Keywords
Flexible endoscopes
Glutaraldehyde
Formaldehyde
Residues
Residual
Washer-disinfector
Toxicology
Allowable limit

A.W. van Drongelen*, A.C.P. de Bruijn, P.J.C.M. Janssen, T.J.H. Orzechowski, W.H. de Jong, R.E. Geertsma

Aldehyde residues on endoscopes – practical values and allowable limits

Summary

Between procedures, flexible endoscopes are reprocessed using disinfectants which frequently contain irritant substances like formaldehyde and glutaraldehyde. The Dutch National Institute for Public Health and the Environment (RIVM) has developed a method for the determination of formaldehyde and glutaraldehyde residues on flexible endoscopes. This method was used on 38 gastroscopes in 13 hospitals to gain an insight into the amount of aldehyde residues on endoscopes used in daily routine. Extraction of the distal end of endoscopes was performed at 40 °C in a jacketed glass tube using water as the extraction fluid. A specific reagent for aldehydes (DNPH) was added, allowing these compounds to be separated and detected using HPLC techniques. The method is sufficiently sensitive to detect and quantify residues of formaldehyde and glutaraldehyde on the distal end of flexible endoscopes. The maximum amounts of formaldehyde and glutaraldehyde found on a gastroscope during this investigation were 11.0 ± 4.4 mg and 68.0 ± 27.2 mg, respectively. There were significant differences between hospitals for both formaldehyde and glutaraldehyde. Moreover, the amounts of residual formaldehyde on endoscopes differed significantly between disinfectants. A toxicological risk assessment was carried out to calculate allowable limits for human local exposure to aldehydes in the intestines by extrapolation from animal exposure data. For endoscopes with a diameter up to 16 mm, this resulted in maximum allowable limits of 316 mg for formaldehyde and 258 mg for glutaraldehyde on the distal 35 cm. These values are considerably higher than the levels encountered in practice, indicating that the current practices for disinfection of flexible endoscopes in Dutch hospitals can be considered to pose a minimal risk for patients with regard to intestinal exposure to residues of disinfectants. However, hospitals and manufacturers of washer-disinfectors should be alert to the potential presence of excessive levels of residues.
(Hyg Med 2006; 31 [10]: 449–457)

Arjan W. van Drongelen, M. Sc.
RIVM
Centre for Biological Medicines
and Medical Technology
P.O Box 1
3720 BA Bilthoven
The Netherlands
Tel +31 30 274 2403
Fax +31 30 274 4422
E-Mail: arjan.van.drongelen@rivm.nl

Adrie C. P. de Bruijn
Paul J. C. M. Janssen, BA.Sc.
Tom J. H. Orzechowski
Wim H. de Jong, DVM, PhD
Robert E. Geertsma, M.Sc.
RIVM
Centre for Biological Medicines
and Medical Technology
P.O Box 1
3720 BA Bilthoven
The Netherlands

Introduction

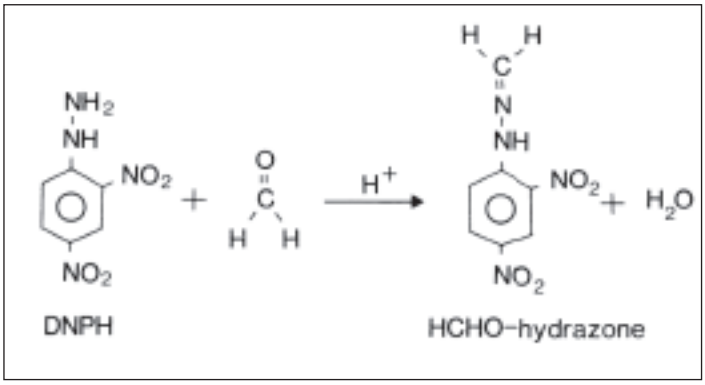
Flexible endoscopes are increasingly used in hospitals for both diagnostic and therapeutic purposes. The main areas of application are the gastrointestinal tract and the lungs. Modern endoscopes are complicated instruments containing a network of channels and valves which create a challenge for cleaning and disinfection. During use, flexible endoscopes become contaminated with pathogens. When cleaning and disinfection of these instruments between two procedures is inadequate, cross-infections can occur [1–3]. Moreover, cells and tissue can remain in the endoscope, which could cause contamination of a subsequent sample, leading to incorrect diagnosis and treatment.

