DOCUMENT OF RECOMMENDATIONS OF THE SPANISH PATHOLOGICAL ANATOMY SOCIETY-IAP REGARDING THE SAFETY MEASURES ADVISED FOR THE MANAGEMENT OF FORMALDEHYDE AND THE USE OF ALTERNATIVE FIXATIVES.

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JUSTIFICATION

The Spanish Society of Pathological Anatomy (SEAP-IAP) produces this document in order to provide useful recommendations for the safe management of formaldehyde by the professionals at the healthcare institutions, especially those of the Pathological Anatomy services, and to provide information for the people in charge of these so mentioned institutions so they adopt the appropriate measures, bearing in mind the regulation in force, and also regarding the basic proposals for the substitution of formaldehyde by alternative fixatives available nowadays.

INTRODUCTION

Formaldehyde is a gas which is volatile at room temperature and soluble in water and polar solvents that is used in multiple applications and industrial processes. In the Pathological Anatomy area it has been used universally as a tissue fixative, not having been substituted up to now by alternative fixatives. This is due to its low price (if the cost derived from the required protection measures, the waste management and the harmful effects on people´s health are not controlled for) and it conserves correctly and for a long time the biological samples, preserving the cellular and tissue morphology adequately.

It´s chemical formula is simple: CH_2O. As a fixative is used in a buffer solution with the 4-10% proportion stabilised with methanol to avoid flocculation. It is flammable and explosive if put at high temperatures or in direct contact with fire.

It presents harmful effects on health, different if they concern acute or persistent situations through time. Thus, it has irritating effects on skin and mucosae (eyes, respiratory tract), but if there is a prolonged exposition it can induce chronic pathologies (dermatitis or allergic conjunctivitis, bronchial asthma). In grave circumstances it could cause collapse, pulmonary oedema an even death.

The permitted levels are of up to 0.3 ppm, since above those concentrations the workers present symptoms of ocular and nasopharyngeal irritation and thoracic oppression. The National Institute of Safety and Hygiene at Work (INSHT) declares a maximal value of VLA-EC of 0.37mg/m3 for professional short period expositions.

Even if in the past it was considered a category 2 carcinogen, the International Agency for Research on Cancer (IARC) has reclassified formaldehyde as a carcinogen substance from the group 2 to the group 1b. At the moment it is therefore included within the category 1B carcinogens with the H350 risk indicator (it causes cancer) instead of the H351 risk indicator 2 (it is suspected it can cause cancer) by the UE 605/2014 Regulation. Also, it is considered as a mutagen agent (teratogenic) of category 2 with H341 risk indicator (it is suspected it can cause genetic defects).

According to the RD 665/1997, the substitution of carcinogenic and mutagenic chemical agent like formaldehyde is the preventive measure with the greatest priority “as long as it is technically feasible”

The new regulation coming into force starting the 1st of January 2016 binds professionals, institutions and official organisms to adapt our infrastructures and work procedures to this new scenario.

There are two approaches to confront this situation:

- To adopt measures that make possible to minimise the risk of the use of formaldehyde on the daily clinical practice.
- Adapt our work procedures substituting formaldehyde by alternative fixatives safer and more ecological.

I.- MEASURES INTENDED TO MINIMISE THE FORMALDEHYDE USE RISKS

A.- STRUCTURAL MEASURES

In this section we must contemplate both the points where the samples are extracted at (biopsies, surgical pieces, foetus autopsies) as well as from the Pathological Anatomy labs themselves.

1.- Biological samples extraction points.

Here we include the endoscopy consultations (Digestive, Respiratory, ORL), Dermatology, Radiology (TAC. RMN, ECO), Urology, Primary Care and any others in which biopsies are extracted for a histological study. The operating theatres of any speciality where surgical pieces are extirpated and delivery rooms are logically also considered.

1.1.- Small size samples.

In order to safely manipulate the formaldehyde transfer to the jars with the biopsies and to avoid spillages, even on the study request sheets that go alongside the jars, it is recommended to use:

- Closed systems of formaldehyde supply.
- Preloaded packages. It is more advisable to use those which prevent the exposition of the formaldehyde during the introduction of the sample on the jar.

1.2.- Surgical pieces.

In the case of surgical pieces it is recommended to:

- To report it to the Pathological Anatomy Service while fresh, immediately after its extraction.
- To use already available commercial systems which conserve the pieces without a fixative (on a vacuum) for hours, so they can be sent afterwards to the Pathological Anatomy Service at the end of the day with the reminding extirpated pieces. Once in the Pathological Anatomy Service the fixative will be added by means of bag filling systems or else as described on the following section.

