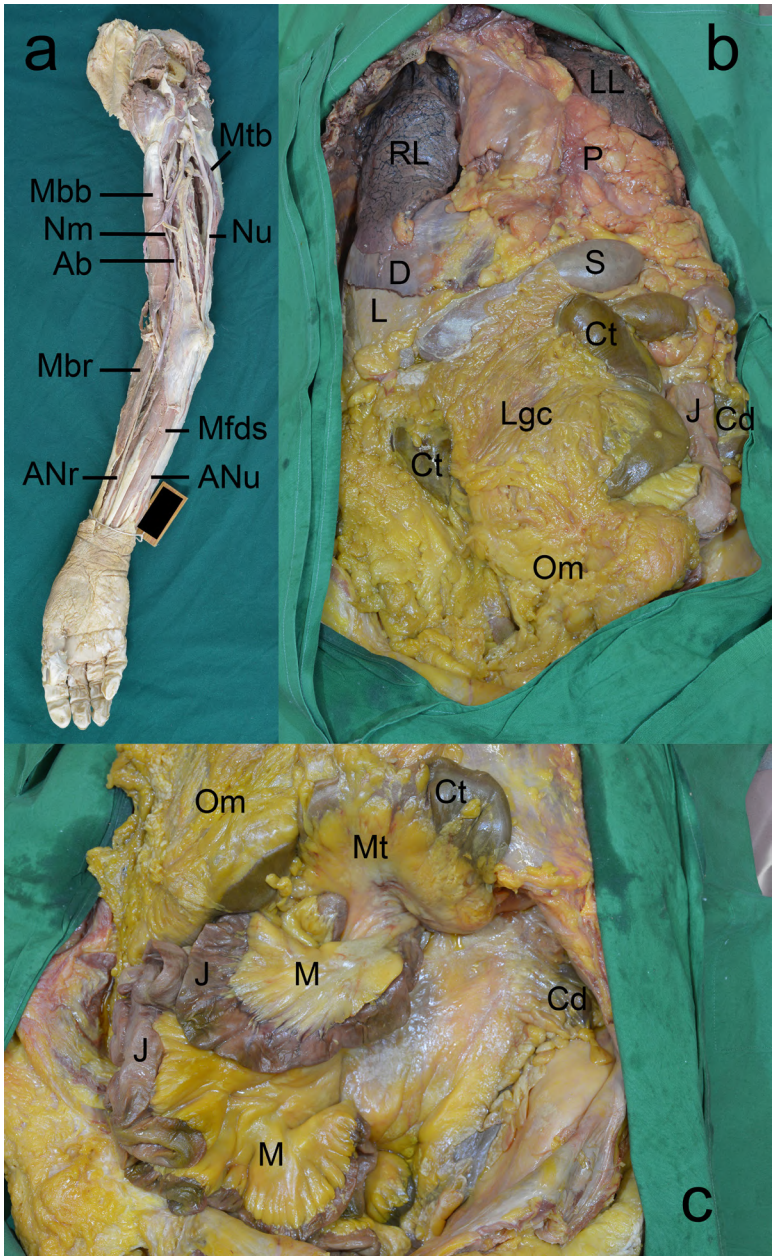

Fig. 2.- Ethanol-glycerin-based soft fixation. **a:** Right upper extremity; **b:** Thoracic and abdominal cavity opened; **c:** Abdominal cavity opened and greater omentum flapped cranially. Fixation quality is good. Organ and tissue colors were quite vivid and exposed structures could be differentiated well.

Mbb Musculus (M.) biceps brachii, Mtb M. triceps brachii, Mbr M. brachiioradialis, Mfds, M. flexor digitorum superficialis, Ab Arteria (A.) brachialis, Nm nervus (N.) medianus, Nu N. ulnaris, ANr A. radialis and N. radialis ramus superficialis, ANu A. and N. ulnaris, RL right lung, LL left lung, P Pericardium, D diaphragm, L liver, S stomach, J Jejunum, Ct Colon transversum, Cd Colon descendens, Lgc Ligamentum gastrocolicum, Om Omentum majus, M Mesenterium, Mt Mesocolon transversum.

sons in 2017. I_M was 0.45 in 2016. For both years, the final evaluation according to TRGS 402 was "Safety precautions are sufficient".

DISCUSSION

Using donated bodies in dissection courses requires fixation and conservation to inactivate possible pathogens and to provide sufficient tissue stability for storage and course times (Brenner, 2014; Balta et al., 2015; Schmiedl, 2017; Waschke et al., 2019).

