Table 1 Effect of *A. longifolia* extract (Ethanolic) in Cyclophosphamide-treated albino rats (after 7 days)

Group	I	II	III	IV	V
Mean RBCs ($10^6/\mu L$)	7.58 ± 0.75	3.95 ± 0.95	7.65 ± 0.21^{a}	6.20 ± 0.26^{a}	6.40 ± 0.34^{a}
Mean Hb (g/dL)	14.60 ± 0.90	8.50 ± 0.52	14.90 ± 0.20^{a1}	9.20 ± 1.36^{a1}	11.4 ± 1.85^{a1}
Mean WBCs ($10^3/\mu L$)	8.10 ± 0.25	3.80 ± 0.35	7.90 ± 0.66^{a2}	5.50 ± 1.24^{a2}	6.90 ± 0.93^{a2}
Mean HCT (%)	48.50 ± 0.86	34.28 ± 1.62	47.12 ± 2.53^{a3}	38.60 ± 2.63^{a3}	41.20 ± 3.76^{a3}

$n = 6$ albino rats per group, tabular value represents mean ± s.e.m.
$P > 0.1$ (comparison of all Groups of I with each of II).
$^{a}P < 0.005$; $^{a1,a2}P < 0.01$; $^{a3}P < 0.05$ (comparison of II with III, IV and V)

Table 2 Recovery period observations after withdrawal of cyclophosphamide (on 22nd day)

Group	I	II	III	IV	V
Mean RBCs ($10^6/\mu L$)	7.58 ± 0.75	5.85 ± 0.45	8.00 ± 0.13^{a}	6.42 ± 0.10^{a}	7.78 ± 0.12^{a}
Mean Hb (gm/dL)	14.60 ± 0.90	9.00 ± 0.53	15.10 ± 0.39^{a1}	12.90 ± 0.23^{a1}	13.40 ± 0.47^{a1}
Mean WBCs ($10^3/\mu L$)	8.10 ± 0.25	6.20 ± 0.33	8.00 ± 0.42^{2}	6.90 ± 0.53^{a2}	7.5 ± 0.33^{a2}
Mean HCT (%)	48.50 ± 0.86	38.90 ± 1.45	48.60 ± 1.10^{a3}	44.50 ± 1.22^{a3}	47.80 ± 1.14^{a3}

$n = 6$ albino rats per group, tabular value represents mean ± s.e.m.
$P > 0.1$ (comparison of all Groups of I with each of II).
$^{a,a1,a3}P < 0.0001$, $^{a2}P < 0.05$ (comparison of II with III, IV and V)

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Pulmonary delivery of spray-dried and crystallised lysozyme

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Maintaining protein stability during formulation is critical. Consequently, the formulation and delivery of proteins present challenges due to their inherent instability. Innovation in biotechnology has resulted in the development of proteins as drugs and several macromolecules are in development for inhalation delivery. Our aims were to assess the effects of spray drying and crystallisation of lysozyme, a model protein, on inhalation performance of the protein in a dry powder form and to investigate the feasibility of using Clickhalers (a dry powder inhaler) as a device for protein pulmonary delivery. Lysozyme was spray-dried and crystallised using different methods to control the protein crystal size. The particle morphology and size distribution of spray-dried particles and crystals were determined using scanning electron microscopy (SEM) and Zetasizer, respectively. The fine particle deposition of lysozyme, from the spray-dried and crystallised forms, was investigated using in vitro lung (Andersen Cascade Impactor) at a flow rate of $57.3\,L\,min^{-1}$. The method of Bradford (1976) was exploited to assay the amount of lysozyme deposited on each stage of the cascade. The particle morphology of dried powder varied from that of crystals. For crystals, SEM revealed tetragonal crystal shape without aggregates. For spray-dried protein, the particles were of a spherical shape with a smooth surface texture as indicated by SEM. The average particle size distributions were 3.35 and 1.36 μm, as determined by Zetasizer, for crystals and spray-dried lysozyme, respectively. The impaction of protein particles on stages of Andersen Cascade Impactor demonstrated that spray-dried lysozyme powder was retained on the mouth piece of the Clickhaler in the concentration of 19.9%, as determined by Bradford method, with a fine particle depositions of about 37.9% (%wt. of particles $< 5\,\mu$m in the aerosol cloud) and 20.5% (%wt of particles $< 3\,\mu$m). For lysozyme crystals, there was no retention of crystals on the mouthpiece of the inhaler. The fine particle depositions were 18% for the protein crystals $< 5\,\mu$m and 12.6% for the protein particles $< 3\,\mu$m in the aerosol cloud. Although the fine particle deposition for crystals was lower than that for spray-dried protein, the spray-dried protein tended to accumulate on the mouthpiece of the dry powder inhaler and this may lead to blockage of the inhaler and accordingly affect the subsequent dosage. Moreover, absorbing moisture by the spray-dried protein can result in microbial growth. For crystals, there was no device retention. Also, the amount of protein crystals recovered, using the Andersen Cascade Impactor, was higher compared to the spray-dried form (83.5% vs 57.4%). This low mass balance may be explained on the basis that some lysozyme denatured on recovery from the impactor, particularly since the protein was used without any additives. Loss was higher in spray-dried protein than in crystals. Our earlier findings (Elkordy et al 2002, 2004) demonstrated that protein crystals better maintained stability, conformational integrity and biological activity. In conclusion, crystallisation shows promise for pulmonary delivery of proteins. It is feasible to use Clickhalers as a pulmonary device for protein inhalation delivery.

