Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
HG1

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/076

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological agents (BAs) must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTIONS OF PART B, AND ALL OF PART C. WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED THE RISK ASSESSMENT MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND EXPLICIT APPROVAL IS ALSO REQUIRED FROM THE UNIVERSITY BIOLOGICAL SAFETY OFFICER. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH, SAFETY & ENVIRONMENT UNIT FOR REVIEW VIA YOUR DEPARTMENTAL BIOLOGICAL SAFETY ADVISOR.
3. It is the responsibility of the Principal Investigator/Supervisor to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities**.

Date Submitted:	19/05/2014	Date Approved:	20/05/2014
Version Number:	1.0	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
Wolfson School of Engineering			
Title of Project			
Title of Project: **Detection of *Salmonella enterica (ATCC® 53647™)* using quartz crystal microbalance.**			
Project Reference Number:			
Person responsible for this work (*Principle Investigator)*			
Name:	Dr Sourav Ghosh	Position:	Lecturer
Department:	Mechanical Engineering	University School:	Wolfson School of Engineering
Person conducting this assessment			
Name:	Dr Igor Efimov	Position:	Research Associate
Department:	Mechanical Engineering	Date Risk Assessment Undertaken:	19/05/2014
Proposed Project Start Date:	20/05/2014	Proposed Project End Date:	31/08/2014

Review History: *required at least once a year or immediately following any significant change to the project. Significant revisions must be detailed on a revision form. The person responsible must ensure that this RA remains valid.*

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date					
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

This provides a useful background for the reviewer and reader. It need only be brief and should provide an overview of the scientific goals.

The scientific goals of the project are listed below:

- Investigate the absorption of pathogen on in-situ cured Polydimethylsiloxane (PDMS) covering the surface of quartz crystal microbalance.
- Explore feasibility of detection using Anharmonic Acoustic Detection Technique (ADT) for transduction of the surface-pathogen binding, into recordable electrical signal.
- Identify design requirements for interpretation of electrical data with desired sensitivity and specificity, for quantitative detection of surface bound pathogen.

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations. This may need cross reference to supporting documentation i.e. protocols.

- Receive samples of bacteria shipped by ATCC (via. LGC) to Goods inwards at the Wolfson School.
- Transfer directly to the Chemical Engineering department microbial laboratory in original packaging.
- Receive samples of bacteria according to the procedure documented in SOP008 "*Receipt of Hazardous Biological Material*" and deliver to the appropriate recipient or other designated personnel.
- Culture and grow the bacteria in the Chemical Engineering department microbial laboratory (performed by Chemical Engineering Laboratory Manager David Smith).
- This cultured bacterium will be first de-activated by *Ultraviolet* radiation and given to the personnel involved in this project in suspension in a 15 mL centrifuge tube.
- The student will prepare a transport kit as follows:
    - The 15 mL centrifuge tube lid will be replaced with a lid which has a hole punctured in. This will be wrapped with parafilm.
    - The 15 mL centrifuge tube will then be placed in a 50 mL centrifuge tube, sealed and placed upright in a centrifuge rack and then in a box.
    - This box will be transferred to the CBE.
- The transport box will be sprayed and wiped with ethanol in the first change of the CBE and then transported to the bench in H34.
- After visual inspection to see for any leaks, the 15 mL centrifuge tube will be placed in the pathogen detection kit.
- The bacterial suspension (using < 5 ul per assay) will be passed over the quartz crystal covered with **Polydimethylsiloxane** (PDMS) and then analysed in the biosensor platform.
- Once the test is finished, the 15 mL centrifuge tube will be replaced in the 50 mL centrifuge tube and placed in the disposal bag. The sample tube and quartz crystal will be placed in the disposal bag.
- The crystal holder will be washed with IMS and then acetone and re-used.

Note 1: This risk assessment is for the bacteria *Salmonella enterica (ATCC® 53647™).*
The microorganism will be deactivated and then suspended in liquid before being handed over to the user to bring to the CBE labs. The user or the designated personnel will not have access to the living organism. This risk assessment is only for the transport of the deactivated bacterial suspension, use for the experiment with equipment and then subsequent disposal of the bacterial suspension along with disposable parts of the equipment. The culture of the microorganism will be risk assessed elsewhere.

Note 2: Procedure for the deactivation of the bacteria.
The ***Salmonella enterica (ATCC® 53647™)*** will be deactivated using *Ultraviolet* radiation in the Chemical Engineering department microbial laboratory. This procedure has been validated - please refer to three papers attached written by (1) Kramer et al (1987), (2) Bintsis et al (2000) and (3) Chang et al (1985).