Anatomical requirements for a fixative comprise fast and homogeneous diffusion through tissue, protein denaturation to prevent autolysis, rapid inhibition of bacterial and non-bacterial degradation processes, fast inactivation of pathogenic microorganisms and structural solidification of

tissues and organs as close to their natural state as possible. However, tissues should not become too rigid and organic tissue components should not be dissolved. Long preservation stability is favorable, as well as a contribution to depersonalization of the body donor by a modest change of complexion and haptic properties (Coleman and Kogan, 1998; Brenner, 2014; Schmiedl, 2017).

The main components of classical fixation solutions contribute to these requirements (Tables 5 and 6). In particular, formaldehyde is a constituent of almost all conservation solutions for donated bodies to be used in dissection courses due to its strong bactericidal, fungicidal and antiviral properties, as well as for its excellent tissue-fixation qualities (Brenner, 2014). The final concentration in fixatives usually varies between 3 and 10% (Waschke et al., 2019). However, health hazards and subsequent legislation continue to restrict utilization of formaldehyde (see below). Alternative fixation protocols with reduction or replacement of formaldehyde must meet the above demands while involving less health hazards.

Fixation techniques

Ethanol-based standard fixation for student dissection courses:

Our protocol used an arterial perfusion solution (Table 3) based on ethanol (86.6%) and containing formaldehyde (2.8%), 2-phenoxyethanol (2.5%) and glycerin (2.1%) as additional components, followed by immersion fixation in 70% ethanol and resulted in a good fixation quality as described in the result chapter. During intraarterial application the fixation solution diffused through tissue over several hours depending on vessel quality and condition of the corpse, i.e., fast enough to stop degradation processes successfully. The volume of fixation solution that can be infused varied with regard to body size but also vessel quality. Application of high volumes improved fixation quality. Subsequent immersion fixation in 70% ethanol postfixed skin and subcutaneous tissue. A storage time of at least 6-12 months assured reliable inactivation of possible pathogenic microorganisms, including a sufficient safety margin (Lischka et al., 1979).

In order to reduce formaldehyde exposure, several anatomical institutes have decreased the formaldehyde concentration of their arterial perfusion solutions to 1.6-4% during the recent past (Rissler and Hauke, 2021). Classical formaldehyde-based fixation (4-8%) yielded specimens with long-lasting stability for dissection, good tissue fixation and antimicrobial effectivity, but tissues became hard and discolored (Hayashi et al., 2016). A reduction of formaldehyde concentration may result not only in reduced health hazards, but also in improved specimen suitability for dissection courses. Requirements for student dissection courses differ to some extent from those for clin-

Table 5. Air concentrations, substance indices, total exposure indices and shift exposure indices.

Location	Time interval	Formaldehyde		Ethanol		2-Phenoxyethanol		BI	I_M
		C_F [mg/m³]	I_F	C_E [mg/m³]	I_E	C_P [mg/m³]	I_P		
18.2.2016									
Room	Long term	0.073	0.197	65	0.068	<0.238	<0.005	0.27	
Person	Long term	0.229	0.619	268	0.279	<0.268	<0.005	0.90	0.45
6.2.2017									
Room	Long term	0.058	0.157	107	0.111	<0.365*	<0.003	0.27	
Person 1	Long term	0.118	0.319	278	0.290	<0.376*	<0.003	0.61	0.35
Person 1	Short term	0.405	1.090	862	0.898	<2.760*	<0.025		
Person 2	Long term	0.107	0.289	388	0.404	<0.336*	<0.003	0.70	0.35
Person 2	Short term	0.135	0.365	352	0.367	<2.550*	<0.023		

Indices were determined according to TRGS 402 and TRGS 900 (TRGS 900 2015; TRGS 402 2016). According to TRGS 402, BI was not determined for short term measurements. C Air concentration, I Substance index for formaldehyde (F), 2-phenoxyethanol (P) and ethanol (E), BI Total exposure index, IM Shift exposure index, * Detection limit with respect to sampling time and flow rate.

Table 6. Main components of fixatives and their characteristics.

