

## Contents

<b>Editorial:</b> By any other name	1
<b>Assessing the reliability of the <i>in vitro</i> pyrogen test system based on human monocyte proinflammatory cytokine release: a comparative study</b> <i>Yukari Nakagawa and Toshimi Murai</i>	3
<b>Cleanroom-dressed operator in unidirectional airflow; a mathematical model of contamination risks</b> <i>Bengt Ljungqvist, Berit Reinmüller and Ove Söderström</i>	11
<b>Which water for pharmaceutical use?</b> <i>Maria Paola Santoro and Claudio Maini</i>	15
<b>Sterile products manufacture – sense and non-sense</b> <i>John Sharp</i>	21
<b>Book Reviews</b>	27
<b>Index to Volume 7</b>	30
<b>Dates for your diary</b>	33

Instructions for authors in this issue

## Contents and Abstracts

### **Assessing the reliability of the *in vitro* pyrogen test system based on human monocyte proinflammatory cytokine release: a comparative study**

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In order to assess the reliability of an *in vitro* pyrogen test system based on proinflammatory cytokine release from human monocytic cells, MM6-CA8, their cytokine-producing responses to various pyrogens were compared with those of cultured human whole blood cells as well as with the pyrogenic responses by the conventional rabbit pyrogen test. MM6-CA8 cells, newly selected by subcloning of a human monocytic cell line, Mono-Mac-6 (MM6),

responded to various pyrogens including endotoxin, peptidoglycan (PG), *Staphylococcus aureus* Cowan I (SAC), and polyribonucleic acid (poly I:C) with a high sensitivity, and produced proinflammatory cytokines such as interleukin (IL) -1, IL-6, and tumour necrosis factor- $\alpha$ . Among these cytokines, IL-6 was produced most sensitively in response to traces of the pyrogens and detected in the largest quantity in the culture medium. The pyrogens were ranked as follows with respect to their cytokine inducibility: endotoxin > PG > poly I:C > SAC in the MM6-CA8 and the human whole blood culture system which is an ex vivo culture test system reproducing pyrogen-induced cytokine production in the human body. In addition, this rank almost agreed with the rank of their pyrogenicity as assessed by the rabbit pyrogen test. These results suggest that the in vitro responsiveness of MM6-CA8 cells to various pyrogens is highly relevant for human pyrogenic reactions, and therefore the in vitro test system is useful and reliable for detecting the presence of materials that are pyrogenic to humans.

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## **Cleanroom-dressed operator in unidirectional airflow; a mathematical model of contamination risks**

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In pharmaceutical manufacturing, people often work in vertical unidirectional air flow. A mathematical model is presented, describing some factors concerning the risks of airborne contamination when a cleanroom-dressed operator is standing in vertical unidirectional flow. The results indicate that aseptic work in a region below the operator's knee always constitutes a risk situation and should be avoided.

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## **Which water for pharmaceutical use?**

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This article explains the most important changes that have occurred recently in water used for pharmaceutical purposes. Monographs on water in the European and the United States Pharmacopoeias are compared, and the new European Pharmacopoeia monograph on "Highly Purified Water" is discussed. The main production methods and new analytical tests adopted by the European Pharmacopoeia for water quality control are also briefly described by highlighting differences with the United States Pharmacopoeia. Finally, the new European Agency for Evaluation of Medicinal Products approved guidelines concerning quality of water for pharmaceutical use are discussed.

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## **Opinion Paper**

### **Sterile products manufacture – sense and non-sense**

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This paper examines a number of statements that have been made about sterility and sterilisation with the object of determining which are rational and tenable. It concludes with a re-assertion of basic principles.

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## Contents

<b>Editorial: What else can I do to show compliance?</b>	35
<b>The ISO contamination control standards – a tool for implementing regulatory requirements</b> <i>Hans H Schicht</i>	37
<b>The use of risk assessment in the pharmaceutical industry – the application of FMEA to a sterility testing isolator: a case study</b> <i>Tim Sandle</i>	43
<b>Transmissible degenerative encephalopathies: problems associated with the sterilisation of medical devices contaminated with the causal agents</b> <i>David M Taylor</i>	51
<b>OPINION PAPER</b> <b>Organism challenge levels at the final filter</b> <i>Maik W Jornitz and Theodore H Meltzer</i>	55
<b>Dates for your diary</b>	59
Correction to Vol 8 No 1 2003 – paper by B Ljungqvist, B Reinmüller and O Söderström	49