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Poster Session 3 – Biopharmaceutics

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The importance of bio-relevant media in the classifying drugs according to the Biopharmaceutical Classification Scheme (BCS)

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To accurately predict the in vivo performance of a drug from in vitro results, it is critical that the in vivo environment encountered by an orally administrated drug is mirrored in vitro. The Biopharmaceutical Classification Scheme (BCS) classifies drugs according to their in vitro solubility and permeability. Four classes exist, of which Class II (low solubility, high permeability) and Class IV drugs (low solubility, low permeability) are dissolution rate limited. However, the solubility parameter of the BCS has been constructed using standard dissolution compendial media, which do not adequately simulate the in vivo condition. Simulated intestinal fluids (SIFs) have thus been devised (Dressman et al 1998), which hopefully bridges some of the gap in achieving in vitro/in vivo correlation. The composition of these media is largely that of bile salt, lecithin and pH, mimicking both the fasted and fed states of digestion. The aim of this study was to examine if the use of SIFs altered the classification of, in the first instance, Class II drugs (ibuprofen), and then was further extended to other BCS class drugs. Phosphate buffer (pH 7.2), fasted state simulated intestinal fluids (FaSSIFs; pH 6.5) and fed state simulated intestinal fluids (FeSSIFs; pH 5.0) were used as dissolution media. Dissolution testing using the paddle method (BP dissolution apparatus II, stirrer speed $50\,rev\,min^{-1}$ and 25°C temperature) was carried out. Paracetamol (Class I) and allopurinol (Class IV) were also investigated. In brief, a drug tablet was added to a dissolution vessel ($n = 3$) and 5-mL samples of media removed, with replacement of 5 mL fresh media, at specific time points over a period of 2 h. A dissolution profile was constructed with percent drug dissolved versus time (min). It was found that the dissolution profiles of the drugs tested varied in the different dissolution media. In general, drug solubility was enhanced upon changing from phosphate buffer to the SIFs, the result most likely being due to the effects of solubilisation and wetting. Examination of ibuprofen solubility showed a pH dependency, which will impact on its dissolution behaviour in the various media tested. A comparison between tablet coating of ibuprofen (film coated and sugar coated) revealed a faster dissolution for film coated compared with sugar coated tablets. For the other drugs, no pH dependency on drug dissolution was observed. Only ibuprofen remained within its proposed BCS class when SIFs were used (based on dissolution and solubility results).

Paracetamol and allopurinol were re-classified to Class IV and III (high solubility, low permeability) drugs, respectively. The presence of bile salt/lecithin mixed micelles in the SIFs probably account for this result. This study thus emphasises a need for physiologically relevant media to predict the in vivo performance of orally administered drugs, and also emphasises that the BCS classification of drugs may change in SIFs.