1.3.- Other situations.

If it was completely indispensable to transfer the formaldehyde from one container or dispenser to the jars where the biopsies or surgical pieces are placed (as in delivery rooms or Obstetrics operation theatres), this process must be performed in a specific area insulated for this task and it must be equipped with an air extraction system (extraction hood). It is also advisable to use the closed dispensation systems with a tap, containing the buffer formaldehyde solution ready to be used.

The jars used must be the appropriated for the transport and conservation of tissue samples and surgical pieces (there are specific commercial packages of different sizes and characteristics available), with watertight closing to prevent spills and surfaces allowing for an unequivocal labelling and identification of the samples.

It is also advisable to implant electronic request systems, since this prevents the use of paper request forms that are often impregnated with formaldehyde due to accidental spills, both at the point where the samples are extracted and at the lab itself.

2.- Sample manipulation at the Pathological Anatomy service.

2.1.- Reception and registration.

Once the jars with the samples have been received at the Pathological Anatomy service they must be examined, after checking the correct identification and filiation data on the jars and the request sheets (or on the electronic application if it was the case), you must:

2.1.1.- Clean the jars in a well ventilated place in the case of spills occurred at the Pathological Anatomy service.

2.1.2.- Fill them until the rapport of the fixative/sample is of at least 4/1 if the sample is submitted fresh as indicated in the previous sections.

2.1.3.- Take them back to the origin point to be submitted again in the right conditions if the spillage (or any other incident) took place at the extraction point.

The jar with the samples must be kept in airtight cabinets with ventilation forced to the outside or at an extraction hood with direct air extraction to the exterior until their cutting.

If the samples arrive fresh, the formaldehyde will be added after the required studies, if there was any, had been carried out (intra-operatory, sample selection for the Biobank, cytological extractions, etc.), also as indicated previously in “other situations”. If the pieces arrive in vacuum bags, available commercial systems will be preferably used for the filling of the fixative.

2.2.- Dissection and cutting

When the macroscopic study of the samples is going to be carried out, the jars must be taken out of the airtight cabinets (or extraction hoods) where they are kept since their reception. “It is recommendable that this process is carried out progressively to prevent having an excessive number of jars on the cutting room/table”. The surgical pieces must be washed under running water before being preserved to minimise the emission of formaldehyde vapours during the macroscopic study, bearing always in mind the current regulation on Production and management of bio-sanitary and cytotoxic waste. The recipients must remain closed at every moment to avoid vapour leakage.

Once measured, it is advisable that the blocks placed on the cassettes are submerged on the buffer solution in a closed recipient with a lid to prevent them from drying and emitting formaldehyde to the surroundings before being introduced in the tissue processor. The use of "continuous load " processors allows the cassettes to pass little time waiting for their inclusion in the paraffin, avoiding this inconvenience.

The cutting rooms should have on top of the specification of habitability, lighting, ventilation, air conditioning and other necessary requirements for the development of the daily healthcare activities, the following structures:

- 2.2.1.- Specific cutting tables (cabinets) with frontal opening and forced air extraction forced to the exterior, with previous filtering (with specific filters as those of aluminium oxide/potassium permanganate), that enables a flow of air from at least 0.7 m/s. These cabinets must have, on top of the preferable triple extraction (inferior, frontal and superior), a specific drain for formaldehyde (with automatic closing),formaldehyde collecting deposit with a maximum level indicator dispositive and container for solid waste impregnated with formaldehyde with an airtight seal. It should also have an electronic control vacuuming sensor alarm and a counter for the hours of use of the filters, with warning systems to carry out their own replacement.

- 2.2.2.- Airtight cabinets with air extraction towards the exterior to keep the jars once the samples have been cut and included, until they are transported to the store (if the inclusion hasn´t been wholly completed) or else they are removed for their elimination as a hazardous waste. The surgical pieces that have been washed before their dissection, after cutting them, must be conserved in their jars after being cut so they must be filled again with formaldehyde following the recommendations that have been pointed out previously, in the same way it was done with the surgical pieces submitted fresh. Once the jars have been filled it´s convenient to always close them immediately to prevent the emission of vapours.

2.3.- Autopsy room

Both if we are dealing with autopsies of adults as well as with foetal or paediatric, these are the infrastructure that the autopsy rooms must have:

- 2.3.1.- A separated space with forced ventilation systems generating negative pressure and with autopsy tables also having extraction systems. Since formaldehyde is heavier than air, it is convenient to inject air from the top of the room and place the aspiration points at its the lowest part.