Chemical agent	Main characteristics
Formaldehyde	strongly bactericidal, fungicidal, insecticidal, antiviral, reversible crosslinking of proteins, very good tissue fixation
Ethanol	bactericidal, fungicidal, antiviral, protein coagulation
Phenol	bacteriostatic, bactericidal, fungicidal, denaturation of proteins
Phenoxyethanol	bactericidal
Glycerin	hydrophilic (moisturizing, emollient, softening agent)

From: Lischka et al., 1979; Lowe and Southern, 1994; Block, 2001; McDonnell, 2007; Koller et al., 2012; Brenner, 2014; Robert Koch-Institut, 2017; Schmiedl, 2017.

ical-anatomical courses. In particular, long-term usability is of importance. In this regard, antibacterial, antifungal, antiviral and anti-insecticidal properties of fixation and storage must be considered, and may limit reduction of formaldehyde concentration much below 2%, or even formaldehyde-free solutions (Waschke et al., 2019).

Using a formaldehyde-free nitrite pickling salt-ethanol-glycol solution for veterinary specimens (dog), tissue and organ consistency, and color remained invivo-like. But conservation of the abdominal organs was only sufficient when the abdominal cavity was opened, and thus the abdominal organs were post-fixed by immersion in the identical storage solution, and storage required cooling (Janczyk et al., 2011).

Ethanol-glycerin-based soft fixation for specialist training courses:

Apart from adverse health effects, formalin-based fixation also has some further disadvantages for specialist training courses, e.g., rigidity of the body, reduced joint flexibility, tissue decoloration, and change in the haptic properties of tissue. This has resulted in the introduction of several alternative conservation protocols, specifically designed for clinical-anatomical courses. They include Thiel solution, ethanol-glycerin-formaldehyde solution, Imperial College London soft preservation solution and saturated saline and nitrite pickling salt solution (Thiel, 1992; Coleman and Kogan, 1998; Thiel, 2002; Hayashi et al., 2016; Balta et al., 2019; Wedel et al., 2019; Shichinohe et al., 2022; Shirai et al., 2022; Suzuki et al., 2022). Also unfixed, thawed, fresh-frozen bodies are used (Hayashi et al., 2016; Shichinohe et al., 2022; Suzuki et al., 2022).

To meet the specific demands of our specialist training courses, an ethanol-glycerin-based soft fixation protocol was devised (Table 3). In contrast with the standard protocol, the soft fixation solution contained less formaldehyde (effective concentration 0.41%), ethanol (43.6%) and 2-phenoxyethanol (1.1%), but more glycerin (31.8%). Addition of thymol and nitrite curing salt provided sufficient conservation properties to the solution. High amounts of glycerin resulted in a softer consistency. The bodies retained sufficient flexibility, and internal organs were less rigid than after our standard fixation. The consistency was suitable to allow realistic procedures in training of surgical intervention techniques, as needed in our specialist training courses. On the other hand, the infection risk inherent in thawed, fresh-frozen corpses was reduced, but not eliminated (Hayashi et al. 2016; Schmiedl 2017). After perfusion with the ethanol-glycerin-based soft fixation solution, the bodies were stored in 30% ethanol, since higher ethanol concentrations (e.g., 70%) resulted in progressive tissue hardening not compatible with soft fixation purposes. Maximum storage times are about 1.5 years; after longer periods, the conservation quality declines.

Wedel et al. (2019) devised an ethanol-glycerol-lysoformin (70%-30%-0.3%) solution for laparoscopic gynecological surgery training, which comes closest to our solution with regard to glycerin content. The authors stated that they could obtain similar results, as with fresh-frozen or Thiel fixed body donors (Wedel et al., 2019). Thiel

fixation and its modifications produced a highly in vivo-like appearance of the fixed bodies and their organs at a macroscopic level, which included coloring, haptic and joint flexibility (Thiel, 1992; Thiel, 2002; Hayashi et al., 2016). However, some of the components are toxic or harmful (Janczyk et al., 2011). Yet another alternative is conservation with saturated saline solution (Coleman and Kogan, 1998; Hayashi et al., 2016). The formaldehyde content was slightly higher compared to our protocol, and the saturated saline solution contained phenol, one other critical chemical, which, however, might be replaced. The authors stated that bodies fixed by this method had a natural color, and that consistency was between Thiel and formalin-fixed specimens and imaging (ultrasound, x-ray), and that histological quality were good (Coleman and Kogan, 1998; Hayashi et al., 2016).