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Enhanced selectivity of protoporphyrin IX accumulation in tumours following topical bioadhesive patch application

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Aminolevulinic acid (ALA) is used in photodynamic therapy (PDT) of neoplastic skin lesions (Donnelly et al 2005). Administration of excess exogenous ALA induces high concentrations of the potent photosensitiser protoporphyrin IX (PpIX) in neoplastic cells. Illumination with light of appropriate wavelength induces PpIX excitation and singlet oxygen production. The singlet oxygen, which is highly reactive and hence travels only short distances in tissue, destroys the target cells selectively. However, it has been shown that topical administration allows ALA to enter the systemic circulation (Moan et al 2003). This may lead to PpIX accumulation at distant sites, inducing skin photosensitivity and other side effects. Due to the rapidly expanding nature of the field, little in the way of sophisticated drug delivery research has been done with ALA. The drug is typically delivered to neoplastic lesions using a topically applied cream (Porphin: Crawford Pharmaceuticals, Milton Keynes, UK). Cream-based delivery is associated with a multitude of problems from both formulation and clinical viewpoints (Donnelly et al 2005). We have previously described a bioadhesive patch containing a defined dose of ALA (McCarron et al 2005). In this study, we compared the release of ALA from this patch (38 mg cm^{-2} ALA) with that from the proprietary cream (20% w/w ALA) across excised normal porcine stratum corneum (stillborn piglets from a local abattoir) in vitro. Having obtained local ethical committee approval, and following clearly documented protocols, we also applied 1 cm^2 of patch to the flanks of Balb/c nude mice at the site of subcutaneously-implanted WiDr tumours. An amount of cream (190 mg) containing an equivalent amount of ALA was applied to another group of tumour-bearing mice. Mice not bearing tumours also had patches or creams applied to 1 cm^2 areas on their flanks. PpIX fluorescence at the application sites was monitored up to 24 h after a 4-h application of creams and patches. The amount of ALA released across normal stratum corneum from the patch was approximately 10 times smaller than that from the cream. The cream induced greater PpIX fluorescence than the patch at the site of application in normal mice. However, the cream also induced high PpIX levels at the opposite flanks of the mice. This latter effect was particularly pronounced in the tumour bearing mice, such that the entire mouse fluoresced under UV light. The maximum PpIX fluorescence at the tumour site was approximately equal for both cream and patch, but the patch did not induce high PpIX levels on the opposite flank of the mouse and the area of fluorescence was well localised to the site of patch application in contrast to the cream, which induced a diffuse pattern of fluorescence. Consequently, the patch was shown to enhance the selectivity of PpIX accumulation in tumours, as ALA released from the patch did not appreciably cross normal stratum corneum or enter the circulation of mice to induce PpIX at distant sites. The patch may thus be considered to be a more efficient delivery system for ALA than the proprietary cream.

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Determination of the intrinsic dissolution rate of a poorly soluble drug using fluorescence spectroscopy

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The intrinsic dissolution rate (IDR) of a pure drug substance can be defined as the rate at which it dissolves from a constant surface area while the temperature, agitation, pH and ionic strength of the dissolution medium are kept constant. It is a useful parameter to determine during the drug development process, as it is an early indicator of bioavailability and formulation limitations. The USP 28 (USP Convention Inc 2005) prescribes IDR calculations based on data up to 10% dissolution. However, for poorly soluble drugs, dissolution may not reach 10% in certain biorelevant media. Furthermore, prolonged run times spanning 72 h may be required to achieve drug concentrations quantifiable by UV spectroscopy. The purpose of this work was to investigate the use of a more sensitive analytical method to quantify very low levels of dissolved drug in vitro in the dissolution media at physiologically relevant dissolution run times. IDR tests were performed on ibuprofen, considered a Class II drug (poorly soluble; highly permeable) according to the Biopharmaceutics Classification System (Lindenberg et al 2004). The tests were carried using the USP28 Rotating Disk Method fitted with 10 mm dies. The simple dissolution media used were 0.1 N hydrochloric acid (HCl) pH 1.2 and 0.003 N HCl containing 0.17% w/v of the surfactant sodium dodecyl sulphate (SDS), pH 2.7. The alternative media comprised an equal-parts mixtures of 0.1 N HCl with whole, semi-skimmed or skimmed milk (pH 2.7). A further medium was prepared comprising of the filtrate from a 1.475% w/v dispersion of casein in 0.01 N HCl, pH 2.7. The HCl-SDS pH 2.7 medium was used to account for the increase in pH and decrease in surface tension seen upon addition of milk to HCl pH 1.2. Samples were withdrawn from the dissolution medium at specified intervals up to 2 h, then analysed by HPLC using fluorescence detection. The ibuprofen release was expressed as the amount of ibuprofen dissolved per unit area plotted versus time. The IDR in the various media were calculated by linear regression of the slopes of dissolution profiles thus generated. The results (Table 1) show that dissolved ibuprofen could be detected and quantified at very low concentrations by using fluorescence spectroscopy in these media, which have pH values well below the pKa of the drug (4.4). Previous attempts (Shah et al 2004) to determine the IDR of ibuprofen using UV spectroscopy failed to detect the drug after 2 h testing in these same media. The ability to determine the IDR of poorly soluble drugs in complex, biorelevant media is of particular value when attempting to predict in vivo performance. This work has demonstrated the applicability of fluorescence spectroscopy in investigating the IDR of a poorly soluble drug over a biorelevant run time.