PART B: Please provide information in one or more of the following sections, as appropriate. Only sections which you complete should be submitted:

Section 1: *micro-organisms (prions, viruses, bacteria, fungi, parasites in ACDP category 2 and pathogens controlled by the Department for the Environment, Food and Rural Affairs).* ***[Work with ACDP category 3 and 4 pathogens is not currently permitted in the University.]***

Section 2: *cell cultures, tissues, blood, body fluids or excreta*

Section 3: *plants and plant material*

Section 4: *animals and animal tissues*

SECTION 1: MICRO-ORGANISMS

B1.1 HAZARD AND RISK IDENTIFICATION: NATURE OF MICRO-ORGANISMS

*This information gives an indication of the **potential** harm that the biological material may cause*

B1.1.1 List all micro-organisms to be used

Name	Strain	ADCP cat*	Source
Salmonella enterica subsp. enterica (ex Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC® 53647™)	**Chi4062**	**Biosafety level 1**	**ATCC (via UK LGC)**
Please note that the bacteria will come to the CBE deactivated using *Ultraviolet* radiation			

see The Approved List of Biological Agents – available on the Health & Safety website

B1.1.2 Has any strain been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form	

B1.2 DESCRIPTION OF RISK TO HUMANS

B1.2.1 The disease(s) caused to humans

Describe the type and severity of effects or disease(s) on human health (including colonisation, infection, allergy, toxin-mediated disease) by each of the agents or strains to be used

Indicate in the adjacent box if Not Relevant (N/R)		**N/R**
Name	Type	Severity

B1.2.2 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected

Name of agent	Risk Category	Justification for Selection
Salmonella enterica (ATCC® 53647™)	**1**	**The microorganism will be deactivated and then suspended in liquid before being handed over to the user to bring to the CBE labs. The user will not have access to the living organism. This risk assessment is only for the transport of the de-activated bacterial suspension, use with the PDMS covering the quartz crystal microbalance and then subsequent disposal of the bacterial suspension along with disposable parts of the equipment. The culture of the microorganism will be risk assessed elsewhere. Due to the deactivation, this material will be considered to be Hazard Group 1.**
		If none proceed to section B1.3

B1.2.3 Infectivity to humans
Describe ALL the route(s)of infection (relevant to the laboratory setting) and the minimum infectious dose(s) if known (e.g. percutaneous, mucocutaneous, inhalation, ingestion)

Name of agent(s)	Route(s) of infection	Minimum infectious dose
Deactivated *Salmonella enterica*	**Not hazardous**	**N/A**

B1.2.4 Drug resistance
Is there any known or suspected drug resistance amongst the strains to be used? Identify & describe.

N/A

B1.2.5 Attenuation or increased virulence
Are the strains attenuated or do they have an increased virulence in any way?

Identify and describe:

Attenuated after exposure to UV radiation.

B1.2.6 Ability to survive
In what form is the agent present e.g. spores or vegetative bacteria, and are there any issues about the agents' robustness, including any resistance to chemical disinfectants?

Identify and describe:

Vegetative bacteria. Susceptible to most common disinfectant.

B1.2.7 Most hazardous procedure?
Identify and describe the most hazardous procedure(s) to be used.

The most hazardous procedure in this risk assessment will be the transport of the deactivated bacterial suspension. This will be performed by the user ensuring that the bacterial suspension has no chance of escaping from the centrifuge tube by placing it in a larger centrifuge tube sealed with parafilm in an upright holder in a box.

B1.3 HUMANS AT INCREASED RISK OF INFECTION

B1.3.1 Are there any pre-existing medical conditions that increase the risk associated with this agents listed in section 1.1 (including immunocompromised workers, pregnant workers, breast feeding mothers, diabetic workers)?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, Occupational Health must be consulted:	

B1.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B1.4.1 Give details of the volumes and concentrations of organisms to be used

Name & Strain	Volume	Concentration
Name: Salmonella enterica subsp. enterica (ex Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC® 53647™) ***Strain: Chi4062***	500 ul	10^6 cfu/mL

B1.5 ENVIRONMENTAL CONSIDERATIONS:

B1.5.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, describe briefly here (A separate risk assessment may be required if the agent to be used poses a significant risk to the environment):	

B1.5.2 Will there be any other environmental risks?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, describe briefly here (NOTE: A separate risk assessment may be required if the agent to be used poses a significant risk to the environment):	

B1.6 OTHER HAZARDS

B1.6.1 Are there any other hazards associated with this work? For example, hazardous chemicals, cryogenic gases ionising radiation.

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, identify these:	
If yes, have these been risk assessed and any necessary approval obtained?	

B1.6.2 Are there any conditions associated with the hazards described in B1.6.1 that require special attention in Section C of this risk assessment? For example, material incompatibilities with disinfectants such as Virkon or hazardous product decomposition associated with high temperatures (ie autoclaving).

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	**N/R**
If yes, provide details and ensure that appropriate control measures are addressed in Section C:	

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (http://www.hse.gov.uk/pubns/misc208.pdf)
The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:

The biological hazard is nil in the CBE as the bacteria has been neutralised by a validated method. In the CBE laboratory the sample will be in a sealed container with minimal risk of aerosol formation.

C1.1.2 Isolation/Segregation

(i) Is/Are the laboratory(s) to be used for this work to be shared with other workers not directly involved in this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**Yes**

If yes, provide details:

Work will be conducted in the CBE laboratories, which is a multiuser facility, with shared equipment. After experiment equipment will be cleaned and decontaminated according to procedures detailed in CBE equipment SOPs. Risk of cross contamination is minimal.

(ii) Is access to the laboratory(s) to be used for this work restricted?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**Yes**

If yes, provide details:

Access to CBE laboratories is restricted to authorised users only. All authorised users have been trained in working in a CL2 laboratory; documented training files for all authorised users are available in CBE offices.