Until 15 years ago, most endoscopes were cleaned and disinfected manually. Since then, equipment has been developed to perform the cleaning and disinfection of flexible endoscopes, guaranteeing a higher and reproducible level of safety. Previously, a method for the evaluation of the cleaning efficacy of washer-disinfectors for flexible endoscopes was reported [4]. Powerful chemical disinfectants are used during the reprocessing, containing chemicals that can be adsorbed onto the endoscope walls. These adsorbed chemicals can be released into a patient during subsequent use, leading to adverse effects, as is illustrated by several cases of post-endoscopic colitis [5–8].

In the period 1998/1999 the Dutch Health Care Authorities investigated the practices for cleaning and disinfection of flexible endoscopes in Dutch hospitals, revealing that the performance of the washer-disinfectors was not validated [9]. Based on this finding, combined with the reported cases of post-endoscopic colitis, the National Institute for Public Health and the Environment was asked to develop a method to determine the amount of residual disinfectant on flexible endo-

scopes. This method was tested on endoscopes in daily practice during hospital visits in order to gain insight into the practical value of the method and to determine actual residue levels on endoscopes. Furthermore, it was decided to perform a toxicological risk assessment of these levels.

Figure 2: Reaction of DNPH and formaldehyde.



Methods

Development of test method

The extraction apparatus was constructed in the shape of a cylinder in which an endoscope could be suspended to extract residues form the outside wall. The selected construction material was glass, in order to prevent adsorption of aldehydes. The internal diameter of the cylinder was 18 mm, allowing the extraction of nearly all types of flexible endoscopes. The length of the cylinder was 40 cm to facilitate easy handling, allowing 35 cm of the distal end of endoscopes to be sampled. To allow extraction at constant (elevated) temperatures, the cylinder was equipped with a jacket for temperature control by circulating water at a constant temperature (Fig. 1).

Formaldehyde and glutaraldehyde are the main ingredients of most disinfectants used in Dutch hospitals, and therefore our investigation focused on these two aldehydes. Water was used as the extraction fluid, because these aldehydes dissolve well in water. Water is also compatible with the materials of endoscopes and is present on the tissues which are investigated with flexible endoscopes. Moreover, the use of water instead of an 'exotic' extraction fluid will increase the acceptability of this method in hospitals. The extraction was performed at 40 °C, because an elevated temperature will enhance the extraction efficacy and this temperature is close to the actual temperature encountered during use of the endoscopes. A water bath was used to obtain water of constant temperature during the extraction. The extraction time was 20 minutes, which is a conservative estimate of the time needed for a medical examination using a flexible endoscope. Moreover, initial testing revealed that prolonging the exposure time had a marginal effect on the efficacy of the extraction. The volume of the extraction fluid was 50 ml, which was the approximate free volume in the extraction cylinder with an endoscope present.

Figure 1: Extraction cylinder

Both formaldehyde and glutaraldehyde are reactive polar chemicals. To facilitate analysis of these chemicals, they were converted into more stable and less polar compounds by reaction with dinitrophenylhydrazine (DNPH) (Fig. 2). Reversed phase liquid chromatography was used for separation, using a water/acetonitil gradient solution. An UV-detector was used for the detection of the derivatives. The detection limit of this method was 0.02 mg/ml for both formaldehyde and glutaraldehyde. The complete protocol for extraction and analysis can be found in a previously published report [10].

Hospital visits

In 12 hospitals, three different gastroscopes were sampled and in one hospital, two gastroscopes were sampled (38 gastroscopes in total). Only endoscopes ready for use were included in order to mimic actual practice. The extraction samples were returned for analysis the same day. The non-parametric Kruskal-Wallis-test was used to evaluate the significance of differences in test results, because this method does not assume a specific distribution of the test results. This was deemed appropriate since a specific distribution cannot be expected in our study design.

The investigation was not set up to test specific disinfectants and disinfection equipment and therefore the data are presented anonymous.

Toxicological risk assessment

The approach described in the international standard EN ISO 10993-17 was used to establish allowable limits for the aldehydes [11]. This standard describes a systematic process through which identified risks arising from toxicologically hazardous substances present in medical devices can be quantified. It also specifies a method for the determination of allowable limits for substances leachable from medical devices.

A literature search revealed that no relevant human data were available for aldehyde exposure allowing extrapolation of intestinal limit values. Therefore, literature was reviewed for studies on animal exposure to aldehydes resulting in local cytotoxicity, because this was determined to be the relevant toxicological endpoint.

Results

Hospital visits

The formaldehyde concentrations in the extraction fluid ranged from ≤ 0.02 to 0.21 mg/ml. The glutaraldehyde concentrations in the extraction fluid ranged from ≤ 0.02 to 1.36 mg/ml. The inaccuracy (95 % confidence level) for the analytical method, performed at our laboratories, was 40 % for both formaldehyde and glu-

taraldehyde. The amount of extraction fluid was 50 ml, giving maximum observed values for formaldehyde and glutaraldehyde of 10.5 ± 4.2 mg and 68.0 ± 27.2 mg, respectively. Four types of disinfectant formulations were encountered in the hospitals, coded as A–D (see Tab. I).