- 2.3.2.- Airtight cabinets with ventilation forced to the exterior for the jars with samples and chemical products.

- 2.3.3.- A formaldehyde supply system with localised extraction as indicated in the previous sections.

- 2.3.4.- A cutting table like the ones described previously.

2.4.- Storage

Once the macroscopic studies of the biopsies, surgical pieces and autopsies have been carried out, the jars are stored correctly closed until the final studies and diagnoses are validated and the waste management procedures can take place. This storage must be made under the following conditions:

- 2.4.1.- In an specific room or space, separated from the working areas.
- 2.4.2.- Both the jars with the fixed samples, as well as the formaldehyde containers must be kept in airtight cabinets with ventilation forced to the exterior.

The store is a place to conserve the samples until they have been eliminated. For that reason, no manipulation or formaldehyde transfer must be performed at any point.

2.5.- Waste management

The management of waste connected to the formaldehyde (fixed samples, impregnated material, already used fixative, etc.) is complex and has a high economic cost. A Sanitary Waste Management Plan must be put in place in every healthcare centre according the law Ley 10/1998 de 21 de abril de residuos (Law 10/1998 of the 21st April of waste)and to the legislation of the Comunidad Autónoma (Autonomous Region) (see as an example the 204/1994 of the Comunidad de Castilla y León).

Since it is compulsory in these cases, the Plan must be known by each and every one of the members of staff of the centre and certified training activities should be carried out for the professionals involved.

The waste impregnated with formaldehyde is considered within the IV type. It must be segregated according to their type and nature (liquid, solid) in accordance to the so mention Plan at the point of origin to prevent mixing products and to allow managing them correctly.

B. –INDIVIDUALS

As a complement to the structural measures, which must always be adopted and which are at the top level of compulsion, it is necessary to use the Individual Protection Equipments (IPEs).

1.- Respiratory system

In order to protect the respiratory system it is necessary to use an auto-filtering mask. Filter plus adaptor. They are category III masks according to the EN140:2000, EN14387:2008 and EN143:2006 and they have a specific filter for organic and inorganic vapours, acid gas or vapours, ammonia and organic derivates (type ABEK).

For the exclusive use of Formaldehyde it is convenient to use type B2P3 filters.

2.- Ocular system

The protection goggles must have an integral frame with panoramic ocular, adaptable to the face, airtight against gases and vapours (and particles smaller than 5 microns) and must be able to protect again splashes. They must comply with the UNE.EN-166:200 ocular protection norm. They must have and anti-fog treatment.

3.- Hands

Nitrile, Butyl or Neoprene gloves must be used, complying with the protection against microorganisms and chemical agents norm: UNE-EN- 374-1,2,3,4; UNEEN-420:2004 and UNE-EN-455-1,2,3,4.

4.- Partial protection

If it was necessary to use other devices such as aprons or over-sleeves they must be resistant to formaldehyde and they must comply with the norm UNEEN-14605:2005+A1:2009.

After carrying out a continuous work in contact with the formaldehyde it is advisable to take a shower at the end of the journey.

SIGNS, CONTROLS AND OTHER DEVICES

In every dependence where there is formaldehyde there must be signs pointing out the hazard and the prohibition to any non authorised person access the premises. The following documentation should also be easily accessible.

- Work procedures and instructions.
- Safety data sheets of the products in use.
- Guiding lines in case of accident and or accidental spill.

Specific emergency kits must also be available and accessible for accident situations. It is important to have showers and eye washers in the case of a splash.

It is also relevant to know the formaldehyde concentration levels at point of exposition. At the moment there are commercial devices that take measurements of the level of toxic products as the formaldehyde that can be used to determine and monitor the environmental quality of the spaces with formaldehyde.

There are also devices available that filter the air of a room or store with great efficiency and electronic systems that neutralise the environmental formaldehyde with great performance (according to the specification of the manufacturing firm), and that in certain occasions can complement the use of other devices (cutting rooms, airtight cabinets at the stores, etc.).

Independently of these option it is compulsory to take measurements of the exposition of the employees during work, according the regulation RD39/1997 the assessment must be made by

a Técnico Superior PRL (Top technician inOccupational Risk Prevention) with a specialization in Industrial Hygiene. Compulsory controls of the Occupational Health of the employees exposed to the formaldehyde must be carried out. “The workers that are in contact with the formaldehyde have an additional payment for hazardous duty”.

II.- PROPOSAL FOR THE USE OF FIXATIVES SUBSTITUTIVE OF FORMALDEHYDE

In the past, different attempts have been made to find fixatives that could substitute formaldehyde without much success, which is the reason why formaldehyde is still being used as a fixative in practically all the Pathological Anatomy labs.

