Title: Toward safer thanatopraxy cares: formaldehyde-releasers use.

Authors: Varlet V, Bouvet A, Cadas H, Hornung JP, Grabherr S

Journal: Journal of anatomy

Year: 2019 Jul 11

DOI: 10.1111/joa.13047

Review Article
Running Author: V. Varlet et al.
Running Title: Formaldehyde-releasers in thanatopraxy

Toward safer thanatopraxy cares: formaldehyde-releasers use

Vincent Varlet, [1,2,✉]
Email Vincent.varlet@chuv.ch
AQ1

Alain Bouvet, [3]

Hugues Cadas, [2]

Jean-Pierre Hornung, [2]

Silke Grabherr, [1,2,3]

AQ2
AQ3

[1] Swiss Human Institute of Forensic Taphonomy, University Centre of Legal Medicine Lausanne-Geneva, Lausanne, Switzerland

[2] Faculty Unit of Anatomy and Morphology, University Centre of Legal Medicine Lausanne-Geneva, Lausanne, Switzerland

[3] Forensic Medicine and Imaging Section, University Centre of Legal Medicine Lausanne-Geneva, Lausanne, Switzerland AQ4

Correspondence

Vincent Varlet, Swiss Human Institute of Forensic Taphonomy, University Centre of Legal Medicine Lausanne-Geneva, Lausanne, Switzerland. T: +41 21 314 38 42; E: Vincent.varlet@chuv.ch

Abstract

Human cadavers constitute very useful educational tools to teach anatomy in medical scholarship and related disciplines such as physiology, for example. However, as biological material, human body is subjected to decay. Thanatopraxy cares such as embalming have been developed to slow down and inhibit this decay, but the formula used for the preservation fluids are mainly formaldehyde (FA)-based. Very recently, other formulas were developed in order to replace FA, and to avoid its toxicity leading to important

environmental and professional exposure concerns. However, these alternative FA-free fluids are still not validated or commercialized, and their efficiency is still under discussion. In this context, the use of FA-releasing substances, already used in the cosmetics industry, may offer interesting alternatives in order to reduce professional exposures to FA. Simultaneously, the preservation of the body is still guaranteed by FA generated over time from FA-releasers. The aim of this review is to revaluate the use of FA in thanatopraxy cares, to present its benefits and disadvantages, and finally to propose an alternative to reduce FA professional exposure during thanatopraxy cares thanks to FA-releasers use.
AQ5

Graphical Abstract

xxxxxx

Accepted: 28 May 2019

Keywords

embalming
formaldehyde
formaldehyde-releaser
human body preservation
thanatopraxy
tissue fixation

Introduction

Within the university course of medical education and related disciplines, human bodies are used as educational tools to teach students, either by prosected specimens presentation or by whole body dissection.

However, in recent years, several concerns have arisen concerning the use of the human cadaver as an educational tool. The arguments against dissection include ethical and financial issues, fears of health hazards, and awareness of people's sensitivities and religious beliefs (Brenner, 2014). Moreover, at the time of virtualization, the imaging tools and practices can offer many alternatives (post mortem computed tomography, post mortem angiography, post mortem magnetic resonance imaging; Grabherr et al. 2017). On the other hand, there are also an increasing number of clinicians arguing for re-enhancing anatomical education by dissection, because tissue resistance, texture

and mechanical properties cannot be mimicked by artificial models (Korf et al. 2008; Balta et al. 2017). As result, virtual models and real human cadavers from donations to medical sciences must co-exist and constitute complementary tools for anatomy and morphology teaching.

However, the use of human bodies as an educational tool requires an appropriate preservation of the cadaver. This is achieved by treating it using thanatopraxy cares with special chemicals, for example, embalming. Embalming practices have been known since antiquity, and were developed mainly for religious reasons (Ancient Egypt culture, for example). These practices were very numerous and embalmer-dependent, which is still the case nowadays (Brenner, 2014; Hambly, 2016; Balta et al. 2019). From this important cultural diversity, a huge variability in embalming fluids has been noticed. One of the most important chemicals used for body preservation is formaldehyde (FA; Musial et al. 2016).

AQ6

However, the toxicity of FA for practitioners and for the environment is well established (Duong et al. 2011; Waschke et al. 2019). Consequently, there is an increasing opposition to FA and other chemicals, whereas ‘green chemistry’ is promoted (Rocha Ferreira et al. 2017). Recently, anatomists and embalmers tried to develop FA-free preservation fluids with mixed results (Balta et al. 2015). In this context, the use of FA-releasers may offer a satisfactory alternative from a professional exposure point of view (Van Dam, 2003; Dissard et al. 2010, 2018). There are over 30 FA-releasers with a wide array of applications. Most are used as preservatives in cosmetics, as antimicrobials in metalworking fluids, paint, lacquers and varnishes, or as durable press chemical finishes in textiles (De Groot et al. 2009). FA appears as a decomposition product of a precursor already containing FA in its composition, or through reaction between reagents with FA as by-product.

The aim of this review is to reevaluate the use of FA in thanatopraxy cares, to present its benefits and disadvantages, and finally to propose an alternative to reduce FA professional exposure during thanatopraxy cares thanks to use of FA-releasers.

Materials and methods

PubMed was searched from September to December 2018 using the keywords ‘embalming’ AND ‘formaldehyde’ OR ‘formaldehyde releaser’, resulting in 155 hits. All the articles and studies that were not directly linked to FA reduction in human embalming cares or FA-

releasers were excluded. The search was therefore extended to engines such as Google in order to enlarge the inclusion of relevant documents (books, open access, European legislation).

FA in embalming fluids

Thanatopraxy refers to the art, the science or the modern techniques allowing the preservation of human cadavers from decomposition by the destruction of pathogenic microorganisms, in order to present them with a living appearance for public and private viewing for the funeral. As result, thanatopraxy refers to cold storages (positive or freezing) or to injections of chemicals (biocides and preservatives). These treatments are used to prepare the body for funeral or to store the body for further use as an educational tool. In this context, embalming constitutes a subcategory of thanatopraxy cares and is rather used to define the old processes used for preserving human bodies in cross-cultural histories through the ages (Trompette & Lemonnier, 2009). For example, embalming in Ancient Egypt was a step of the mummification process, including evisceration, exsanguination, drying and balms, oils administrations before bandages wrapping (Singh Batra et al. 2010). Today, embalming is used to define the perfusion of the body by embalming fluids during thanatopraxy operations.

AQ7

The three goals of thanatopraxy are sanitization, presentation and preservation. Sanitization and preservation are achieved by the use of embalming fluids, whereas presentation is made thanks to mortuary cares including post mortem grooming and make-up.