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**Yes**

If yes, list the sharps:

Needles to inject into tubing

If yes, justify there use – is there an alternative? **There is no alternative for the needles as they are needed to inject into tubing**
If yes, describe there use and disposal: **Sharps bins are available**
If yes, describe any additional precautions employed to reduce risk: **Handle with care and wear hand gloves while using the needles.**

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker i.e. do the work procedures generate aerosols or splashes that pose a risk to workers?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**Yes**
If yes, specify the type(s) and when they will be used: **All bacterial culture work will be performed in a BSC in the Chemical Engineering laboratory under their operating procedures. Preparation of the quartz crystals will be carried out in the CBE Fume Hood as per SOP026 "*Use and Maintenance of the Captair M321 Fume Cupboard*".**	
(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**No**
If yes, specify:	

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored? **Preparation of the quartz crystals and biosensor analysis will be carried out in CBE.**
How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills. **The bacteria will be transported to the CBE labs as per the following procedure:** o **The 15 mL centrifuge tube lid will be replaced with a lid which has a hole punctured in. This will be wrapped with parafilm.** o **The 15 mL centrifuge tube will then be placed in a 50 mL centrifuge tube, sealed and placed upright in a centrifuge rack and then in a box.** o **This box will be transferred to the CBE.**

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills **No transport outside the laboratory once the testing has been done at the CBE.**

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?						
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)						**No**
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):						
Description of material to be shipped (*indicate in available boxes*). Is this:						
Category A		UN2814		UN2900		*Packaging instruction 602 or 620 must be followed*
Or?						
Category B		UN3373		*Packaging instruction 650 must be followed*		
Or?						
Non-hazardous		*Should be packaged to protect sample*				

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly?

The bacteria samples will be shipped by ATCC (via LGC) to Goods inwards at the Wolfson School. They will be taken over to the Chemical Engineering laboratory in the original packaging. The procedure for the safe receipt of packages containing potentially biohazardous material and their delivery to the appropriate recipient or other designated personnel is documented in SOP008 "*Receipt of Hazardous Biological Material*". This SOP is intended to minimise the consequences that could result from failure of packaging methods and materials used to ship biohazardous materials.

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**No**
(ii) Where will these rotors/buckets be opened?	
(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge	

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages.

37 C static incubators for microbial culture.

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:
70% IMS and 1%Virkon will be used

Have these disinfectants been validated for use with the recipient biological material?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**Yes**
If yes, describe the procedure: **For hazard group 1 and 2, biological agents it is normally sufficient to rely on the manufacturer's data providing the recommended concentrations and contact times are used. Hence, 1% Virkon is used per manufacturer's instructions and according to local Code of Practice and SOP006 "*Preparation of disinfectant for use within CBE laboratories*". Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10 mins.**	

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored? **Side fastening *Howie* type lab coats will be worn at all times within the CBE facility. They are stored outside the laboratory in a dedicated change area. Guidance on the proper use of PPE will be taken from CBE SOP037 "*Use of Personal Protective Equipment*".**
(ii) What type of gloves will be worn and where will they be stored? **Autoclave gloves, stored near the autoclave will be worn at all times when operating the autoclave as directed by SOP025 "*Use and Maintenance of Systec VX-95 autoclave*".** **Cryogenic gloves, stored in the CBE autoclave room are worn at all times when using liquid nitrogen storage containers as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*".** **Disposable latex powder free gloves for general use will be worn at all times when in the CBE facility, as directed by SOP037 "*Use of Personal Protective Equipment*".**
(iii) Describe any other PPE to be used: **Laboratory safety glasses will be worn as directed by relevant SOPs when working within the CBE.** **Face shield (primarily for handling liquid nitrogen) will be worn when retrieving cell vial from storage in the CBE as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*".** **Full length aprons will be worn when retrieving cell vial from liquid nitrogen stores in the CBE facility, as directed by SOP013 "*Use and Maintenance of Liquid Nitrogen Stores*" and when operating the autoclave as directed by SOP025 "*Use and Maintenance of Systec VX-95 Autoclave CBE045*".**

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located **Designated eye wash stations and hand washing facilities are available in the change room of each laboratory; other hand basins are situated directly inside the analytical laboratory and in the main change area as entering and exiting the facility.**

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?		
Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	**Virkon Decontamination according to SOP003 "*Disposal of Biological Waste*"**	**According to manufacturer's instructions, see section C2.1.9**
Solid waste	**Autoclave Decontamination according to SOP003 "*Disposal of Biological Waste*"**	**Treatment cycle is validated according to SOP024 "*Maintenance of Systec VX-95 Autoclave CBE044*". Annual validation is conducted by an external contractor.**

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box			
Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor
Liquid waste	**N/R**	**N/R**	**N/R**
Solid waste	**Consumables**	**Minimum 121°C for 15 mins (under clinical vacuum) CYCLE#4**	**Designated Autoclave tape monitors**
Location of autoclave	Servicing details	Location of back-up autoclave	Designated area for storage of unsterilised waste
	Annual	**CBE/045- In autoclave room H31**	**Second change**

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
All the hazardous liquid waste will be poured into an empty bottle, sealed and transported to the school safety officer for safe disposal.
To the drain?
After 1% Virkon decontamination for 24 hours, waste is poured down the drain followed by copious amounts of water. Refer to SOP003 "*Disposal of Biological Waste*". **In the occurrence of a contamination, flask will be treated with 3% Virkon overnight before being disposed of, refer to SOP003 "*Disposal of Biological Waste*".**
As solid waste? **No**
Other? **N/A**