Instructions for authors in this issue

## Contents and Abstracts

### **The ISO contamination control standards – a tool for implementing regulatory requirements**

Hans H Schicht  
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The elaboration of international standards for cleanroom technology is a joint effort of ISO, the International Standards Organization and CEN, the Committee for European Normalisation. A brief review of the objectives and guidance principles for the work of ISO/TC 209, the Technical Committee

responsible for the development of these standards, is followed by an assessment of their present status of development and their impact on technical practice. Attention is also focused on the interface with the regulatory requirements for pharmaceutical cleanrooms. With the exception of air cleanliness classification, where harmonisation between the ISO and GMP determinations is still unsatisfactory, the ISO standards serve as a most potent tool for converting the GMP philosophies into technical practice and for establishing performance and quality criteria that are harmonised on a worldwide scale.

Paper based upon a presentation given at the European Compliance Academy / Concept Heidelberg education course FDA and European GMP compliance for cleanrooms, Copenhagen, 16/18 December 2002.

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## **The use of risk assessment in the pharmaceutical industry – the application of FMEA to a sterility testing isolator: a case study**

Tim Sandle

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The use of risk assessments is a growing trend within the pharmaceutical industry. One such analytical method for performing risk assessments is FMEA (Failure Mode and Effects Analysis). This article uses a FMEA approach to examine a sterility testing isolator by studying it for severity of risk, occurrence of risk and method of detection of the risk, thereby showing the usefulness of FMEA for this type of study and for wider application to other areas of testing and manufacturing.

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## **Transmissible degenerative encephalopathies: problems associated with the sterilisation of medical devices contaminated with the causal agents**

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The ill-defined agents that cause transmissible degenerative encephalopathies (TDEs) like Creutzfeldt-Jakob disease (CJD) are difficult to inactivate; as a result, they have been transmitted accidentally. With the recent emergence of variant CJD (vCJD), and its clear association with bovine spongiform encephalopathy, there has been concern regarding its potential transmission through surgical and other medical procedures. This is based upon the increasing knowledge that, compared with sporadic CJD, which mainly involves infection of neural tissues, the new variant form also appears consistently to infect lymphoreticular tissues such as the spleen and lymph nodes. Current thinking on the inactivation of TDE agents is discussed.

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### **Opinion paper**

#### **Organism challenge levels at the final filter**

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Aseptic processes of the biopharmaceutical industry widely use membrane filters of a specific pore size to sterilise the fluid drug product. Sterilising grade filters, commonly 0.2µm rated, are able to retain a minimum of 10<sup>7</sup> cfu/cm<sup>2</sup> *Brevundimonas diminuta* and are qualified to do so. In view of the ability of sterilising grade filters to retain such challenge levels, the maximum allowable pre-filtration bioburden level, 10 cfu/100ml, described in the CPMP Notes for Guidance (486/95) issued in April 1996, does not seem to be plausible. This paper describes the various guidelines and their scientific sense.

In aseptischen Prozessen in der Biopharmazeutischen Industrie werden hauptsächlich Membranfilter mit spezifischen Porengrößen zur

Sterilisierung flüssiger Produkte eingesetzt. 0.2µm Sterilfilter werden durch Bakterienbeaufschlagungstest qualifiziert. Die Filter müssen in der Lage sein 10<sup>7</sup> cfu/cm<sup>2</sup> *Brevundimonas diminuta* zurückzuhalten. In Anbetracht dessen, ist der in der CPMP Notes for Guidance (486/95), April 1996, beschriebene maximale erlaubte Bioburden nicht plausibel. Dieser Artikel beschreibt unterschiedliche Guidelines und deren Sinn.

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## Contents

<b>Editorial:</b> Summertime – and the living is easy?	63
<b>Measurement of air-borne pyrogens by the <i>in vitro</i> pyrogen test (IPT) based on human whole blood cytokine response</b> <i>I Kindinger, S Fennrich, B Zucker, G Linsel, S von Aulock and T Hartung</i>	65
<b>Keep your powder dry – establishing Dry Powder Inhaler (DPI) performance</b> <i>S Creeke, A Gill, P Seeney and R Smith</i>	71
<b>People as a contamination source: cleanroom clothing systems after 1, 25 and 50 washing/sterilizing cycles</b> <i>B Ljungqvist and B Reinmüller</i>	75
<b>Establishing a system-risk analysis for the production of parenterals</b> <i>H Justicia-Palomares</i>	81
<b>Letter to the editor</b>	85
<b>Dates for your diary</b>	87