Health hazards of fixatives and exposure limits

Within the EU, occupational exposure to hazardous substances is regulated at EU level (Regulation (EC) 1272/2008, 2008; Commisssion Regulation (EU) 605/2014, 2014; Commission Regulation (EU) 2018/669, 2018; Commission Delegated Regulation (EU) 2021/849, 2021). Subsequent translation into national law is executed in Germany through a cascade of acts, ordinances and technical regulations (DIN EN 689, 1995; DIN EN 482, 2015; TRGS 900, 2015; TRGS 402, 2016; Chemikaliengesetz, 2021; Gefahrstoffverordnung, 2021; Arbeitsschutzgesetz, 2022). Specifically, TRGS 900 assigns occupational exposure limit values. Likewise, occupational exposure to hazardous substances is regulated in many other countries in Europe, Asia, Australia/Oceania and America (NORMA Oficial Mexicana NOM-010-STPS-2014, 2014; Industrial Safety and Health Act, 2018; Safe Work Australia, 2019; Ontario Regulation 833, 2019; Control of Substances Hazardous to Health Regulations 2002, 2020; Health and Safety Executive, 2020; Ministry of Employment and Labor Notice No. 2020-48, 2020; Occupational Safety and Health Act, 2020; Alberta Regulation 191/2021, 2021; Suva, 2021; Hygieniska gränsvärden, 2021; Industrial Chemicals Act, 2021; BEK nr 1054, 2022; Part 1910 - Occupational Safety and Health Standards, 2022; Regulation S-2.1 r.13, 2022; Sociaal Economische Raad, 2022; Workplace Safety and Health Act 2006, 2022; Worksafe, 2022)

Our standard fixation solution contains three chemical agents that are considered hazardous: formaldehyde, ethanol and 2-phenoxyethanol. The soft fixation solution additionally contains thymol as a regulated chemical (Regulation (EC) 1272/2008, 2008; Commission Regulation (EU) 2018/669, 2018; Chemikaliengesetz, 2021; Commission Delegated Regulation (EU) 2021/849, 2021; Gefahrstoffverordnung, 2021). Health hazards associated with those four substances are summarized in Table 2. To determine the health hazards of a fixation solution, all of its components have to be taken into account. If there is no reliable evidence to the contrary, risks of mixtures of chemical substances are assumed to be additive in many countries (TRGS 402, 2016; Ministy of Employment and Labor Notice No. 2020-48, 2020; Hygieniska gränsvärden, 2021; The Japan Society of Occupational Health, 2021; BEK nr 1054, 2022; Part 1910 - Occupational Safety and Health Standards, 2022).

Among the chemicals in our solutions, both number and severity of adverse health effects associated with formaldehyde clearly rank first. This applies in particular to the hazards mutagenicity and carcinogenicity, which are only found in formaldehyde. Carcinogenicity of formaldehyde in humans has long been suspected (Pabst, 1987), but in 2012 the IARC saw sufficient evidence of carcinogenicity in humans to reclassify formaldehyde from group 2A (probably carcinogenic to humans) to group 1 (carcinogenic to humans) (IARC, 2012). “Sufficient evidence of carcinogenicity in humans” means that a causal association between exposure to the agent and human cancer has been established (IARC, 2019). According to the body of evidence, the IARC concluded that formaldehyde causes nasopharyngeal cancer and leukemia in humans. Furthermore, the IARC Working Group reported a positive association between formaldehyde exposure and sinonasal cancer in humans, sufficient evidence for carcinogenicity of formaldehyde in experimental animals, and mechanistic evidence of cancer induction by formaldehyde *in vitro* and *in vivo* in

humans and experimental animals (IARC, 2012). The IARC, a research agency under the auspices of the World Health Organization (WHO), evaluates the carcinogenic risks of numerous agents, classifying substances on the strength of evidence for carcinogenicity (IARC, 2019). It does not quantify the amount of risk increase due to exposure or threshold levels. IARC classifications are solely based on scientific grounds and have no direct consequences for using the chemical in a specific country or setting.

However, subsequent legislative bodies in several countries adapted their hazard categorization and exposure limits for formaldehyde. In 2014, the EU adapted the hazard category from cat. 2 (suspected human carcinogen) to cat. 1B ("presumed to have carcinogenic potential for humans, classification is largely based on animal evidence"), but did not go as far as to classify formaldehyde as a cat. 1A carcinogen ("known to have carcinogenic potential in humans, classification is largely based on human evidence") (Regulation (EC) 1272/2008, 2008; Commisssion Regulation (EU) 605/2014, 2014).