C1.2.16 Solid Waste Disposal
Describe the waste category and disposal route. *(For guidance refer* to http://www.environment-agency.gov.uk)

Colour Code	Categorisation	*Hatch relevant box(es)*	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)		Yellow Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
Purple/Yellow Special case, contact DSO	Sharps (contaminated with cytotoxic/cytostatic material)		Purple/Yellow lidded Sharps bin>clinical waste disposal (incineration @ 1000C)
Yellow	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal)		Yellow rigid one way sealed tissue bins>clinical waste disposal (incineration)
Yellow	Animal body carcasses or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal		Yellow rigid one way sealed tissue bins > clinical waste disposal (incineration)
Special Case – Contact DSO	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
Orange	Infected or potentially infected lab wastes that have been pre treated before leaving the site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > orange clinical waste bags > clinical waste disposal (incineration)
Yellow	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site		Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > yellow one way sealed tissue bins > clinical waste disposal (incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, describe the procedure and describe where this aspect of the work will be conducted:	
(ii) Is shedding of infectious materials by the infected animals possible or expected?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:	

(iii) Who will perform the inoculations of animals/vectors? What training have they received?	
Indicate in the adjacent box if Not Relevant (N/R)	**N/R**
Provide details of the training required:	

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a bioreactor/fermenter be used to culture a biological agent?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, describe the size, and type of the bioreactor/fermenter.	
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray.	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**N/R**
If yes, describe:	

C1.2.19 Other Control Measures Required?

N/R

C1.3 Emergency Procedures

C1.3.1 Describe the procedures in place for dealing with spillages *(specify disinfectants and any special containment for large volumes)*

Within the BSC: **N/R**
Within the laboratory but outside the control measure e.g. BSC, spill tray **Local Procedures described in CBE SOPs which specifically detail spillage prevention and response measures will be employed** **1- SOP006 - "*Preparation of disinfectant for use within CBE laboratories*"** **2- SOP038 – "*Biological Spill Response*"** **Labelled biological spill kits are located in the CBE unit and signs are posted throughout the CBE unit to enable workers to locate the nearest biological (and chemical) spill kit and also to advise on spill response and reporting procedures.**
Outside the laboratory e.g. during transport **No transport outside the laboratory once the testing has been done at the CBE.** **If there are any movements, they are likely to be contained within the University campus using local procedures: SOP038 – "*Biological Spill Response*".**

Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation)

Procedures to respond to accidental exposure are detailed in CBE SOP038 "Biological Spill Response" and the CBE CoP. These are detailed in spill response posters located in the CBE laboratories.

Designated hand washing facilities are located in laboratory change areas and immediately inside the analytical lab where the cryostorage unit is located in the CBE facility.

Eye wash stations are readily available in each laboratory change area and within laboratories that do not have a change area.

A first aid kit is located outside the laboratory unit. Signs are posted throughout the laboratory unit to enable workers to locate the nearest medical kit. Contact details for first aiders are posted in laboratories.

Any sharps injury is to be reported and treated by local first aider immediately. List of first aiders is available in the CBE unit corridor.

Essential and emergency contact details are posted in the CBE laboratories.

C2 ASSIGNMENT OF CONTAINMENT LEVEL

The laboratory Containment Level is directly related to each of the 4 Hazard Groups; organisms categorised as HG1 (lowest hazard rating) should normally be handled in CL1 facilities (minimum level of containment), and likewise up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the presence of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work? (see COSHH Schedule 3, Part II for a list of criteria)

Containment level 1 is required for this work. However, all procedures will be carried out under containment level 2 (CL2). This is for reasons other than worker protection, including the need to ensure research material is protected and to maintain quality.

C2.2. Describe extra controls or derogation from certain controls
:

N/R

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
H34, Analytical Laboratory	**Centre of Biological Engineering**	**Holywell Park**	**C.J. Hewitt (Biological Safety Officer)** **R.Temple (Department Safety Officer)** **K.Sikand (Laboratory Manager)**

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Khobragade	**S.O**		**PhD Student**
Efimov	**I**		**Research Associate**
Ghosh	**S**		**Principal Investigator**

C4.2 Information, Instruction and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity. Instruction should include the 'Local Rules' or 'Local Codes of Practice' which focus on the working instructions to be followed by all persons involved in the work activity to control or prevent exposure to hazardous biological agent(s). These should be written and readily available to all workers working at Containment Level 2. A formal record of training should be kept for all individuals working at Containment Level 2.

Access to CBE laboratories is restricted to authorised users. In order to obtain authorised user status, operators must satisfy minimum training requirement set by CBE management and Health and Safety Committee. Basic training modules include a detailed review of the current Code of Practice (CoP), this document details specific aspect of class 2 working in relation to handling biological agents, waste management, training requirements of lab equipment and emergency procedures including spill responses.

All training is documented in a personal training file, which is held in the CBE office at all times. Prior to being granted access to CBE labs, each training file must be reviewed and signed off by both lab management and the departmental safety officer (DSO).

Once authorised access has been granted, it is the responsibility of the operator to identify specific training needs prior to the start of new projects. SOPs and risk assessments relevant to project equipment and/or procedures can be used as training aids. Training files are live documents and must be continually updated to record all training acquired.