Typical modern embalming fluids contain a variety of preservatives, biocides, moisturizing agents and dyes. The compositions are very diverse and should conform with the aims of embalming: thorough and complete preservation; softness of tissues, colour of muscles and organs as in unembalmed bodies; distension (and the colouring) of the vasculature (Brenner, 2014). The final formulated fluid must answer to the biological and physicochemical properties and characteristics compiled in Fig. 1.

Properties and characteristics of embalming fluid.

To achieve these goals and thanks to the bactericidal, fungicidal and insecticidal properties of FA, preservation with FA-based fluids constituted since the beginning of 1900s the best alternative to dangerous and toxic concoctions of heavy metal salts.

Formaldehyde, CH_2O, is a highly reactive aldehyde gas formed by oxidation or incomplete combustion of organic material, and can be produced secondarily in air from photochemical reactions. FA is known to react with proteins, lipids and nucleic acids (Srinivasan et al. 2002; Hoffman et al. 2015).The properties of FA as tissue fixative and preservative come from these reactions and generated by-products.

FA as tissue fixative

Formaldehyde acts by amino formylation, i.e. cross-linking several proteins chemically by inserting a methylene bridge ($-CH_2-$) between the nitrogen atoms of adjacent proteins, amines and related nucleophiles, resulting in fixation or tanning type action (Bedino, 2003; Thavarajah et al. 2012). Methylene bridging is sterically controlled, and occurs most often between lysine and various other amino acids (arginine, cysteine, asparagine, glutamine). Except amino formylation, other bridgings are also possible (hydroxyls, indoles and imidazoles), but their stability is random. The lysine bindings with cysteine are relatively stable but reversible, those with arginine, asparagine and glutamine are stable but susceptible to acid hydrolysis, and those with tyrosine appear to be very stable and acid-resistant. It seems in general that the stronger FA treatment is, the more acid-resistant the binding, even if there is a variability among protein cross-linkages.

These polymerization properties have been used in industrial applications. Bakelite (the first plastic) is the trademark name for FA and phenol polymer, formed under acidic conditions. As several embalming fluids combine both FA and phenol, this kind of polymerization may occur during thanatopraxy, but it is not clear whether these chemicals react within the fluid itself or within the corpse to form such a resin (Brenner, 2014). Another example is Formica, the trademark name for FA and urea polymer formed under neutral pH conditions. Formica is a resin used as glue, fire retardant and water repellant. As these resin formations can take place either using acid-catalysis (e.g. oxalic acid, hydrochloric acid or sulphonate acids) or base-catalysis, such resin formation may take place when considering

the long storage-times of the cadavers. When controlled, these reactions could positively contribute to the embalming, otherwise they could promote uncontrolled hardening and undesirable rigidity.

To avoid these reactions, FA is used in aqueous dilutions such as formaline. Formaline is constituted by 9–16% methanol, 33–49% FA and water up to 100%. Given the fact that a quantity of 4–5 g of FA is required to react with and fix 100 g of soluble protein and that the average protein content in the human body is about 16%, an 80-kg body should contain about 13 kg of proteins requiring 0.5–0.6 kg of pure FA, i.e. 1.4–1.7 L of formaline (37% FA)~~;~~ (Mayer, 2012; Brenner, 2014). Based on an average amount of 10 L injected, the final FA concentration of the embalming fluid would be about 5–6%. Several formulations were developed with higher FA concentrations according to the aim of the fixation (educational tool, microscopic fixations, etc.) but, taking into account the toxicity of FA, the current formulations try to reduce FA amount (Coleman & Kogan, 1998; Tissier & Migné, 2001; Anderson, 2006; Levine et al. 2006; Whitehead & Savoia, 2008; Kalanjati et al. 2012; Gahukar et al. 2014). Indeed, an increase of FA amount in embalming fluids leads to an increase of free FA in body. This FA vapour needs to be evacuated before using the embalmed bodies as an educational tool before dissection (Keil et al. 2001; Sugata et al. 2016).

AQ8

AQ9

AQ10

AQ11

FA as preservative

Formaldehyde is classified as a high level (8% FA in 70% alcohol) or intermediate-to-high level (4–8% FA in water) disinfectant (Mayer, 2012). It has a broad spectrum of action on microorganisms, including putrefactive organisms. FA in solution constitutes by itself an aseptic medium for microbial growth. Furthermore, by reacting with proteins, it destroys the existing living organisms and forms new polymers (resins), which are stable and unfit as food for organisms. FA reacts with DNA less frequently than with RNA (single-stranded DNA) because the hydrogen bonds holding DNA in its double helix are more stable (McKeen, 2012; Yamada et al. 2019). FA can bind covalently to adenine and guanine components of RNA and adenine, guanine and thymine of DNA to form cross-links (stable methylene-bis-adenosine from adenine, for example) and with other protein to form molecular adducts unsuitable for life.

As result, FA concentration should be sufficiently high to prevent microbial growth, but sufficiently low to avoid strong hardening and to prevent too much toxicity.

Indeed, if FA is toxic for putrefactive organisms, it is also toxic for humans working with it.

FA disadvantages

Formaldehyde solution (formaline) is considered a hazardous compound, notably by its toxic vapours. FA is rapidly metabolized, can be produced endogenously in humans or formed through the metabolism of many xenobiotic agents. At air levels of 0.5–2 ppm, FA may function as an irritant, and cause mild eye and mucous membrane irritations (Kim et al. 2011; Kundu & Gangrade, 2015; Kuriachan et al. 2017). Acute exposure to FA may reversibly diminish the sense of smell. Acute and chronic skin exposure may produce irritation and peeling, as well as an allergic contact dermatitis. Additionally to these pathological affections, an important variety of carcinomas have been associated with FA exposure, such as sinonasal, nasopharyngeal, other head and neck, respiratory cancer, lymphohaematopoietic, and brain and central nervous system cancers (Lu et al. 2010; Songur et al. 2010). FA was classified as a confirmed risk factor in cancer by the International Agency for Research on Cancer (IARC) in 2012 based on strong scientific evidence for sinonasal tumours, and more limited evidence for other tumours such as haematopoietic system (IARC Monographs, 2012).

AQ12

In the anatomical context of the dissection laboratory, the adverse effects of FA have been studied extensively (Fritzsche et al. 2012; Gahukar et al. 2014; Arya et al. 2016; D'Ettorre et al. 2017; Elshaer & Mahmoud, 2017; Scheepers et al. 2018). Cytogenetic damages have also been reported in anatomy workers even below the respective occupational exposure limit (Bono et al. 2010; Musak et al. 2013; Costa et al. 2015). As a result, it was clearly put in evidence that FA can lead to important health issues, in the short term (skin and respiratory sensitization) and longer term (cancer).