The differences between the hospitals for both formaldehyde and glutaraldehyde are significant ($p = 0.003$ and 0.005, respectively).

Toxicological risk assessment

For glutaraldehyde exposure, two rat studies successfully reproduced the colitis seen in patients after endoscopic examination [5,12]. However, these studies provided no adequate data for calculating allowable limits according to EN/ISO 10993-17. Therefore, a more elaborate dose-response study with intranasal application was selected as the basis for the Tolerable Contact Levels (TCLs) [13]. In this study, a volume of 0.04 ml of three different concentrations of formaldehyde and glutaraldehyde in water was instilled nasally into rats followed by histological examination of the nasal epithelium three days later. The Non-Irritating Levels in this study were 10 and 40 mmol for glutaraldehyde and formaldehyde, respectively. The Non-Irritating Level was converted into a TCL for humans by applying a modifying factor, taking into account both inter-individual variations among humans and interspecies variation (animal-to-human extrapolation). Usually for inter-and intraspecies variation a modifying factor (safety factor) of 10 is used for each variation, resulting in a total factor of 100. Variation is mainly due to differences in kinetics and metabolism of the toxic compound inducing systemic toxic effects. Such differences in kinetics and metabolism, however, are not relevant for local effects like irritation due to direct cytotoxicity. Moreover, local irritation is a toxicological endpoint known to vary to a limited degree only. For these reasons, a total modifying factor of 2 was considered to be sufficient. This yielded TCLs of 1.8 and 1.5 mg /cm2 for formaldehyde and glutaraldehyde respectively, using a nasal surface area for adult rats of 13 cm2. It was assumed that residues migrate from the surface area of the endoscope to an equal area of epithelial tissue. This is a worst-case assumption, because actual

Table I: Aldehyde residues on flexible endoscopes (each line represents a single endoscope).

Hospital	Formaldehyde (μg/ml)	Glutaraldehyde (μg/ml)
Disinfectant A		
1	0.15	0.03
	0.20	0.04
	0.07	⩽ 0.02
2	0.04	0.07
	0.11	0.32
	0.08	0.28
3	0.17	0.54
	0.16	0.86
	0.21	0.60
Disinfectant B		
4	⩽ 0.02	0.24
	0.03	0.28
	0.03	0.04
5	⩽ 0.02	0.18
	0.05	0.06
	⩽ 0.02	0.19
6	0.05	0.08
	0.05	0.03
	0.05	0.11
Disinfectant C		
7	0.08	0.08
	0.13	0.09
	0.07	0.03
8	0.05	0.03
	0.19	0.04
	0.04	⩽ 0.02
9	0.13	0.84
	⩽ 0.02	0.11
	0.06	1.36
Disinfectant D		
10	⩽ 0.02	0.15
	⩽ 0.02	0.24
11	0.03	0.98
	0.03	0.98
	⩽ 0.02	0.69
12	⩽ 0.02	0.09
	⩽ 0.02	0.15
	⩽ 0.02	0.39
13	⩽ 0.02	0.21
	⩽ 0.02	0.28
	⩽ 0.02	0.76

data on the transfer of aldehydes from the instrument's surface to the intestinal epithelium were lacking. Calculating the surface area of the distal 35 cm of a gastroscope with a diameter of 16 mm this resulted in maximum allowable limits of 316 mg for formaldehyde and 258 mg for glutaraldehyde.

Discussion

The analytical method is not suitable for the detection of small differences in concentrations, due to its relative inaccuracy of 40 %. However, for our purposes it was adequate. The extraction procedure developed was easy to perform in hospitals and the equipment was easy to handle and assemble. The test interferes minimally with the routine use of the endoscopes.

The levels found within a single hospital differed considerably, although the treatment of endoscopes within one department can be assumed to be equal for all endoscopes. Type, age and state of the endoscopes could cause such differences, as well as the time between disinfection and extraction.

Despite this intrahospital variation, significant differences could be demonstrated between hospitals for both formaldehyde and glutaraldehyde. This shows that the differences in the disinfection processes and the composition of the disinfectants will also play a role. For two disinfectants (B & D), the manufacturers stated that their products contained no formaldehyde. Disinfectant D consistently yielded values near the detection limit, whereas disinfectant B yielded higher values several times. No further actions have been undertaken to explain these results.