Table 1 Amount of ibuprofen dissolved from 10 mm dies after 60 minutes (n = 6)

Medium	Amount dissolved (mg cm^{-2})	
	Mean	s.d.
HCl pH 1.2	0.42	0.08
HCl-SDS pH 2.7	0.41	0.06
HCl-whole milk	2.34	0.05
HCl-semi-skimmed milk	0.32	0.06
HCl-skimmed milk	0.24	0.10
HCl-casein (filtered)*	0.34	0.04

*n = 5.

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Enterohepatic recirculation of norfloxacin – a whole body physiologically based pharmacokinetic model

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The extent of tissue penetration, distribution and the size of the therapeutic window of antibiotics are among the most important issues confronting antibiotic therapies. Modelling is an important applied tool in drug discovery and development for the prediction and interpretation of drug pharmacokinetics. Whole body physiologically based pharmacokinetic (WBPBPK) models are

increasingly used to predict pharmacokinetic behaviour of drugs, since they provide a method to model the in vivo delivery of drugs and to provide quantitative information about the physiological events involved in delivery. Norfloxacin is a fluoroquinolone antibiotic used mainly in genitourinary infections. However, it is known to be widely distributed and achieves good penetration into most tissues. Norfloxacin undergoes enterohepatic recirculation and has an active renal tubular excretion pathway. The aim of this study is to develop a WBPBPK model incorporating the process of enterohepatic recirculation to study norfloxacin kinetics in rats. Using published values of physiological parameters and tissue concentration and arterial blood data measured over time, a WBPBPK model for norfloxacin is derived, comprising of 15 compartments, including the venous and arterial compartments. Initially, it is assumed that each tissue is represented by a single, well-stirred compartment and tissue affinities (Kp) were estimated from the model using the area and open loop methods. Comparisons were made with Kp values estimated at steady state. Permeability versus perfusion rate limitation of the norfloxacin distribution process for each tissue was tested. These alternative assumptions were judged based on fits of the tissue concentration data to the WBPBPK model by using nonlinear least squares regression. Additionally, data were available following the administration of three different intravenous bolus doses (25, 50 and 100 mg kg^{-1}) to the rats, which showed that norfloxacin has dose ranging pharmacokinetics. It has been reported that norfloxacin has nonlinear pharmacokinetics, which was investigated using the developed WBPBPK model. In conclusion, the study demonstrates that the model is able to adeptly represent the physiological processes involved in norfloxacin kinetics, allowing valid deductions to be made.

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Multiple-application dosing: effect on the percutaneous absorption of aluminium from commercially available products