AQ13

More extensive reviews about the biological effects and toxicity of FA are easily available in the literature (Patton, 2015; Zhang, 2018). Until now, there has been no ‘decision of non-inclusion’ in Annex I or Ia of Directive 98/8/EC, for the relevant product-type PT 22 ‘Embalming and taxidermist fluids’ revised in 2012 (EU Regulation 2012). However, EU

is aware that it is important to find substitution products to FA in this kind of fluids for both regulatory, safety and quality reasons (Technical notes for Guidance, 2013).

Besides toxicity and tissue hardening, FA has several other disadvantages for embalming purposes. It rapidly coagulates the blood, converts the tissues to grey when it mixes with blood, fixes discolourations, dehydrates tissues, constricts capillaries, deteriorates with age, and has an unpleasant odour. To counterbalance these inconveniences, FA-based embalming fluid formulations were developed as aqueous dilutions with wetting agent and substances with strong diffusive physicochemical properties, such as glycerine, anticoagulant substances such as sodium citrate, dye such as eosin and, according to the case, specific additional solvent or products for precise goals such as colour control (use of chloral hydrate) or odour (perfumes; Mishra et al. 2016). However, the best formulation is difficult to achieve because each reagent is physicochemically different (solubility, viscosity, pH, ionic strength, behaviour in dilution, etc.).

In glycerine-based formulations, ethanol or methanol must be added to inhibit microbial growth maintaining low FA concentration in embalming fluid, as glycerine has no antiseptic properties. Some formulations require pH buffering to avoid the inconveniences of a delayed acidification of the body over time, which could transform FA into formic acid. As time goes by, this kind of solution could generate skeletal decalcification and colour smoothing.

Another problem with the FA-based embalming fluids can derive from the reaction with lipids, which is less described in literature (Jones, 1969; Fox et al. 1985). FA reacts with the double bonds of unsaturated fatty acids. The resulting complex contains a free carbonyl group, which probably originates from the FA. The reaction occurs over a wide pH range, and takes place in the absence of oxygen or moisture. In this reaction, FA may act as an oxidizing agent, with production of aldehyde groups in the lipids, leading to physicochemical body changes during storage.

Therefore, the use of alcohols in FA-based embalming fluids is practical for their germicide properties but also because alcohols can dissolve lipids (Demiryürek et al. 2002). According to the class of alcohols (primary, secondary or tertiary) and the dilution, the embalming fluid has different characteristics. The germicide effect is more important in heavy molecular weight alcohols so that methanol is less efficient than ethanol, and less efficient than isopropanol. The lipid extractive

property is also related to the class of alcohols, and is more important in tertiary alcohols than primary alcohols. If the alcohols constitute satisfactory preservative agent, their tissue penetration is variable and they can contribute to tissue hardening in long-term storage. As result, aqueous dilutions are necessary according to the embalming requirement. The mixture of alcohols and FA allows to combine the benefits of FA as tissue fixative and preservative with the benefits of alcohols (quick fixation, reduction of toxic FA vapours, better glycogen and nucleic acids preservation, etc.), and to limit the disadvantages of FA (toxicity, tissue hardening, etc.) and of pure alcohol-based solutions (tissue shrinkage, hardening, deposition of pigment in blood, total or partial lysis of red blood cells, increased flammability, etc.; Cox et al. 2006; Moelans et al. 2011; Shian et al. 2016).

Alternative to FA

Therefore, to avoid the inconveniences of FA-based embalming fluids, trials have been performed and specific FA-free formulations were commercialized to this purpose.

However, FA substitution must lead to similar final fluid properties and characteristics. As previously presented in Fig. 1, FA is a good preservative and fixative agent, active at neutral pH. It has an antimicrobial activity and tissue fixative properties, which must be controlled to avoid hardening. The use of another substance to guarantee the effects of FA must not destabilize the embalming fluid properties.

Consequently, metallic salts could be used as was done in the past, even if they also support a certain toxicity (Bryant & Peck, 2009; Hambly, 2016). Zinc chloride ($ZnCl_2$) could be useful as a preservative and penetrating agent, but should be used in mixture with moisturizing agents and dyes because, when used alone, it leads to rigidity and gives a grey hue to the body. Moreover, zinc chloride shows good tissue fixative properties (Van Toor et al. 2006; Goodarzi et al. 2017). However, it also has a significant toxicity for embalming operators (acidic pH, irritant to caustic agent) and may reduce the final compliance of the embalming fluid to the optimal embalming fluid properties. Aluminium sulphate [$Al_2(SO_4)_3$] could also be useful as an alternative due to its antimicrobial effect, but it is not soluble in water and has coagulant and tanning properties.

AQ14

Chloral hydrate [$CCl_3CH(OH)_2$] is already used in embalming fluids, usually about 20% (w/w; Guimaraes da Silva et al. 2004). This

substance due to its carbonyl function acts as a preservative and tissue fixative, and prevents body discolouration. However, the best preservative agents are dialdehydes, such as glutaraldehyde, glyoxal, butanedialdehyde and ethanedial (Kiernan, 2000; Bedino, 2003; Balta et al. 2015; Cisne de Paula et al. 2018). Antimicrobial and fixative properties of dialdehydes mainly due to their carbonyl functions are distinct from their toxicities due to the number of carbon atoms separating the two carbonyl functions (dialdehyde with an even carbon atom number is less toxic than an odd number; Camiener, 1993; LoPachin & Gavin, 2014). Among them, glyoxal seems to be the best candidate for FA substitution.

AQ15

Other preservative substances have been investigated, such as diethylacetal [$CH_3CH(C_2H_5O)_2$], formulated at 30–33% in mixture with tannins (7–9%) and potassium carbonate, such as salts/acids mixed with citric and ascorbic acids, and sodium and carbonate bisulphite formulated in equal parts in proportions up to 40% (Dessart et al. 2006), polyhexamethyleneguanidine hydrochloride (Anichkov et al. 2011), fluorine (Blake & Falder, 2004) or iodine derivatives such as polyvinylpyrrolidine-iode (Dermot & Barrow, 2000) formulated in weak amounts (0.05–2%) with usual embalming fluids additives, or the use of N-vinyl-2-pyrrolidone in dilution (from 4 to 21.5% of body weight; Haizuka et al. 2018). Other mixtures have also shown interesting properties to replace FA and phenol, such as ethanol –glycerine–thymol mixture (Hammer et al. 2012), aliphatic alcohols-mono and diethylene glycols (Goyri-O'Neill et al. 2013) or based on phenoxyethanol (Frolich et al. 1984).