For this project, S.O. Khobragade and I. Efimov will partake in practical aspects of the work and where needed help and supervision will be provided by S.Ghosh. He will be assisted in the labs by CBE Researchers.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Khobragade	**Has training on file.** **Use of the crystal sensor will be taught by S. Ghosh**
Efimov	**Has training on file.** **Use of the crystal sensor will be taught by S. Ghosh**

C4.4 Other people who may be at risk from the activity e.g. cleaners, maintenance workers or other workers in shared laboratory

Details:
None. Cleaners and maintenance workers are not authorised to enter the laboratory area. All laboratory cleaning is undertaken by authorised personnel only. Access for non-laboratory workers is subject to local permit to work procedures. If access is needed, for essential maintenance of equipment for example, a clean down and decontamination of laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker fully supervised according to SOP004 "*General Laboratory Housekeeping*" and the local CoP.

C5 OCCUPATIONAL HEALTH

C5.1 Vaccination

Is an effective vaccination available for any of the pathogens associated with this work? Advice can be obtained from the Occupational Health Adviser (OHA) if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

C5.2 Health Surveillance

Is health surveillance required? (Health surveillance is typically applied if working with a hazardous substance that: a) produces an identifiable disease or adverse health effect that can be related to exposure; b) there is a reasonable likelihood that the disease or effect may occur under the conditions of work, and c) there are valid techniques for detecting indications of the disease or effect).

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**No**

C6.1.2 Does This Work Have Ethical Approval? If Yes, Provide Details

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)			**N/R**
Approval number:			
Date obtained:		Ethics committee name:	

C6.1.3 Are other registrations/notifications required for this work? For example HSE notification under COSHH, Home Office notification under anti-terrorism, crime and security act etc

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	**No**
If Yes, give details:	

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

C7.1.1 Are there any licensing requirements for this work?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. Current procedures to be followed:

- If you wish to import any animal products that you know are not infected with an animal pathogen, or have good reason to expect that they are not infected with an animal pathogen, from within or outside of the EC you must apply for a Research Sample Licence using the Defra form IAPPO1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/iapppo1.htm

- If you wish to import such an animal product but it is known or suspected of being infected with an animal pathogen then you must use DEFRA form IM137. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/inttrade/im137.htm

- If you wish to import an animal pathogen listed under the Specified Animal Pathogens Order then you must use DEFRA form PATH1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/path1.htm

In all cases the instructions for their submission is stated on the forms themselves.

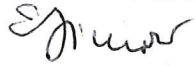
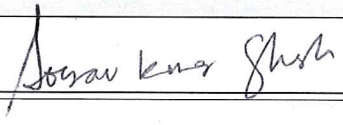
ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

8. DECLARATION

*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and University Biological Safety Officer*

I, the undersigned:

- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all individuals working on the activity
- will ensure that no work will be carried out until this **assessment has been completed and approved** and that all necessary control measures are in place
- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

Name: Person conducting assessment	**Signature:**	**Date:**
I.Efimov		
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
S.O.Khobragade		19/05/2014
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
S.Ghosh		

9.APPROVAL

For work involving **Hazard Group 1** biological agents: Review and approval is required by authorised and designated members of CBE staff before the work begins

For work with **Hazard Group 2** biological agents: Explicit approval is required from the Departmental Biological Safety Advisor and the University Biological Safety Officer before work begins.

If the biological agent has been **Genetically Modified** this form, (approved by the relevant authority, as above) should be submitted with the GMO risk assessment to the Departmental Biological Safety Advisor and both forms forwarded to the LU GM Safety Committee for final approval.

Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
A.Chandra, RA	A. Chandra	20 May 2014
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
R.Temple		
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**
C.J. Hewitt		

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UV Inactivation of Pathogenic and Indicator Microorganisms

JOHN C. H. CHANG, SUSAN F. OSSOFF, DAVID C. LOBE, MARK H. DORFMAN, CONSTANCE M. DUMAIS, ROBERT G. QUALLS, AND J. DONALD JOHNSON*

Department of Environmental Sciences and Engineering, School of Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

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Survival was measured as a function of the dose of germicidal UV light for the bacteria *Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Bacillus subtilis* spores, the enteric viruses poliovirus type 1 and simian rotavirus SA11, the cysts of the protozoan *Acanthamoeba castellanii*, as well as for total coliforms and standard plate count microorganisms from secondary effluent. The doses of UV light necessary for a 99.9% inactivation of the cultured vegetative bacteria, total coliforms, and standard plate count microorganisms were comparable. However, the viruses, the bacterial spores, and the amoebic cysts required about 3 to 4 times, 9 times, and 15 times, respectively, the dose required for *E. coli*. These ratios covered a narrower relative dose range than that previously reported for chlorine disinfection of *E. coli*, viruses, spores, and cysts.

Disinfection of water and wastewater with UV radiation appears to be a potential alternative to chlorine. Low concentrations of chlorine residuals are toxic to aquatic life (29), and some of the by-products of chlorination have been shown to be mutagenic (18). Germicidal UV radiation does not produce undesirable by-products and is effective in inactivating a variety of microorganisms (13, 30).