Besides the strength of evidence for carcinogenicity, EU CLP categorization takes into account additional considerations, including, e.g., the level of concern for human carcinogenicity (Regulation (EC) 1272/2008, 2008). As a legislative body, the EU classification directly and indirectly (through national legislation) regulates usage, safety measures and occupational exposure limits, and thus it is relevant for individual institutions (Regulation (EC) 1272/2008, 2008; TRGS 900, 2015). All countries screened (Table 1) have imposed occupational exposure limits for formaldehyde. However, permissible concentration levels vary substantially among countries. Presently, the strictest legal regulations are found in Japan, with an exposure limit of 0.12 mg/m³, which is only one third of the EU exposure limit (0.37 mg/m³; both values for long-term/time weighted average) (Industrial Safety and Health Act, 2018; Directive 2004/37/EC, 2022). Even much lower is the recommendation of the American National Institute for Occupational Safety and Health of 0.02 mg/m³ (The National Institute for Occupational Safety and Health, 2022). Should a level like that become a legal standard, it would be very difficult to continue with fixation solutions containing formaldehyde.

The main hazard of ethanol is its physical property of high flammability. Its flash point is at 12°C. Ethanol vapors form explosive mixtures with air at concentrations between 2.5 and 13.5 vol% (Carl Roth GmbH, 2022). Since ethanol-based fixation, and in particular storage of whole bodies, requires large volumes of concentrated ethanol solutions, precautions regarding fire and explosion protection are necessary. These include sufficient ventilation and precautions against electrostatic discharge (Carl Roth GmbH, 2022) in all relevant rooms, but in particular in the mortuary. To ensure vapor concentrations remaining safely below the lower explosion limit, measurements of ethanol vapor concentrations and alarms signaling ventilation failure are expedient and intact good quality electric installations and leak-tight cuvettes should be standard. The legal requirements might vary according to local regulations.

Further health hazards of the four regulated chemical agents of our fixatives include several forms of acute toxicity, organ damage and/or irritation; further skin sensitization for formaldehyde and environmental toxicity for thymol (Table 2). In contrast to formaldehyde, not even all of the screened countries have long-term/TWA limits for ethanol, and only few assigned short-term values. To date, occupational exposure limits for 2-phenoxyethanol exist only in some EU countries, Switzerland and some Canadian provinces, and they vary greatly.

Exposure measurements

To evaluate occupational exposure in a regular dissection course setting, exposure measurements were conducted during normal dissection course hours. Exposure during the dissection course can vary, depending on the precise dissection situation. In particular, opening the body cavities is likely to result in greater exposure due to evaporation of fixation fluid which has accumulated inside the cavities and the exposure of large surface areas of organs and tissues saturated with fixative. Therefore, short-term exposure measurements were made on the opening of the

body cavities (15 min) and long-term exposure measurements were obtained on the same course day, i.e., working on corpses with newly opened body cavities.

Occupational exposure to hazardous substances is regulated at several levels. Firstly, limits of air concentrations [mg/m³] are imposed for each substance, and measured exposure is related to these limits (C_i, I_i) (TRGS 900, 2015; TRGS 402, 2016). Secondly, if exposure to a mixture of hazardous substances occurs during a working shift, hazards are considered additive; thus a total exposure index (BI) is determined, which must not exceed 1 (TRGS 402, 2016). Finally, time periods with varying exposure during a working shift are computed in a time-weighted manner, resulting in a shift exposure index (I_M) (TÜV NORD Umweltschutz Gmbh & Co. KG, 2017). Comparable procedures for calculating a time-weighted average of exposures and/or considering exposure to multiple substances exist also in other countries (Ministry of Employment and Labor Notice No. 2020-48, 2020; Grenzwerteverordnung, 2021; Hygieniska gränsvärden, 2021; Suva, 2021; The Japan Society of Occupational Health, 2021; BEK nr 1054, 2022; Part 1910 - Occupational Safety and Health Standards, 2022; Worksafe, 2022).

Both room-related and person-related (long-term) exposure measurements remained well below the limits for all three regulated substances of our fixation solution, i.e., $I_i \leq 1$ (Table 4). Also, BI and I_M remained below the limits (≤ 1). Exposure limits may be exceeded for short time periods by a substance-specific exceedance factor, which was two for all of our substances, and thus all short-term exposure limits were met (TRGS 900, 2015). Exposure measurements and deducted indices are the bases for the final evaluation according to TRGS 402, which relates to sufficiency of safety precautions (TRGS 402, 2016). For our dissection course situation, the final evaluation was “Safety precautions are sufficient” (TÜV NORD Umweltschutz Gmbh & Co. KG, 2017).