Nowadays there are different types of alternative fixatives whose composition is based in different types of alcoholic dilutions of Glyoxal (ethanol, 2-dione) or Propylene glycol. Due to their high content in ethanol they must be managed with caution since they are flammable solutions.

In Spain there are already some services of Pathological Anatomy that have been using these alternative fixatives for some years already and whose experience, together with the information available on the scientific bibliography, provides us with data to keep in mind when the time comes to write down these recommendations.

The information obtained in these scientific articles indicates that these fixatives have the great advantage of being less toxic products, so their manipulation and management as products is highly safe. “Their initial cost may be higher than that of the formaldehyde, but bearing in mind the global cost including the waste management, the infrastructures and the protection measures required by the use of formaldehyde, the final expense tilts the balance in favour of these formaldehyde-free fixatives”.

The macroscopic aspect of the biopsies and the surgical pieces is somewhat different regarding the fixation with formaldehyde, a fact we have to get used to while making macroscopic studies. As it happens with formaldehyde, the tissue samples shrink so the dimensions of the damages are diminished, maybe in a more evident manner with the use of these alternative fixatives and the consistency of the tissue is somewhat more elastic.

When it is necessary to conserve the surgical pieces for a long period of time it is recommendable to use liquid-tight containers with formaldehyde, sophisticated conservation techniques such as those employed with the anatomic specimens (plastination), or else to use alternative non formaldehyde based fixatives with a high ethanol content, that have demonstrated to preserve the tissues and anatomical pieces for periods higher than two years without problem.

The microscopic image of the tissue samples and the cellular blocs fixed with these products has a great quality and it doesn’t present the retraction artefacts that were observed in the past. Perhaps, the tissues show a greater eosinophil staining, a situation that is corrected

without problems adapting the staining protocols of each lab. A relevant fact that has been communicated is that with some of these fixatives (the ones which contain Glyoxal) the granulations of the eosinophil leucocytes, that are essential in some specific types of biopsies (Dermatology, Haematology), are not appreciated well.

The new processors used in combination with formaldehyde fixatives, which employ microwave technology, seem to increase even more the quality of the molecular integrity and the preservation of the tissues, thus their use seems to be recommendable.

The especial stains (PAS, Thicrhomatic, Alcian blue) and those silver based (reticuline, metanamine-silver, etc.) are correctly performed without the need to change the protocols when they are carried out in the automatic dyers or manually. The interference of these fixatives with the use of decalcification methods routinely used hasn´t been described either.

Despite the fact that these are techniques increasingly less used nowadays, it is not known if the dyes for fats (like Oil Red O or Sudan) in tissues fixed on these alternative products (but not included in paraffin) alter the results of this special dyes, although, being alcohol based fixatives these techniques could actually be specifically affected.

Regarding the results obtained with the inmunohistochemical techniques a great uncertainty has arisen. They are satisfactory in general and it is possible to superimpose them with those of the samples fixed on formaldehyde and included in paraffin, even if some labs describe that certain nuclear antigens may need an adjustment of the antigenic recuperation protocols since the inmunostaining tends to be weaker.

Nevertheless, given that there exist numerous clones and primary antibody types, different systems of antigen recuperation, of visualization methods and different inmunostaining commercial platforms, as well as fixation and paraffin inclusion protocols, these procedures must be adjusted in each case to obtain the optimal results in each lab.

The special pharmacologic-diagnostic kits with the approval of the FDA or similar organisms that limit their use to very particular situations for which they have been designed and approved (tissue fixed on formaldehyde and included in paraffin) deserve a special mention. If the specification is for tissue samples included in paraffin, there is nothing more to it than to adjust the protocols, if it was necessary. If it is specifically indicated that the they must be applied on tissues fixed on formaldehyde and included in paraffin (FFPE), on top of adjusting the protocols equally, it is necessary to establish a specific validation programme in each lab so the IVD (in vitro diagnosis) marking for clinical use doesn't get lost.

However, given the healthcare repercussion that it has, we indicate that the study of the HER-2 through inmunohistochemistry or on site non fluorescent hybridation seem to not be modified with the use of alternative fixatives, in the same way as it occurs with that of the Oestrogen and Progesterone Receptors.

The cytogenetic studies don't suffer relevant alterations. Perhaps, they must soften the enzymatic digestion conditions so the morphologic preservation of the cellular nuclei seems to

be better with the non formaldehyde based fixatives. Neither the use of techniques using histocatheter (EBER, Kappa, Lambda) presents problems depending on the communicated experience for groups of our country.