AQ16

AQ17

Other strategies could be developed based on vegetal tannins (tannic acid at levels from 1 to 10% in mixture with zinc and aluminium salts is used in wood preservation; Cisne et al. 2018), saturated salt solutions (Hayashi et al. 2014, 2016; Burns et al. 2018), sugars derivatives (oxopolysaccharides with free carbonyl functions) and honey (Sharquie & Najim, 2004; Kuriachan et al. 2017) , syntans (polymers of unsaturated carboxylic acids or their salts such as acrylic methacrylic, crotonic acids and acrylamide, acrylonitrile, etc. formulated from 3 to 6%), resins such as polyacrylates (Kato et al. 2009; Fernandez, 2016) and polymethylacrylates (but their physicochemical properties remain different from those required for embalming fluid). Epoxy compounds have been also tested in concentrations close to 4%, and seem to lead to a weaker hardening and better life-like colour than dialdehydes (Sung

et al. 1996; Alsharif et al. 2017; Ajileye et al. 2018). 1,4-Bis (hydroxymethyl)-2,5-piperazinedione ($C_6H_{10}N_2O_4$) was also tested in embalming fluid for its biocid and preservative properties, but its price is consequent (Berke & Rosen, 1991). As result, if the individual properties of these substances seem very interesting for preservative and tissue fixative, their behaviour in mixture, their price and the intermediate reaction required to release FA are still not sufficiently controlled and optimized to imagine FA substitution.

AQ18

AQ19

AQ20

Finally, ionic liquids represent a very nice alternative (Dessart et al. 2006). They are pure liquid organic salts at ambient temperature, and certain are composed with organic cations such as quaternary ammonium or imidazolium and aromatic or non-aromatic compounds. These cheap and weakly toxic solvents have been tested in comparison to FA with very good results for 1-methyl-3-octyl-oxymethyl-imidazolium-tetrafluoroborate for example (Majewski et al. 2003; Pernak et al. 2005). This opens up a wide field for future investigations on this new class of solvents in medicine by using them as a pure solvent or co-solvent.

New alternative: FA-releasers

FA-releasers for embalming fluids

Formaldehyde-releasers constitute a class of chemicals that are able to generate FA, directly through decomposition of a precursor already containing FA in its composition, or indirectly through reaction between reagents with FA as by-product. There are over 30 FA-releasers with a wide array of applications (Table 1).

Table 1 List of FA-releasers

Bioban CS 1135®	**Imidazolidinyl urea (IU)**
Bioban CS 1246®	MDM hydantoin
Bioban P-1487®	Methenamine
2-Bromo-2-nitropropane-1,3-diol (Bronopol)	N,N′-Methylenebis(5-methyloxazolidine)
Diazolidinyl urea (DU)	4,4′-Methylenedimorpholine

Dihydroxydimethylolethyleneurea, methylated	N-Methylol-chloracetamide
1,3-Dimethyl-4,5-dihydroxyethyleneurea	Methylol urea
Dimethylhydantoin formaldehyde resin	Paraformaldehyde
Dimethyloldihydroxyethyleneurea (DMDHEU)	Polyoxymethylene melamine
Dimethylolethyleneurea	Polyoxymethylene urea
Dimethylolpropyleneurea	Benzylhemiformal/Preventol D2®
Dimethylol urea	Propyleneglycol hemiformal
DMDM hydantoin (DMDMH)	**Quaternium-15 (Q-15)**
Ethylene urea	Sodium hydroxymethylglycinate
Forcide 78® I	Tetramethylol acetylenediurea
Forcide 78® II	Tris(N-hydroxyethyl) hexahydrotriazine
Glyoxalurea	Tris(hydroxymethyl)-nitromethane

Those that are in bold could be useful in embalming applications.

Only chemicals for which adequate clinical data are available to identify them as FA-releasers beyond doubt have been compiled. Most are used as preservatives in cosmetics; as antimicrobials in metalworking fluids, paint, lacquers and varnishes; or as durable press chemical finishes in textiles (Flyvholm & Andersen, 1993). However, only a few of them could be used as suitable FA-releasers in embalming fluids. Indeed, their FA-releasing properties are strongly linked to pH. Most of them can liberate FA in acidic media, which have to be avoided in order to perform the best body perfusion during embalming.

AQ21

Formaldehyde-releasing could constitute a positive approach to lower FA exposure for embalmers and thanatopraxy operators, but still guaranteeing the body preservation by FA. During embalming, FA is weakly present and the strategy is to permit FA-release during the body storage. The main advantage is control FA emission over time, and the main drawback is the lack of data concerning the kinetics of FA release (Silverman & Holladay, 2015). Only six FA-releasers have been investigated more deeply to understand the FA-release kinetics: 2-

bromo-2-nitropropane-1,3-diol (Bronopol), dimethyloldihydroxyethyleneurea (DMDHEU), DMDM hydantoin (DMDMH), dimethylol urea (DU), imidazolidinyl urea (IU) and quaternium-15 (Q-15; Fig. 2).
AQ22

Formaldehyde (FA) molecules produced from FA-releasers.

Analytical challenge of FA measurement

Formaldehyde-releasing has put in evidence an additional challenge to the analytical FA monitoring because both FA and FA-releasers are chemically sensitive (SCCNFP, 2002). The parameters of the extraction/analysis method can induce biased results because FA release is driven by specific pH, temperature, ionic strength, dilution, and eventually the presence of other solvents or embalming fluid ingredients. The biochemical composition of the media is also of importance through cross-linkages and reactions implying FA.

Indeed, the reactivity of FA constitutes a first problem. It has been shown using pure FA aqueous dilutions that the parameters of extraction and analysis can promote FA reactions with the matrix

compounds leading to an underestimated FA quantification. In a comparison between the free FA detected and the FA added of 1.0 g and 0.5 g in water and in different cream bases, a higher percentage of free FA was detected from 0.5 g samples than from 1.0 g samples, whereas no correlation was seen between the viscosity of the cream base and the release of FA. As result, it seems that if the influence of the viscosity is not significant, only a part of FA released would be extracted and quantified. The other part of free FA may be still dissolved in the samples or may have reacted with other molecules as is the case with amino acids in amino formylation.

The second problem comes from the fact that FA release from FA-releasers is also chemically driven. Artificial FA release from FA-releasers can be observed during the extraction driven by specific conditions. As result, extraction and analytical procedures can induce a FA misestimation. It can be an overestimation when the analytical procedure promotes FA-releasing or an underestimation when the physicochemical parameters promote FA-releasers stability.

As result, new methods were developed to minimize the misestimation of FA amount due to extraction and analytical parameters. Indeed, the quantification of free FA in the presence of FA-releasers usually gives too high and non-reproducible values with the commonly applied methods, such as the ‘acetylacetone method’ and 2,4-dinitrophenylhydrazine (2,4-DNPH) derivatization, as the FA-releasers are degraded by the reagents in these methods (Brandao et al. 2018).