Using internationally accepted methods for establishing allowable limits, it was possible to establish limit values based on extrapolation of data from animal studies. The values are considerably higher than the levels extracted from the gastroscopes in the hospitals. This suggests that the use of endoscopes in Dutch hospitals should be safe with regard to aldehyde exposure of patients. Nevertheless, hospitals and manufacturers of washer-disinfectors should be aware of this potential problem, and measures should be taken to avoid the presence of excessive levels of residues. The test method presented in this article could be used to perform onsite evaluations in hospitals. Our derivation of the allowable limits for formaldehyde and glutaraldehyde on flexible endoscopes is, as far as we know, the first derivation of these limits. An important caveat to the limit values we derived is that their value is not applicable to patients with an allergy to glutaraldehyde or formaldehyde, as in such patients values which elicit allergic reactions are unknown.

Conclusions

- The combination of the extraction method and the analytical method is sufficiently sensitive to quantify the levels of formaldehyde and glutaraldehyde on flexible endoscopes.
- The extraction method interferes minimally with the treatment schedule of patients in a hospital.
- The levels of residual formaldehyde and glutaraldehyde on the distal 35 cm of endoscopes vary widely within and between hospitals.
- The allowable limits for exposure of human intestines are estimated to be 316 mg for formaldehyde and 258 mg for glutaraldehyde on the distal 35 cm of endoscopes.
- The maximum levels extracted from endoscopes in hospitals (10.5 ± 4.2 mg and 68.0 ± 27.2 mg, respectively for formaldehyde and glutaraldehyde) were considerably lower than these limit values, indicating that the current practices for disinfection of flexible endoscopes in Dutch hospitals are considered to pose a minimal risk for patients with regard to intestinal exposure to residues of disinfectants.
- Hospitals and manufacturers of washer-disinfectors should be aware of the potential presence of excessive residues. The test method presented in this article could be used to control this aspect.

Literature

1. Agerton T, Valway S, Gore B et al. Transmission of a highly drug-resistant strain (strain W1) of Mycobacterium tuberculosis. Community outbreak and nosocomial transmission via a contaminated bronchoscope. JAMA 1997; 278: 1073–1077.
2. Michele T, Cronin W, Graham N et al. Transmission of Mycobacterium tuberculosis by a fiberoptic bronchoscope. Identification by DNA fingerprinting. JAMA 1997; 278: 1093–1095.
3. Bronchoscopy related Infections and Pseudo infections New York, 1996 and 1998. MMWR Weekly 1999; 48: 557–560.
4. Orzechowski, TJH, de Bruijn, ACP, and Wassenaar, C. Validation of a cleaning test for flexible endoscopes Zentralsterilisation - Central Service 2003; 11: 165–178.
5. Durante L, Zulty J, Israel E et al. Investigation of an outbreak of bloody diarrhea: association with endoscopic cleaning solution and demonstration of lesions in an animal model. Am.J.Med. 1992; 92: 476–480.
6. Dolce P, Gourdeau M, April N, Bernard P. Outbreak of glutaraldehyde-induced proctocolitis. Am.J.Infect.Control 1995; 23: 34–39.
7. Asselah T, Touze I, Boruchowicz A, Collet R, Maunoury V, Colombel J. [Acute hemorrhagic colitis induced by glutaraldehyde after colonoscopy]. Gastroenterol.Clin.Biol. 1996; 20: 213–214.
8. Jonas G, Mahoney A, Murray J, Gertler S. Chemical colitis due to endoscope cleaning solutions: a mimic of pseudomembranous colitis. Gastroenterology 1988; 95: 1403–1408.
9. Cleaning and disinfection of scopes too flexible? (in Dutch). The Hague: Dutch Health Care Inspectorate (IGZ). 2000.
10. Van Drongelen, AW, Orzechowski, TJH, de Bruijn, ACP, Hogendoorn, EA, and Wassenaar, C. RIVM-report 605148011: Method for the determination of aldehyde residues on flexible endoscopes (in Dutch). Bilthoven: RIVM 2003.
11. EN/ISO 10993-17 Biological evaluation of medical devices; Part 17: Establishment of allowable limits for leachable substances. Brussels: European Committee for Standardization (CEN) 2003.
12. Abemayor E, Falkenstein D, Rotterdam H, Raicht R. Glutaraldehyde colitis: confirmation in a rat model. Am.J.Gastroenterol. 1990; 85: 1269.
13. St Clair MB, Gross E, Morgan KT. Pathology and cell proliferation induced by intranasal instillation of aldehydes in rat: comparison of glutaraldehyde and formaldehyde. Toxicol.Pathol. 1990; 18: 353–361.