Looking in particular at formaldehyde exposure, room-related measurements were 0.073 mg/m³ (2016) and 0.058 mg/m³ (2017), person-related (long-term) measurements amounted to 0.229 (2016), 0.118 and 0.107 mg/m³ (2017). The occupational exposure limit of formaldehyde has been 0.37 mg/m³ since 2015, and has remained unchanged since then (TRGS 900, 2015, 2022). Thullner et al. (Thullner et al., 2015) conducted exposure measurements at five anatomical institutes using different fixation protocols and with different course settings. They found air concentrations of formaldehyde varying between 0.16-0.79 mg/m³ for room related and 0.41-1.14 mg/m³ for person-related measurements. As in our course situation, person-related measurements were higher than room related measurements (average approx. x2). Formaldehyde air concentrations after our standard fixation were lower than those determined by Thullner et al. (2015). This is probably mainly due to a low formaldehyde concentration in our standard fixation solution and storage of fixated body donors in 70% ethanol without formaldehyde addition, leading to a diffusion of formaldehyde into the alcohol solution. Formaldehyde concentration and/or absolute formaldehyde content in fixation and storage solution constitute the primary determinant for the magnitude of formaldehyde evaporation from the body donors during the dissection course (Thullner et al., 2015; Waschke et al., 2019). It may be easier to reduce or completely omit formaldehyde in preservation/storage solution than in fixation solution. In a survey, 8 of 23 anatomical institutes stated that they use formaldehyde-free storage solutions, and most of them abandoned the formaldehyde component recently (Rissler and Hauke, 2021). In order to reduce formaldehyde exposure, Waschke et al. (2019) recommended considering the protocols for perfusion fixation, storage and body humidification during the dissection course separately, and reducing the amount of formaldehyde in all phases of the embalming and dissection process. Additionally, some institutes have successfully applied neutralizing chemicals, e.g., monoethanolamine and urea solution, to decrease formaldehyde evaporation from the corpses (Coskey and Gest, 2015; Kawata et al., 2019; Otsuka et al., 2022).

Further, technical and structural measures can reduce exposure very effectively. These include basic constructional aspects of the dissection hall (e.g. size and height in relation to the number of

corpses), ventilation (e.g. room vs./plus single table extraction systems, air vent skirting, air flow, air extraction volumes) and temperature regulation (e.g. air conditioning/cooling/heating, illumination) (Thullner et al., 2015; Waschke et al., 2019). At our facility, size and height of the dissection halls are quite large, and an effective ventilation and air conditioning system is installed, allowing high air extraction volumes at low room temperature even in summer. Therefore, single table suction devices are not necessary.

Additional recommendations for exposure reduction are, e.g., small number of donated bodies per room, exclusion of very overweight body donations from the dissection course, uncovering of bodies prior to course start, small number of students per dissection table, and reduction of thermal loads in the room (Thullner et al., 2015; Waschke et al., 2019).

Limitations of the study

Exposure measurements were undertaken on two days, which were chosen as days with maximum exposure situations, since it was our intention to find out if we could conduct the course safely with the present precautions. To map the complete exposure situation during a whole dissection course longitudinally, it would be necessary to perform more measurements throughout the course year and during different dissection situations. Additionally, air concentrations in relationship with fixed bodies of different size and body condition would be interesting to monitor. Our present study focused on occupational exposure of teaching staff and students during the dissection course. Future studies focusing on technical staff should be carried out to measure exposure in the fixation laboratory, in the storage hall and during preparatory works for the dissection course. Exposure during specialist training courses is lower compared to the dissection course, because of the lower concentrations of formaldehyde, ethanol and 2-phenoxyethanol in our soft fixation solution, of a smaller number of corpses in the room – and often only selected body parts are used. Nevertheless, it would be interesting to establish measurements for different clinical anatomy courses.

CONCLUSION

The two different ethanol-based fixation protocols presented here offer suitable alternatives for student dissection courses and specialist training courses, respectively. Fixative properties of the solutions were sufficient for long-term usability during the dissection course (ethanol-based standard protocol) or the specific demands of the specialist training courses (ethanol-glycerin-based soft fixation). Dissectability of the bodies was good with respect to the requirements of student and specialist training courses, respectively. Exposure to formaldehyde and other hazardous substances in the dissection course was compatible with current legislation and health protection requirements.

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