As the high-performance liquid chromatography method with post-column derivatization adopted within the EU is too complicated for routine analysis, other extraction designs have been developed, such as gas-diffusion microextraction with simultaneous acetylacetone derivatization, or closed container diffusion (CCD) method consisting of absorbent filter loaded with 2,4-DNPH located in the headspace above the sample that must trap and derivatize free FA released from FA-releasers present in the sample (Karlberg et al. 1998; Brandao et al. 2018). Using these extraction methods, FA is gently trapped on absorbants minimizing artefactual reactions in the matrix. Then, FA is derivatized directly on these absorbants and later analysed.

FA-releasing kinetics

For example, after 1 year of storage, cream base products fortified with various levels of FA-releasers have led to the following average FA recovery yields, measured similarly with CCD and EU methods: DU = 15.9% (sample pH 4.4), IU = 8.5% (sample pH 4.4) and

Bronopol = 3.5% (sample pH 6; Karlberg et al. 1998). Other FA-releasing kinetics were done in aqueous samples with phosphate buffer (final buffer solution at 0.1 M at pH 7.4) and monitored by nuclear magnetic resonance (Kireche et al. 2010). Reactions between FA released and amino acids have been put in evidence, but free FA was still noticeable after several days of storage. Methenamine at 0.130, 0.259 and 0.389 M has not led to a detectable amount of FA after 1 day of storage. This result is conformed to the fact that FA could be released from methenamine under acidic conditions and not at pH 7.4. With DMDMH, FA release was quick and the initial amount of FA-releaser did not seem to be very influent even if a greater amount was obtained for the weakest concentration. Indeed, after 1 day of storage, about 31, 24 and 21% of the theoretical amount of FA was generated, respectively, from DMDMH solutions at 0.096, 0.193 and 0.289 M at neutral pH. The polymerization of FA at highest concentrations could be a possibility to explain this result.

With Bronopol, from an aqueous dilution at 0.273 M, about 3.1, 3.7 and 19% of the theoretical amount of FA was generated, respectively, after 1, 7 and 40 days of storage. FA release from Bronopol was also more extensively investigated in other studies, but FA was analysed by 2,4-DNPH derivatization, a method known to overestimate FA release by degradation of FA-releaser during the analysis (Kajimura et al. 2008). However, individual factors were investigated, such as pH, temperature and viscosity. Aqueous Bronopol solutions (0.1%, w/v) with 0.005 M sodium phosphate buffer (pH 6) incubated for 90 min in water baths of 25, 40 and 60 °C have resulted in temperature-dependent FA release (Kajimura et al. 2008). The highest temperature led to the most important FA release. Aqueous Bronopol solutions (0.1 and 0.01% w/v) buffered at pH 2, 4, 6 and 8 with diluted 0.005 M sodium phosphate and stored 50 days at 25 °C resulted in pH-dependent FA release. The alkaline pH led to the most important FA release. More precisely, a sharp increase of FA is noticed from the 1st to 3rd day, and a gradual increase from the 3rd to 50th day of storage. There was a corresponding decrease of Bronopol at these times but without pH changes. Finally, homemade lotion and jell formulated with two levels of Bronopol (0.1 and 0.05%, w/v) have shown similar FA release at 25 °C over 50 days. However, and conversely to aqueous solutions, FA release from Bronopol in homemade cosmetics was not found to be pH dependent. These results suggest that FA release from Bronopol is also influenced by other matrix compounds. This information can be easily explained by the reaction of FA released and amino acids for example. The knowledge of the behaviour of FA-releasers is crucial for further use in

the formulation of embalming fluids considering the complex composition of the human body.

As result, each FA-releaser should be considered individually according to the required final characteristics of the formulated embalming fluid. Considering that a minimal amount of 4.5 g of FA is required to formulate 10 L of embalming fluid ready to be used, various amounts of FA-releasers must be chosen to reach the same embalming properties of FA if added directly in the fluid. The FA-release is matrix-, pH-, temperature- and quantity-dependent (Doi et al. 2010). For example, if a body storage of 1 month is required and for a production of 10 L of embalming fluid, FA could be substituted by 50–60 g of DMDMH or minimal amount of 80 g of Bronopol if the pH is maintained at about 7 if FA release is assumed to be linear. In case of more acidic pH 6 and for longer storage, a higher Bronopol amount would reach the required FA concentration. Alternatively, IU or DU could be used if pH was maintained even more acid (about 4), but this does not conform to the neutral pH usually required for good embalming practices. However, these suggestions should be very carefully investigated because some of them can lead to other problems. For example, Bronopol could decompose into nitrites, and in the presence of certain tertiary amines and amides can form carcinogenic nitrosamines (Balta et al. 2015).

Conclusion

Formaldehyde has been widely used in thanatopraxy and histology practices for centuries. It has the best compromise in terms of preservation, tissue fixative and desiccative retardant properties. However, this compound reveals an important toxicity, and is concerned by environmental and regulatory pressures in order to find an alternative. Potential candidates could be found through technology transfer from the tanning industry, for example. However, complete substitution of FA by other compounds has led to mixed results. Another alternative should be the use of FA-releasers in embalming fluid formulations. These compounds can release FA over time, but they require the control of several parameters such as pH and temperature. However, they open new investigation routes to develop embalming fluids in order to guarantee the preservation and the safety of embalmed bodies as well as the life-like aspect of dead bodies for grieving families and relatives.

Conflicts of interest

The authors do not declare any conflict of interests.

Authors' contributions

VV: concept and study design, literature search and interpretation, manuscript writing. AB: concept and study design, literature search. HC: interpretation data, manuscript editing. JPH: interpretation data, manuscript editing. SG: interpretation data, manuscript editing.

References

Ajileye AB, Esan EO, Adeyemi OA (2018) Human embalming techniques: a review. Am J Biomed Sci 10, 82–95.

Alsharif MHK, Musthafa M, Elamin AY, et al. (2017) A brief review on the principles of human cadaver preservation and monitoring of microbial degradation. Forensic Med Anat Res 5, 19–31.

Anderson SD (2006) Practical light embalming technique for use in the surgical fresh tissue dissection laboratory. Clin Anat 19, 8–11.

Anichkov NM, Danilova IA, Vasiliev OD, et al. (2011) Use of a polyguanidine solution for fixing biological and anatomical specimens. Neurosci Behav Physiol 41, 18–21.

Arya D, Chauhan K, Prajapati B, et al. (2016) Health hazards due to formaline exposure in the department of anatomy. World J Pharm Pharmaceut Sci 5, 2016–2024.