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Aluminium has been widely used as an antiperspirant in both cosmetic and pharmaceutical products for over fifty years. Recent interest in the topical application of antiperspirants has been provoked by a range of studies that have focused on the alleged link between aluminium absorption and Alzheimer's disease, and the perceived risk of other formulation ingredients, such as parabens (i.e. Darbre 2003). Recent studies in our laboratories suggested that penetration of aluminium appears to depend upon both the method of application to the skin and the nature of the formulation. The level of aluminium observed penetrating across the skin from these products is well below safety limits, and the level ingested via other routes (Flarend et al 2001). It is the purpose of this study to expand upon the previous research by using a multiple application protocol, which aims to replicate consumer use of such products more accurately than conventional finite or infinite dose in in vitro percutaneous absorption experiments. Several shop-bought products were examined (i.e. Driclor and Vaseline Intensive Care Roll-ons, Arrid Ultra Dry and Sure for Men Sticks). Experiments were conducted over a 24-h duration using porcine skin in Franz-type diffusion cells. Products were applied to the skin surface at the beginning of the experiment, and after 4, 8 and 12 h. Total ion analysis was by atomic absorption spectroscopy (AAS). The results of these experiments suggest that formulation type (particularly viscosity) is a significant factor in the total amount of aluminium absorbed across the skin. Further, multiple dosing results in an initial steady-state region which then flattens. This suggests donor phase depletion, despite the multiple dosing protocol employed. However, comparison with single-application experiments (Moss et al 2004) demonstrates that the rate of absorption is higher where a multiple application has been used. Any decrease observed in initial rate may be due to saturation of the skin reservoir layer, the stratum corneum. This would suggest that, despite multiple applications, this layer eventually becomes saturated and thus may alleviate concerns of multiple dosing. Such an argument does not, however, account for formulation factors and possible damage to the stratum corneum from, for example, ethanol or surfactants present in some of these products. Therefore, while the experiment is still an in vitro estimate and cannot fully replicate in-use consumer use, it does address more accurately the issue of consumer/clinically relevant experimental designs in percutaneous absorption. In this case, the protocol would indicate that multiple dosing does not substantially increase the percutaneous absorption of aluminium from topically applied products.

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Effects of pharmaceutical excipients on in vivo drug metabolism

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Many new drug entities are poorly soluble, requiring the use of co-solvents or excipients to produce intravenous formulations. Evidence in the literature demonstrates that some of these excipients have the ability to affect drug metabolism. However, in vivo studies, using excipient concentrations relevant to pre-clinical studies, are lacking, and it is often difficult to know how results from in vitro studies may be reflected in vivo. In early pharmacokinetic studies, it is desirable to avoid any alteration of drug metabolism by formulation additives, as this could mask the drug's true pharmacokinetic nature (Bittner & Mountfield 2002). This work therefore aims to investigate the effects of some commonly used pharmaceutical excipients on drug metabolism in vivo. ^{14}C breath tests were carried out in groups of rats using erythromycin, aminopyrine and NDMA as substrates. Excipients were given as either a single intraperitoneal dose 20 min before, or a single intravenous dose immediately before, administration of the breath test substrate. $^{14}CO_2$ exhalation half-life was determined from exhaled $^{14}CO_2$ and statistically compared between control and excipient treatment groups to determine any excipient effects on metabolic rates of the substrates. DMSO at high dose (4 mL kg^{-1}) prolonged the $^{14}CO_2$ exhalation half-life of all three substrates. $^{14}CO_2$ exhalation half-life was increased by 270% for aminopyrine, 255% for erythromycin and 55% for NDMA, demonstrating inhibition of metabolism by CYP3A (erythromycin), CYP2E1 (NDMA) and mixed function oxidases (aminopyrine). However, at the usual pre-clinical doses of 0.2 and 0.4 mL kg^{-1}, no effects of DMSO on metabolism were apparent. Propylene glycol, at the standard pre-clinical dose of 0.8 mL kg^{-1}, increased the $^{14}CO_2$ exhalation half-life in the NDMA breath test by 55%, but did not significantly affect the metabolic rates of aminopyrine or erythromycin. This suggests a specific effect of propylene glycol on metabolism by CYP2E1. Solutol HS15, at the commonly used dose of 100 mg kg^{-1}, did not significantly affect the $^{14}CO_2$ exhalation half-lives of aminopyrine or erythromycin, an interesting contrast to its reported ability to inhibit CYP3A in vitro (Bravo Gonzalez et al 2004). These results demonstrate that excipients can affect the pharmacokinetics of some drugs in vivo after a single acute administration of doses commonly used in pre-clinical studies. Their potential to do this should be borne in mind when selecting excipients for use in intravenous drug formulations, as inappropriate selection could lead to a false impression of drug half-life, and affect metabolism of concomitantly administered drugs. The results also demonstrate that excipients that can inhibit metabolism in vitro do not necessarily do this in vivo, at commonly used doses.