A number of pathogenic microorganisms, particularly viruses and cysts, are much more resistant to chlorine than is *E. coli* (18). Yip and Konasewich (30) suggested that the doses of UV light necessary to kill pathogens, including viruses, bacterial spores, and protozoa, are much more comparable to the doses of UV light necessary to kill indicator bacteria than is the case for chlorine. If so, the UV levels necessary to meet coliform standards may be relatively more effective than chlorination in killing pathogens. To date, much has been published on the inactivation of microorganisms by UV light. Because of the difficulties and subtleties of measuring UV dose (9), many of the dose values reported are not comparable. Dose is normally defined as intensity times exposure time. Sensitivity to UV disinfection can vary for a certain species of microorganism according to strain, growth medium, stage of the culture, and influences of the plating medium on repair of sublethal damage (9, 15). No comparison has included the coliform group from field samples which actually serves as the basis for present standards. Comparisons of UV resistance must be done by careful determination of UV dose and standardized growth conditions.

The objective of this study was to (i) accurately define the UV dose required to inactivate certain human pathogens, including bacteria, viruses, spores, and cysts, and (ii) evaluate the validity of using coliforms as indicators of UV disinfection efficiency.

MATERIALS AND METHODS

Bacteria. The bacteria used in this study were obtained from the American Type Culture Collection, Rockville, Md.: *Escherichia coli* (ATCC 11229), *Streptococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Shigella sonnei* (ATCC 9290), *Salmonella typhi* (ATCC 6539), and *Bacillus subtilis* (ATCC 6633). Except for the *B. subtilis* spores, bacteria were grown in nutrient broth until they were well into the stationary phase (20 to 24 h at 35°C). Samples of the broth culture were then filtered with a 0.45-μm-pore Millipore filter and rinsed with sterile buffered water. Cells were resuspended in sterile buffered water and filtered through a 1.0-μm Nucleopore polycarbonate membrane to remove aggregated groups of bacteria. The filtrate was used in the UV inactivation studies. The process of washing and irradiation was completed as quickly as possible to minimize the time in buffered water. Vegetative bacteria were enumerated on spread plates with nutrient agar (Difco Laboratories, Detroit, Mich.).

B. subtilis spores were produced in Schaeffer medium (10, 16). After sporulation, the suspension was heated to 80°C for 10 min and then sonicated for 2 min at 70 W in an ice bath. The spore suspension was washed by centrifugation, and the pellet was resuspended in distilled water. This procedure was repeated until microscopic examination showed individual spores free of cell debris. After the last centrifugation, the spores were again sonicated to ensure that there was no aggregation and stored in distilled water at 4°C. The viable spores were enumerated by using pour plates with Difco nutrient agar.

Viruses. Poliovirus type 1 (LSc2ab) and simian rotavirus (SA11) were used in this study. Poliovirus was propagated and assayed in MA104 cells, a continuous Rhesus monkey kidney cell line. SA11 was propagated in secondary African green monkey kidney cells and assayed in MA104 cells. Assays of both viruses were accomplished by cytopathic plaque formation in cell monolayers (6, 28). Monodispersed virus stocks were obtained by Freon extraction of the cell culture medium supernatant from 100% infected cultures, centrifugation at 105,000 × *g* for 2 h to pellet the virus, suspension of the virus pellet in phosphate-buffered saline, and filtration through a Tween 80-treated 0.08-μm-pore polycarbonate filter.

Amoebic cysts. A sample of *Acanthamoeba castellanii* (ATCC 30234) trophozoites was obtained from the American Type Culture Collection. The methods developed by Neff et al. (19) for production of amoebae and induction of synchro-

* Corresponding author.

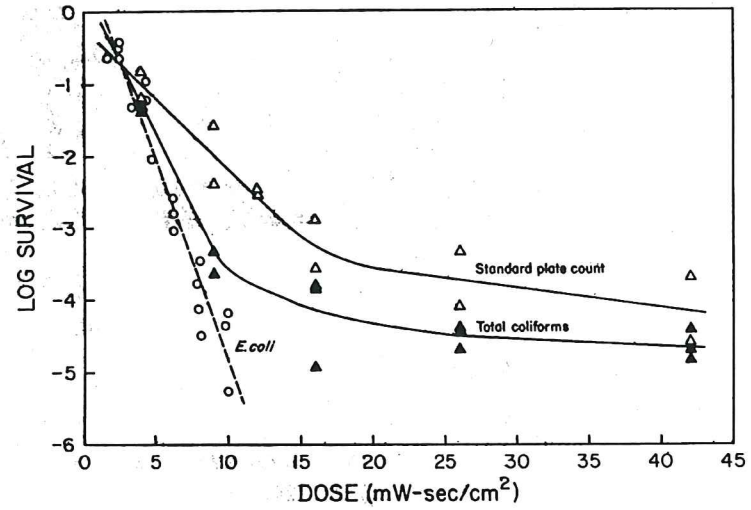

FIG. 1. Survival versus UV dose for cultured *E. coli*, filtered (10-μm pores) total coliforms, and microorganisms growing on standard plate count agar from a secondary effluent. Each datum point represents one irradiation and three replicate platings.

nous encystment were used. The amoebae were allowed to encyst for 1 week at 25°C with constant aeration. Encystment was greater than 90%.

A plaque assay developed by Rubin et al. (25) was used to enumerate amoebic cysts. A 0.1-ml sample of the cyst suspension together with 0.15 ml of a 24-h broth culture of *E. coli* were spread on Fulton plating medium agar. The plates were incubated at 30°C for 4 to 5 days, and the plaques on the bacterial lawn were counted.