Balta JY, Cronin M, Cryan JF, et al. (2015) Human preservation techniques in anatomy: a 21st century medical education perspective. Clin Anat 28, 725–734.

Balta JY, Cronin M, Cryan JF, et al. (2017) The utility of cadaver-based approaches for the teaching of human anatomy: a survey of British and Irish anatomy teachers. Anat Sci Educ 10, 137–143.

Balta JY, Twomey M, Moloney F, et al. (2019) A comparison of embalming fluids on the structures and properties of tissue in human cadavers. Anat Histol Embryol 48, 64–73.

Bedino JH (2003) Embalming chemistry: glutaraldehyde versus formaldehyde. Champion Expanding Encyclopedia of Mortuary Practices 649, 2614–2632.

Berke PA, Rosen WE (1991) Glycine anhydride dimethylol as a biocide and preservative. US5707993A.

Blake DC, Falder SB (2004) Preservative and embalming fluids. WO2006059126A1.

Bono R, Romanazzi V, Munnia A, et al. (2010) Malondialdehyde−deoxyguanosine adduct formation in workers of pathology wards: the role of air formaldehyde exposure. Chem Res Toxicol 23, 1342.

Brandao PF, Ramos RM, Rodrigues JA (2018) GDME-based methodology for the determination of free formaldehyde in cosmetics and hygiene products containing formaldehyde releasers. Anal Bioanal Chem 410, 6873–6880.

Brenner E (2014) Human body preservation – old and new techniques. J Anat 224, 316–344.

Bryant CD, Peck DL (2009) Encyclopedia of Death and the Human Experience, 1st edn. Thousand Oaks, CA: SAGE Publications, 1 160 p.
AQ23
Burns DM, Bell I, Katchky R, et al. (2018) Saturated salt solution cadaver-embalming method improves orthopaedic surgical skills training. J Bone Joint Surg Am 100, e104.

Camiener GW (1993) Safe dialdehydes useful as decontaminants, fixatives, preservatives and embalming agents. US5429797A.

Cisne de Paula R, Babinski MA, Pereira-Sampaio MA, et al. (2018) The use of fixative solutions throughout the ages: a comprehensive review. Acta Sci Anat 1, 21–28.

Cisne R, Souza AM, Pereira-Sampaio MA, et al. (2018) Tannic acid solution: a better fixative solution than formalin for elastin and collagen – toxic and morphological assessment. Anat Rec 301, 1544–1550.

Coleman R, Kogan I (1998) An improved low-formaldehyde embalming fluid to preserve cadavers for anatomy teaching. J Anat 192, 443–446.

Costa S, Carvalho S, Costa C, et al. (2015) Increased levels of chromosomal aberrations and DNA damage in a group of workers exposed to formaldehyde. Mutagenesis 30, 463.

Cox ML, Schray CL, Luster CN, et al. (2006) Assessment of fixatives, fixation, and tissue processing on morphology and RNA integrity. Exp Mol Pathol 80, 183–191.

De Groot AC, Flyvholm MA, Lensen G, et al. (2009) Formaldehyde-releasers: relationship to formaldehyde contact allergy. Contact allergy to formaldehyde and inventory of formaldehyde-releasers. Contact Dermat 61, 63–85.

Demiryürek D, Bayramoğlu A, Ustaçelebi Ş (2002) Infective agents in fixed human cadavers: a brief review and suggested guidelines. Anat Re 269, 194–197.

Dermot C, Barrow J (2000) Embalming fluid.. US20030206884A1.

Dessart L, Serpentini CL, Benoît-Marquié F, et al. (2006) Fluides d'embaumement: une vie après le formol ? L'actualité chimique 299, 26 –35.

D'Ettorre G, Cricuolo M, Mazzotta M (2017) Managing formaldehyde indoor pollution in anatomy pathology departments. Work 56, 397–402.

Dissard D, Jay JC, Gilotin D, et al. (2010) Composition and method for organ preservation. US20150010488A1.

Dissard D, Jay JC, Gilotin D, et al. (2018) Composition and method for tissue preservation and embalming. US9861094B2.

Doi T, Kajimura K, Taguchi S (2010) Survey of formaldehyde (FA) concentration in cosmetic containing FA-donor preservatives. J Health Sci 56, 116-122.

Duong A, Steinmaus C, McHale CM, et al. (2011) Reproductive and developmental toxicity of formaldehyde: a systematic review. Mutat Res 728, 118–138.

Elshaer NSM, Mahmoud MAE (2017) Toxic effects of formalin-treated cadaver on medical students, staff members, and workers in the Alexandria Faculty of Medicine. Alexandria J Med 53, 337–343.

European Commission (2013). Technical notes for guidance – Guidance document on the evaluation of efficacy of embalming products (PT22). CA-July-13-Doc.6.2.d

European Union (2012) Regulation No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. L167:1–123.

Fernandez JD (2016) Post mortem reconstitution of circulation. United States Patent Application 20160140878.

Flyvholm MA, Andersen P (1993) Identification of formaldehyde releasers and occurrence of formaldehyde and formaldehyde releasers in registered chemical products. Am J Indust Med 24, 533–552.

Fox CH, Johnson FB, Whiting J, et al. (1985) Formaldehyde fixation. J Histochem Cytochem 33, 845–853.

Fritzsche FR, Ramach C, Soldini D, et al. (2012) Occupational health risks of pathologists – results from a nationwide online questionnaire in Switzerland. BMC Public Health 12, 1054.

Frolich KW, Andersen LM, Knutsen A, et al. (1984) Phenoxyethanol as a nontoxic substitute for formaldehyde in long-term preservation of human anatomical specimens for dissection and demonstration purposes. Anat Rec 208, 271–278.

Gahukar S, Ramteke U, Majumdar D, et al. (2014) Prevalence of formaldehyde in indoor air of gross anatomy laboratory and cadaver storage room of a medical college. J Environ Occup Sci 3, 181–185.

Goodarzi N, Akbari Gh, Razeghi Tehrani P (2017) Zinc chloride, a new material for embalming and preservation of the anatomical specimens. Anat Sci 14, 25–30.

Goyri-O'Neill J, Pais D, Feire de Andrade F, et al. (2013) Improvement of the embalming perfusion method: the innovation and the results by light and scanning electron microscopy. Acta Med Port 26, 188–194.

Grabherr S, Egger C, Vilarino R, et al. (2017) Modern post-mortem imaging: an update on recent developments. Forensic Sci Res 2, 52–64.

Guimaraes da Silva RM, Matera JM, Maciel Ribeiro AAC (2004) Preservation of cadavers for surgical technique training. Vet Surg 33, 606–608.

Haizuka Y, Nagase M, Takashino S, et al. (2018) A new substitute for formalin: application to embalming cadavers. Clin Anat 31, 90–98.