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A reproducible, robust meal for gastric emptying studies

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There is a need for a medium calorie meal that can be used in pharmacokinetic studies and shows reproducible emptying under varied physiological conditions. Nutrient liquid meals often used in enteral feeding have been used to study antacids (e.g. May et al 1984). This group showed reproducible gastric emptying of 375 mL of Tc-^{99m} labelled Clinifeed ISO in healthy subjects. This item is now discontinued but a similar item, Clinutren ISO, is available. The purpose of this study was to produce a new robust, reproducible model of gastric emptying using Clinutren ISO. Gastric emptying rates were studied in the fasted and postprandial states. The trial was conducted as single centre, randomised, two-way, crossover, within subject study in eight healthy male subjects aged 22–32 years. The study was approved by the North Glasgow Universities NHS Trust Ethics Committee and ARSAC. Clinutren ISO (400 mL; Nestle Ltd, UK) was used to simulate a homogenous meal with an energy content of 1680 kJ. This was radiolabelled by the addition of technetium-99 m diethylene-triaminapentacetic acid (^{99m}Tc-DTPA). Each subject ingested the Clinutren ISO either after an overnight fast or 3 h after a standard breakfast (scrambled egg, two rashers of bacon, 100 g hash browns, two slices of white toast and butter and 200 mL of whole milk; calorific value ~1000 kcal). Scintigraphic imaging was performed with the subject in a standing position. Anterior and posterior static acquisitions of 25 s duration were collected immediately after administration of the radio-labelled Clinutren ISO (t = 0) then every 5 min for 30 min and then every 10 min until 180 min, or 90% of the meal had left the stomach. Subjects were free to move around between images. The gastric emptying

times were extremely reproducible. There was no statistical difference between the mean gastric emptying times, measured by t_{50} (time taken for 50% of the Clinutren ISO to leave the stomach and t_{90} (time taken for 90% of the Clinutren ISO to leave the stomach) in the fed and fasted state (Table 1). A robust and reproducible model of the gastric emptying of a new nutrient liquid meal is described, which shows similar emptying kinetics in the fasted and the post-prandial conditions.

This study was completed as part of a MRC CASE studentship funded by GSK.

Table 1 Mean gastric emptying times

Fasted t_{50} (min)	Fed t_{50} (min)	Fasted t_{90} (min)	Fed t_{90} (min)
60 ± 6.6	63 ± 7.8	120.6 ± 13.2	135 ± 19.2

Data are means $\pm$ s.d, n = 8.

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Poster Session 3 – Pharmacology

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The Gram-negative bacterial flora of the cotton production environment and relevance to occupational lung disease

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To grow cotton commercially, a temperate climate is required with ample water supply. Similar conditions favour the growth of the majority of bacteria; couple this with the nutrition provided by the large quantities of plant and insect sugars present on the fibres and the ideal environment for the proliferation of microbial populations is created. Many of these microbes are Gram-negative bacteria, which release endotoxin (known as lipopolysaccharide or LPS in the purified form) from their outer membrane. Inhalation of endotoxin primarily targets alveolar macrophages causing a decrease in lung function (Rylander 1992). The contaminated cotton fibres enter the cotton mill, where associated bacterial cells and fragments thereof are released with dust created during cotton processing. Prolonged exposure to this environment can result in the textile worker's lung disease byssinosis, a chronic and irreversible condition. This study involved the quantification and identification of Gram-negative bacterial cells in numerous cotton samples from diverse countries. The aim was to investigate the diversity of cotton bacteria, as well as to identify differences in viable counts and hence assess risk to respiratory health and how this varies across the world. Ten cotton lint samples were provided by the Liverpool Cotton Research Corporation from countries within Africa, the Middle East and North and South America. Bacteria were extracted from the fibres by vigorous shaking in phosphate-buffered saline, followed by spread plating onto over-dried tryptic soy agar plates seeded with cycloheximide ($50\,\mu g\,mL^{-1}$) and vancomycin ($15\,\mu g\,mL^{-1}$) to prevent the growth of fungi and Gram-positive bacteria, respectively. Plates were incubated for 18–24 h at 37°C (±2°C) and colony-forming units then counted manually. Colonies appearing morphologically different were subcultured for a further 18–24 h. Isolates were then identified using microscopy and Analytical Profile Index (API) strips. Viable cell counts on the samples varied significantly from a high of $2.2 \pm 0.3 \times 10^5\,CFU\,g^{-1}$ on cotton from Zimbabwe, to $7.1 \pm 2.1 \times 10^2\,CFU\,g^{-1}$ on the cotton sample from Tajikistan. The mean viable count was significantly higher on cotton samples from countries within Africa compared with those within Asia ($1.4 \pm 0.3 \times 10^5\,CFU\,g^{-1}$ compared with $2.4 \pm 0.6 \times 10^3\,CFU\,g^{-1}$) ($P < 0.05$). This is of importance as African countries exhibit high levels of occupational lung disease (Abebe & Seboxa 1995) and these regions lack the means to effectively monitor and protect their workforce. Five genera of bacteria were identified on the samples: *Aeromonas, Enterobacter*, *Pantoea*, *Psuedomonas*, and *Serratia*, all common bacteria found in soil, water or on plants. The most widely distributed genus was *Enterobacter*, identified in cotton from seven countries (Table 1). This is also significant, as endotoxin from different bacterial genera varies in its ability to induce an inflammatory response upon inhalation, and that from *Enterobacter* has been shown to be one of the most potent (Helander et al 1980). Hence, a significant health risk is posed to cotton workers across the world.