Field samples. Samples of secondary effluent were collected from the Sandy Creek Wastewater Treatment Plant, Durham, N.C. A portion of the samples was gently filtered through a nylon screen with approximately 10-μm pores to remove most of the larger particles capable of harboring embedded bacteria. These samples were irradiated within 2 h. Coliform survivors were enumerated by the one-step total coliform membrane filtration procedure (1), since no significant difference was found for the survival of UV-irradiated coliforms as enumerated by either the standard membrane filtration or most-probable-number total and fecal coliform procedures (21). Standard plate count microorganisms were enumerated according to standard methods (1).

Irradiation. For accurate dose-survival data, samples were irradiated in an apparatus which provided a nearly collimated beam (22). Measurements of incident intensity (I_0) at the liquid surface at 254 nm were made with an International Light IL-500 radiometer with an SEE-240 detector calibrated by reference to a National Bureau of Standards lamp. Stirred suspensions of 0.5-cm depth were irradiated in small petri dishes. The average intensity in the stirred suspension (I_{avg}) was calculated as follows (12):

$$I_{avg} = I_0(1 - e^{-A_eL})/A_eL$$

where A_e is the absorbance per centimeter (base e) and L is the path length. Spectrophotometric absorbance at 254 nm was measured with a Cary 219 spectrophotometer.

All dose-survival experiments with bacteria and cysts were done with six dose levels, two replicate irradiated suspensions, and three platings of each dilution. For viruses, two platings were used. Survival data were treated according to Chick's Law as log N_s/N_0 versus dose, where N_0 is the initial concentration of organisms and N_s is the density of survivors.

RESULTS AND DISCUSSION

Vegetative bacteria. Most of the vegetative bacteria (*E. coli*, *S. aureus*, *S. sonnei*, and *S. typhi*) exhibited similar resistance to UV light (Fig. 1 and 2) and required about the same dose for 3 log units (99.9%) of inactivation. The exception to this was *S. faecalis*, which required about a 1.4 times higher dose for three log units of inactivation. Several of the survival curves, including the one for *S. faecalis*, and especially those for the *B. subtilis* spores and the *A. castellanii* cysts (Fig. 3), exhibited an initial lag in slope or "shoulder." Such curves have elsewhere been attributed to multiple-hit kinetics or related phenomena (9). The multiple-

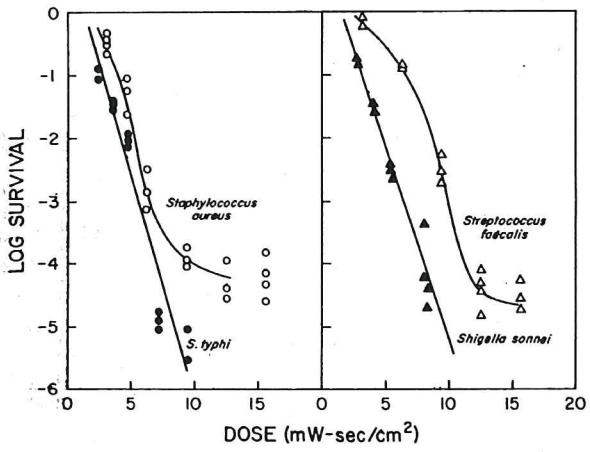

FIG. 2. Survival versus UV dose for *S. sonnei*, *S. typhi*, *S. faecalis*, and *S. aureus*. Each datum point represents one irradiation and three replicate platings.

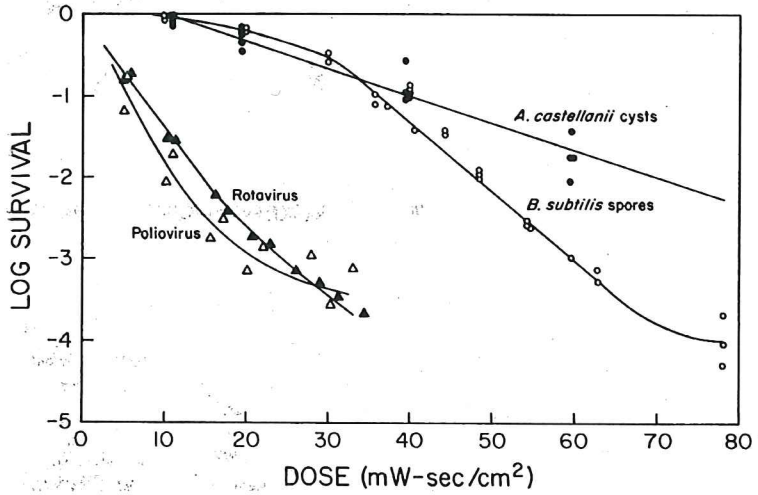

FIG. 3. Survival versus UV dose for poliovirus type 1, rotavirus SA11, *B. subtilis* spores, and *A. castellanii* cysts. Each datum point represents one irradiation and two (for viruses) or three replicate platings.

hit theories can apply both to aggregates of cells or single cells (9).