Hambly TR (2016) Dr Hambly's Historical Guide to Embalming AQ24 Cookbook. Lulu.com. 84 p. ????: ????.

Hammer N, Löffler S, Feja C, et al. (2012) Ethanol-glycerin fixation with thymol conservation: a potential alternative to formaldehyde and phenol embalming. Anat Sci Educ 5, 225–233.

Hayashi S, Homma H, Naito M, et al. (2014) Saturated salt solution method: a useful cadaver embalming for surgical skills training. Medicine (Baltimore) 93, e196.

Hayashi S, Naito M, Kawata S, et al. (2016) History and future of human cadaver preservation for surgical training: from formalin to saturated salt solution method. Anat Sci Int 91, 1–7.

Hoffman EA, Frey BL, Smith LM, et al. (2015) Formaldehyde crosslinking: a tool for the study of chromatin complexes. J Biol Chem 290, 26404–26411.

International Agency for Research on Cancer (IARC) (2012) A Review of Human Carcinogens. F. Chemical Agents and Related Occupations. IARC Monographs. 615 p. ????: WHO Press.

Jones D (1969) The reaction of formaldehyde with unsaturated fatty acids during histological fixation. Histochem J 1, 459–491.

Kajimura K, Tagami T, Yamamoto T, et al. (2008) The release of formaldehyde upon decomposition of 2-bromo-2-nitropropan-1,3-diol (Bronopol). J Health Sci 54, 488–492.

Kalanjati PV, Prasetiowati L, Alimsardjono H (2012) The use of lower formalin-containing embalming solution for anatomy cadaver preparation. Med J Indonesia 21, 203–207.

Karlberg AT, Skare L, Lindberg I, et al. (1998) A method for quantification of formaldehyde in the presence of formaldehyde donors in skin-care products. Contact Dermat 38, 20–28.

Kato M, Ohnishi K, Kitayama E (2009) Corpse-treatment composition and corpse-treatment method. US 2009/0282659A1.

Keil CB, Akbar-Khanzadeh F, Konecny KA (2001) Characterizing formaldehyde emission rates in a gross anatomy laboratory. Appl Occup Environ Hyg 16, 967–972.

Kiernan JA (2000) Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. Micros Today 8, 8–13.

Kim KH, Jahan SA, Lee JT (2011) Exposure to formaldehyde and its potential human health hazards. J Environ Sci Health, Part C 29, 277 –299.

Kireche M, Gimenez-Arnau E, Lepoittevin JP (2010) Preservatives in cosmetics: reactivity of allergenic formaldehyde-releasers towards amino acids through breakdown products other than formaldehyde. Contact Dermat 63, 192–202.

Korf HW, Wicht H, Snipes RL, et al. (2008) The dissection course – necessary and indispensable for teaching anatomy to medical students. Ann Anat 190, 16–22.

Kundu S, Gangrade P (2015) Study of the toxic effects of formaldehyde vapours within the dissection hall on the first year Indian medical students. Int J Anat Res 3, 1179–1190.

Kuriachan D, Suresh R, Janardhanan M, et al. (2017) Analysis of fixative properties of three eco-friendly substances: a comparison with formalin. Oral Maxillofac Pathol J 8, 79–84.

Levine RL, Kieves S, Cathey G, et al. (2006) The use of lightly embalmed (fresh tissue) cadavers for resident laparoscopic training. J Minim Invasive Gynecol 13, 451–456.

LoPachin RM, Gavin T (2014) Molecular mechanisms of aldehyde toxicity: a chemical perspective. Chem Res Toxicol 27, 1081–1091.

Lu K, Collins LB, Ru H, et al. (2010) Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. Toxicol Sci 116, 441–451.

Majewski P, Pernak A, Grzymislawski M, et al. (2003) Ionic liquids in embalming and tissue preservation. Can traditional formalin-fixation be replaced safely? Acta Histochem 105, 135–142.

Mayer RG (2012) Embalming: History, Theory, and Practice, 5th edn, 728 p. New York, NY: McGraw-Hill Medical.

McKeen L (2012) The Effect of Sterilization on Plastics and Elastomers, 3rd edn, 480 p. Amsterdam: Elsevier.

Mishra SR, Passey J, Singh R, et al. (2016) Embalming fluid – modified composition for hot and humid places. Int J Anat Res 4, 2531 –2535.

Moelans CB, Oostenrijk D, Moons MJ, et al. (2011) Formaldehyde substitute fixatives: effects on nucleic acid preservation. J Clin Pathol 64, 960–967.

Musak L, Smerhovsky Z, Halasova E, et al. (2013) Chromosomal damage among medical staff occupationally exposed to volatile anesthetics, antineoplastic drugs, and formaldehyde. Scand J Work Environ Health 39, 618.

Musial A, Gryglewski RW, Kielczewski S, et al. (2016) Formalin use in anatomical and histological science in the 19th and 20th centuries. Folia Med Cracov 56, 31–40.

Patton A (2015) Formaldehyde: Synthesis, Applications and Potential Health Effects. 1st edn, 182 p. New York, NY: Nova Science Publishers.

Pernak A, Iwanik K, Majewski P, et al. (2005) Ionic liquids as an alternative to formalin in histopathological diagnosis. Acta Histochem 107, 149–156.

Rocha Ferreira J, Cardoso Rezende L, Barbosa AS, et al. (2017) Economic, human and environmental health benefits of replacing formaldehyde in the preservation of corpses. Ecotoxicol Environ Saf 145, 490–495.

Scheepers PTJ, Graumans MHF, Beckmann G, et al. (2018) Changes in work practices for safe use of formaldehyde in a university-based anatomy teaching and research facility. Int J Environ Res Public Health 15, 2049.

Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (2002) Opinion concerning the determination of certain formaldehyde releasers in cosmetic products. SCCNFP/586/02, final. Adopted by the SCCNFP during the 22nd plenary meeting of 17 December 2002.

Sharquie KE, Najim RA (2004) Embalming with honey. Saudi Med J 25, 1755–1756.

Shian SW, Soenggono A, Pramod SV (2016) Comparison of tissue preservation using formalin and ethanol as preservative formula. Althea Med J 3, 359–363.

Silverman RB, Holladay MW (2015) The Organic Chemistry of Drug Design and Drug Action. Elsevier. eBook ISBN: 9780123820310
AQ25 https://doi.org/10.1016/c2009-0-64537-2.

Singh Batra AP, Signh Khurana B, Mahajan A, et al. (2010) Embalming and other methods of dead body preservation. Int J Med Toxicol Legal Med 12, 15–19.

Songur A, Ozen OA, Sarsilmaz M (2010) The toxic effects of formaldehyde on the nervous system. Rev Environ Contam Toxicol 203, 105–118.