We gratefully acknowledge the support of the Liverpool Cotton Research Corporation and the British Cotton Growing Association.

Table 1 Geographic distribution of cotton bacteria

Bacterial genera	Number of countries
Enterobacter	7
Pseudomonas	4
Serratia	3
Aeromonas	2
Pantoea	2

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The fungal profile of cotton fibres, associated toxins and wider implications for indoor health

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Cotton plant material is an ideal substrate for the proliferation of fungal colonies. Many of these are soil-borne saprophytes, which survive on plant debris. Therefore, these organisms are available to contaminate cotton fibres while they are exposed to field weathering before harvest. Further to this, several fungi can also develop while organic material is stored. This results in the unavoidable introduction of these organisms to the industrial environment. Many common filamentous fungi are capable of producing mycotoxins, which are secondary metabolites of low molecular weight, able to cause a number of effects including kidney failure, damage to the central nervous system and some are known carcinogens (Fischer & Dott 2003). This study involved the quantification and identification of fungal cells on numerous cotton samples from diverse countries. The aim was to enumerate the cotton fungal cells, as well as to identify the genera present, to assess potential health risks. Ten cotton lint samples were provided by the Liverpool Cotton Research Corporation from countries within Africa, the Middle East and North and South America. Fungal cells were extracted from the fibres by vigorous shaking in phosphate-buffered saline, followed by spread plating onto over-dried malt extract agar plates seeded with chloramphenicol ($30\,\mu g\,mL^{-1}$) to prevent the growth of bacteria. Plates were incubated for four days at 25°C (±2°C), then colony forming units were counted manually. Colonies appearing morphologically different were subcultured as before for 4 days. Isolates were then mounted using tape-lift procedures and stained with lactophenol blue solution; identification was carried out using macroscopic and microscopic morphology with comparison to taxonomic reference material. Viable cell counts on the samples varied significantly, from a high of $9250 \pm 820\,CFU\,g^{-1}$ on cotton from Benin to $281 \pm 29\,CFU\,g^{-1}$ on the cotton sample from Uzbekistan. The mean viable count was significantly higher on cotton samples from countries within Africa, compared with those within Asia ($4233 \pm 668\,CFU\,g^{-1}$ compared with $1033 \pm 67\,CFU\,g^{-1}$) ($P < 0.05$). Five fungal genera were identified in the samples; these were *Aspergillus, Cladosporium*, *Fusarium*, *Penicillium* and *Rhizopus*, all common soil and plant fungi. The most widely distributed genus was *Aspergillus*, identified in every cotton sample involved in the study, with *Aspergillus niger* the most common species, followed by *A. flavus*. These *Aspergillus* species are able to produce several mycotoxins, the most potent of which is aflatoxin, a carcinogen that has been shown to produce lung neoplasms upon inhalation (Bennett & Klich 2003). *Fusarium* and *Penicillium* are also potential toxin producers (Table 1). The presence of such fungi in organic material has significant implications for the health of workers in a number of occupational environments, and further research into these organisms and the toxins they produce is required.

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Table 1 Toxins produced by cotton fungi

Fungal taxa	Toxin
Aspergillus flavus	Aflatoxin B1 and B2
Aspergillus niger	Malformin C, ochratoxin A
Fusarium	Fumonisin, trichothecenes
Penicillium	Citrinin