These data are coherent with the results obtained in the molecular studies published and with the ones made in our labs. The quality of the DNA is superior to that obtained from samples fixed in formaldehyde. Due to that, the quantitative PCR or of sequencing studies could present also better results, although we don´t know if any fixatives, having an alcoholic base, may contain rests which could interfere with the activity of the polymerase DNA and interfere in the results of the amplification.

The information available on the quality of the RNA is not homogenous as in the case of the DNA. The differences are identified depending on whether it comes from total RNA, messenger or ribosomal. In any case, the discordances are important since there are studies that indicate that the quality of the RNA is very high while in others, including our experience, it is not so different from that obtained with samples fixed on formaldehyde.

This is a point of great importance given that if it is confirmed that it is possible to carry out expression studies of RNA in fixed studies (on these alternative fixatives) and included in paraffin, a great scientific and healthcare leap forward would take place which would revolutionise even the concept of the current Biobanks. The bibliography describes that the RNA fragments conserved with these fixative are of a greater length.

In the same way that we have commented in connection with the pharmacological-diagnostic kits of inmunohistochemistry or of onsite hybridation, the molecular biology kits approved for the in vitro diagnosis must be validated through specific programmes.

However, in these cases where a pharmacologic-diagnosis or protein targeting detection must be carried out with kits only valid for tissue fixed on formaldehyde and included in paraffin, it is possible to perform the study on sample submitted fresh to the Pathological Anatomy lab, where the fixation process is adequately performed and monitored or else it is possible to fix them directly in formaldehyde, paying attention to the precautions exposed until a consensus based on the scientific evidences provided by the International Scientific Societies is reached.

Although the proteomics studies are not used, until the moment, in the Pathological Anatomy labs as a healthcare activity, the published studies indicate that the quality of the isolated proteins studied by means of two-dimensional gels and mass spectrometry seems to be clearly superior compared to that of samples fixed in formaldehyde.

Finally, based on the data we have at our disposition nowadays, we believe it would be advisable that the SEAP-IAP starts a methodological, rigorous and publishable work by studying the divers parameters (detection of all kind of biomarkers based on DNA, RNA, proteins, pharmacological-diagnosis with IVD marking, etc.) in a significant and representative number of samples, comparing the performance of that detection in tissues fixed with formaldehyde with that of diverse alternative fixatives.

FINAL CONSIDERATIONS

1. Formaldehyde is a toxic product considered a carcinogen (1B) and mutagen (2) that is routinely used as a fixative in the Pathological Anatomy services. With the new regulation coming into force on the 1st of January 2016, the health institutions in general, and the Pathological Anatomy services in particular, must adapt to this new situation through these two strategies: minimising the risks of the formaldehyde use though the adoption of preventive and protective, structural and individual measures, or also working with alternative fixatives and devices that facilitate the transport of the samples in the best conditions to the Pathological Anatomy lab. Evidently there may be intermediate or transitional situations.
2. We recommend the study of the biopsy circuits in each centre by a multidisciplinary group, lead by the Pathological Anatomy that involves the Management Head Office, General Services and Infrastructures Head Office, Nursery Head Office, Occupational Hazard Prevention Unit and the Waste Management and other clinical services. It is important to detect deficiencies in protection and analysing rigorously the measures applied in each centre by the Occupational Hazard Prevention Services.
3. Inmunohistochemistry, cryogenics and molecular biology trial validation studies must be carried out to ensure that the results obtained with these new fixatives are reliable, reproducible and apt for clinical diagnosis. *It is therefore recommended to progressively replace the formaldehyde fixation by alternative fixatives, maintaining the use of the formaldehyde for biopsies and surgical pieces with tumour related pathology, until new standards internationally recognised are reached and the validation of the different especial techniques, inmunohistochemical, cytogenetic and molecular becomes possible, supported by the work groups of the scientific societies, in our case the Quality Control Programme of the SEAP-IAP*.
4. It is also convenient to start replacing other toxic products other than formaldehyde, by other less harmful ones. This is the case of the Xileno that can be substituted by Isopropanolol. It is necessary to insist on the aspects related to the compulsory and certified training for the professionals who do their duties in contact with formaldehyde or on the availability of informative documentation (welcome plan); also on the prevention measures, signalling and infrastructure maintenance. It is equally important to recommend that each lab has Normalised Work Procedures and Technical Instructions for each process for its correct functioning.
5. This new scenario implies a change in the functional and structural organization of the Pathological Anatomy services that will have to adapt in a short period of time.

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