Srinivasan M, Sedmark D, Jewell S (2002) Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol 161, 1961–1971.

Sugata Y, Miyaso H, Odaka Y, et al. (2016) Levels of formaldehyde vapor released from embalmed cadavers in each dissection stage. Environ Sci Pollut Res 23, 16176–16182.

Sung HW, Cheng WH, Chiu IS, et al. (1996) Studies on epoxy compound fixation. J Biomed Mater Res 33, 177–186.

Thavarajah R, Mudimbaimannar VK, Elizabeth J, et al. (2012) Chemical and physical basics of routine formaldehyde fixation. J Oral Maxillofac Pathol 16, 400–405.

Tissier C, Migné V (2001) Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used in taxidermy and embalming processes (Product type 22). INERIS-DRC -01-25582-ECOT-CTi/VMi-n°01DR0175.

Trompette P, Lemonnier M (2009) Funeral embalming: the transformation of a medical innovation. Science Studies 22, 9–30.

Van Dam AJ (2003) DMDM-hydantoin: the promising result of a search for an alternative in fluid preservation of biological specimens. Collection Forum 18, 104–115.

Van Toor I, Verplancke V, Van Glabbeek F, et al. (2006) Zinc chloride embalming technique and silicone plastination. FASEB J 20, A885 –A886.

Waschke J, Bergmann M, Bräuer L, et al. (2019) Recommendations of the working group of the Anatomische Gesellschaft on reduction of formaldehyde exposure in anatomical curricula and institutes. Ann Anat 221, 179–185.

Whitehead MC, Savoia MC (2008) Evaluation of methods to reduce formaldehyde levels of cadavers in the dissection laboratory. Clin Anat 21, 75–81.

Yamada M, Funaki S, Miki S (2019) Formaldehyde interacts with RNA rather than DNA: accumulation of formaldehyde by the RNA-inorganic hybrid material. Int J Biol Macromol 122, 168–173.

Zhang L (2018) Formaldehyde: Exposure, Toxicity and Health Effects. Issues in Toxicology n°37, 389 p. Croydon, UK: The Royal Society of Chemistry.

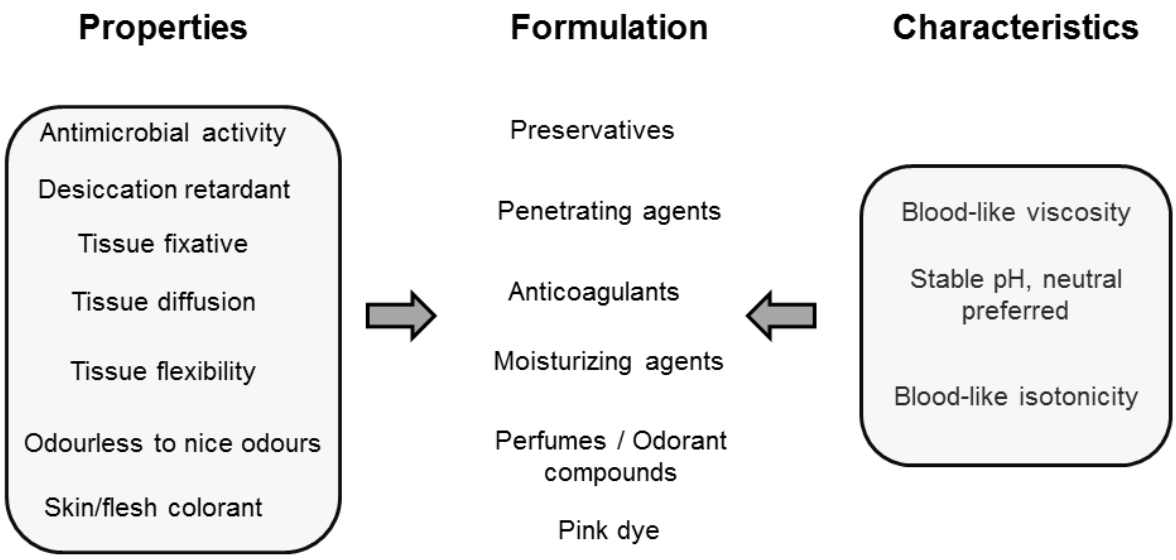

Figure 1. Properties and characteristics of embalming fluid

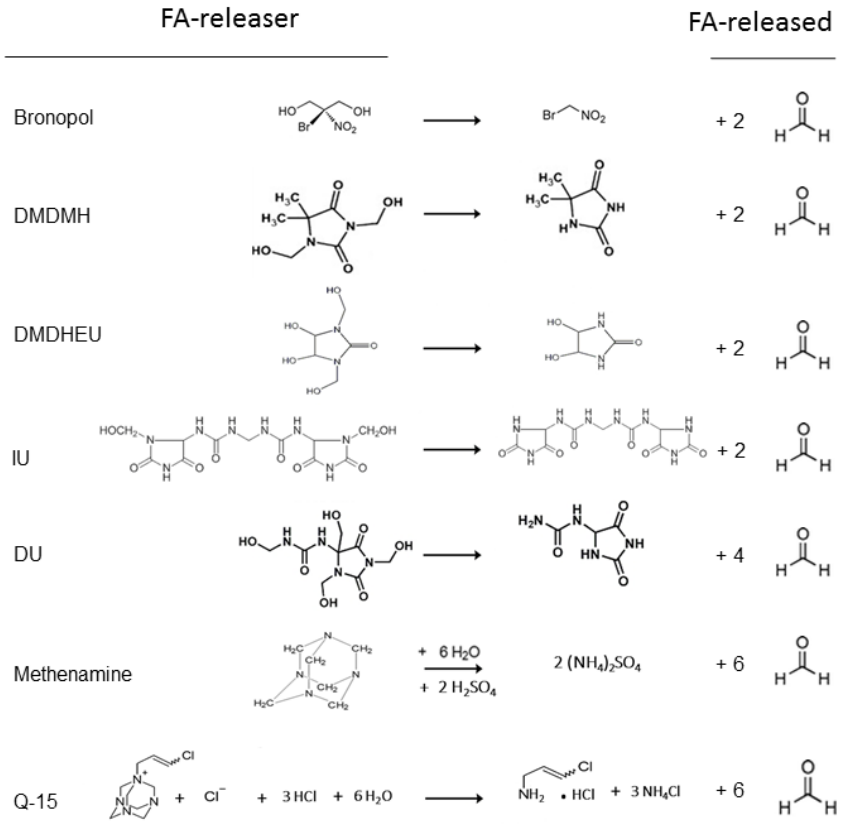

Figure 2. Formaldehyde molecules produced from formaldehyde releasers