Instructions for authors in Vol 8 No 2 (loose insert)

## Contents and Abstracts

### **Measurement of air-borne pyrogens by the *in vitro* pyrogen test (IPT) based on human whole blood cytokine response**

Ilona Kindinger\*, Stefan Fennrich\*, Bert Zucker†, Gunter Linsel†, Sonja von Aulock\* and Thomas Hartung\*  
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†Free University of Berlin, Germany

The impact of environmental micro-organisms as well as their fragments and components, especially endotoxins, on human health is increasingly

recognised. Different syndromes have been described in connection with inhaled air-borne microbiological contamination, e.g. sick building syndrome, humidifier lung, organic dust toxic syndrome (ODTS) and "Monday illness". Air-conditioning systems intensify this problem, as does the collection of biological waste in households, which represents a substantial source of air-borne pollution.

In 1995 we described a new method for the detection of pyrogenic contamination<sup>1,2</sup>. This sensitive test (in vitro pyrogen test: IPT) uses the natural reaction of the immune system to detect a broad spectrum of pyrogens in human blood. Safety tests in injectable drugs represent the main application and the test has been successfully validated for inclusion into the European Pharmacopoeia.

Here, the test was adapted to the detection of environmental air-borne pyrogens. Air was drawn through a filter which was then incubated directly with diluted human whole blood. The release of the inflammatory cytokine, interleukin-1 $\beta$  (IL-1 $\beta$ ), was measured by ELISA. In animal stables, up to 3 x 10<sup>6</sup> endotoxin equivalent units (EEU) were found per cubic meter of air.

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## **Keep your powder dry – establishing Dry Powder Inhaler (DPI) performance**

Sarah Creeke, Andrew Gill, Phil Seeney and Rod Smith  
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In the days of muzzle loaders the most important thing was to keep your gunpowder dry. In 1649 Oliver Cromwell wanted his troops to stay prepared for action and is often quoted as saying "Put your trust in God; but be sure to keep your powder dry." Over 350 years later with dry powder inhalers the sentiment is the same. It may be a great active device, but only if you keep your powder dry.

This article discusses the importance of moisture in determining the performance of a dry powder inhaler (DPI) system, the concept of a moisture budget for a DPI, the sources of moisture, how these can be understood and protection of a DPI from the harmful effects of moisture. It outlines an approach to investigation of product and packaging integrity and how assessment of container integrity could be used to quickly determine

potential product stability. This would conventionally take many months in an environmental chamber.

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## **People as a contamination source: cleanroom clothing systems after 1, 25 and 50 washing/sterilizing cycles**

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Tests and comparative studies have been performed on selected clothing systems in a dispersal chamber installed at KTH. Results are reported from tests carried out with clothing systems which have passed through 1, 25 and 50 washing/sterilization cycles, respectively. Results from a comparison of two types of cleanroom underwear used in combination with the cleanroom coverall show lower levels of released airborne contaminants when long-sleeved cleanroom undershirts were used with long-legged cleanroom underpants.

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## **Establishing a system-risk analysis for the production of parenterals**

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System-risk analysis (SRA) is a potent tool included in the Total Quality Management System (TQMS) that evaluates the exposure of parenteral drug manufacturing to potential risks. A complete SRA increases our knowledge about our facilities and the influence of different factors on the quality, efficacy and security of parenteral products.

SRA allows us to describe, enumerate and evaluate the risks and their effect on the manufacturing process. It identifies the possible causes and, as a

consequence, enables us to implement corrective and preventive actions that can be included in routine procedures and operations.

El Sistema de Análisis de Riesgos (SRA) es una potente herramienta de trabajo dentro del Sistema de Gestión de Calidad Total (TQMS) que nos permite evaluar los riesgos a que esta expuesta la fabricación de medicamentos parenterales. Un SRA completo nos permite conocer nuestras instalaciones y los factores que pueden influir en la calidad, seguridad y eficacia de los medicamentos parenterales. Nos permite enumerar, describir y evaluar los riesgos y su influencia en el proceso de fabricación. Identifica las posibles causas de los riesgos permitiendo implementar acciones correctoras y preventivas, y la inclusión de las mismas en los procedimientos y operaciones de rutina.

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## **Continuous microbiological monitoring in the pharmaceutical industry – an option or an impossible task?**

Peter Koger

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In Europe there seems to be a tendency towards continuous microbiological monitoring, ie. air sampling for the presence of viable microorganisms throughout the production cycle, even when no firm requirements in this direction yet exist. However when investigating the practicalities of such an approach, staff quickly find out they are facing some distinct problems. Many instruments on the market are not equipped or able to sample for more than the minimum required 1m<sup>3</sup> (1000 litres of air – in active microbiological sampling) or for a longer time period. In addition, problems exist regarding the viability of the microorganisms captured, due to dehydration and impaction speed, etc. This article discusses some of the issues involved.

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## **Think global ... but act local!**

Jordi Botet and Jordi Fàbrega

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“Think global ... but act local” is a well established marketing principle meaning that even if you have a clear idea of where you want to go you should not forget where you are and also that to resolve a big problem you have to solve many smaller ones.

Surprisingly enough, this principle often applies perfectly to our pharmaceutical world, but for opposite reasons: the fight for everyday “local” problems doesn’t allow for “global” solutions.

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## **Preservation and preservative efficacy testing: European perspectives**

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This paper considers the use of antimicrobial preservative systems in pharmaceutical products in Europe. It concentrates mainly on pharmaceutical products containing chemical active ingredients that are intended for human use. In addition, it is limited to consideration of liquid and semi-liquid preparations (and does not consider the preservation requirements of solid dosage forms such as tablets and capsules).

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## **Regulatory issues regarding container-closure systems with respect to leachables and extractables**

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The United States Food and Drug Administration (FDA) has issued guidances that address packaging requirements for drug products. The

FDA's May 1999 Container Closure Guidance has stimulated the requirements for extractable and leachable testing of container/closure packaging components. In addition, industry-based working groups have been established to assess extractable concerns and other scientific issues.

Primary container/closure systems, as well as other packaging components, have the potential to interact with the dosage form. Extractable testing studies are recommended even if containers or closures meet compendial suitability tests. Extensive testing for extractables should be performed as part of the qualification of the container/closure components.

Inhalation and injection drug products have the highest requirements. The identity and concentration of leachables in inhalation and nasal drug products must be monitored throughout the dosage form's shelf life since the product consists of the dosage form and container closure system.

Container/closure prescreening assures suitability for use with the dosage form and establishes appropriate methodology to test leachables using validated methods.

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## **Short Communication**

### **Prospective follow-up of sevelamer's efficacy and tolerance in haemodialysis patients**

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The aim of this prospective survey was to study the effects of the phosphate binder, sevelamer (Renagel®) on serum phosphate, calcium, parathyroid hormone and LDL cholesterol in stable haemodialysis elderly patients. Tolerance and compliance after one year of treatment were evaluated. Sevelamer was found to reduce serum phosphorus from  $2.2 \pm 0.4$  to  $1.7 \pm 0.4$  mmol/l, and LDL cholesterol from  $2.84 \pm 1.03$  to  $2.16 \pm 1.03$  mmol but did not increase serum calcium. Parathyroid hormone values remained unchanged. The dose of sevelamer was four to six capsules of 403 mg sevelamer. Higher doses than six capsules per day of sevelamer were reached with difficulty even with high serum phosphorus values because of tolerance and compliance problems in such elderly patients taking many

other drugs. Fifty percent of our patients were not compliant with only half of the recommended dose.

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## Contents

<b>Editorial:</b>	89
<b>Continuous microbiological monitoring in the pharmaceutical industry – an option or an impossible task?</b> <i>Peter Koger</i>	91
<b>Think global ... but act local!</b> <i>Jordi Botet and Jordi Fàbrega</i>	95
<b>Preservation and preservative efficacy testing: European perspectives</b> <i>Brian R Matthews</i>	99
<b>Regulatory issues regarding container-closure systems with respect to leachables and extractables</b> <i>FL DeGrazio</i>	109
<b>SHORT COMMUNICATION</b>	
<b>Prospective follow-up of sevelamer's efficacy and tolerance in haemodialysis patients</b> <i>C Heiz-Valle, T Cao Huu, P Monfort, A Perrin, MA Hoffman and M Kessler</i>	113
<b>Dates for your diary</b>	117

Instructions for authors in Vol 8 No 2

## Contents and Abstracts