Our data agree well with the comparable survival data for *E. coli*, *S. typhi*, *Shigella paradysenteriae*, and *S. aureus* by Sharp (27), although his data did not extend below the −2 log survival level. The *Streptococcus* sp. used by Sharp appeared to be more sensitive than the one we used, however. Much of the early work has been summarized by Zelle and Hollaender (31), but the results are reported as the dose necessary for 90% inactivation, a dose level which may be unduly influenced by the initial lag in slope evident in much of the data (9). The most widely cited comparison of a wide variety of organisms is that of Nagy (17). Those data are difficult to compare with other data because the bacteria were irradiated on the surface of agar plates, and the data were summarized in terms of the dose necessary to completely destroy the microorganisms, a value dependent on the initial density of the organisms on the plate surface. A report of the sensitivity of *Legionella pneumophila* to UV radiation (2) indicates that it may be considerably less resistant than *E. coli*.

In comparing chlorine disinfection data, the concept of dose (14) is not as straightforward as it is for UV. However, a number of references have been summarized recently (18) in which comparisons are made on the basis of concentration (C) times time (t) for a survival level of 1%. The resistances to chlorine (HOCl) of several enteric bacteria are roughly comparable (18). At neutral pH, *S. typhi* strains are somewhat more resistant to HOCl than is *E. coli*. Thus, when UV light and chlorine disinfection are compared on the basis of a given level of inactivation of nonaggregated *E. coli*, the inactivation of vegetative enteric bacteria appears to be generally comparable.

Total coliforms and standard plate counts. The survival curve for the total coliforms in the filtered secondary effluent sample was relatively similar to that for the cultured *E. coli* at the higher levels of survival (Fig. 1), but the curves diverged at the lower levels of survival. Experiments involving the removal of particles both smaller and larger than 10 μm (20) and fractionation of particle sizes (S. F. Ossoff, Master's Technical Report, University of North Carolina at Chapel Hill, 1984) have shown that the tendency of wastewater coliform survival curves to level out at low levels of survival is caused by a small fraction of cells protected by their association with larger particles. Microscopic particle size measurements indicated that 10-μm filtration removed most, but not all, of the aggregates and particles capable of harboring protected coliform cells.

The total coliforms represent a group of bacteria, some unknown proportion of which may be *E. coli*. The fact that the bacteria were cultured did not appear to create dramatic differences between the survival curves of the cultured bacteria and those of the natural coliforms except at high levels of inactivation. These results suggest that the total coliforms can serve as an adequate indicator of disinfection for at least the vegetative bacteria used in this study. The microorganisms enumerated by the standard plate count represent a diverse assemblage. The survival curve for the standard plate count microorganisms (Fig. 1) is shifted toward somewhat higher doses than the curve for filtered total coliforms. The standard plate count and coliform survival curves suggest a relatively small range in UV resistance of the portion of the total microbial population enumerated by these methods. The standard plate count may be as good a measure of bacterial pathogen disinfection efficiency as the coliform tests.

Viruses. The survival curves of the two viruses studies are presented in Fig. 3. The curves for rotavirus and poliovirus are very similar, and these viruses exhibit three to four times more resistance to UV irradiation than do the vegetative bacteria (Fig. 4).

The viral disinfection data agree reasonably well with those of Hill et al. (8), who used eight enteric viruses. Estimating a corrected dose from their measurements of incident intensity, we found the dose necessary for −3 log survival ranged from about 28 to 42 mW−s/cm² (30 mW−s/cm² for poliovirus type 1). Morris (13) found the dose necessary for 98% inactivation of poliovirus to be about 2.5 times that for *E. coli*. The conclusion of Yip and Konasewich (30) that enteric viruses are somewhat more sensitive to UV light than *E. coli* was not supported by this study.

In terms of comparison of our data to those on chlorine, Clark et al. (4) found that *E. coli*, *S. typhi*, and adenovirus type 3 were all inactivated at approximately the same

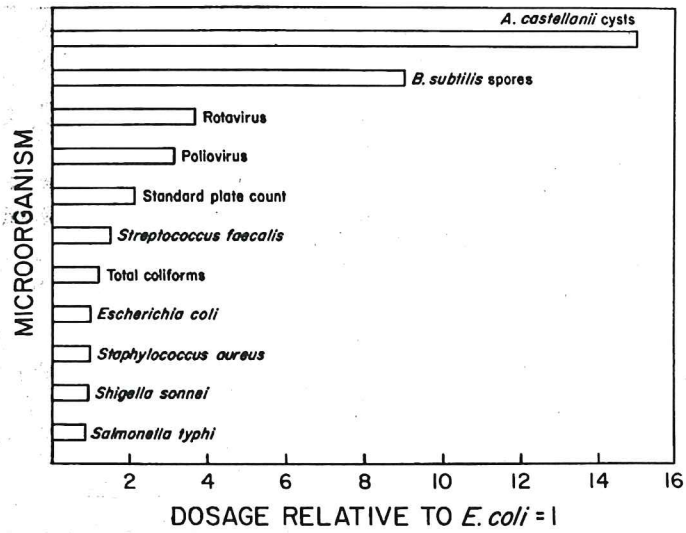

FIG. 4. Relative UV doses required for 99.9% inactivation of various microorganisms compared to that for *E. coli*.

concentration of free chlorine. This report of the low resistance of adenovirus type 3, however, appears anomalous among a number of reports concerning other enteric animal viruses (7, 18). In another study (5), the doses of free chlorine necessary to produce 99% inactivation at pH 6 and 5°C for six types of enteric animal viruses ranged from 3.5 to 43 times that necessary to inactivate *E. coli*. For poliovirus type 1, the dose was about 25 times that for *E. coli*. These relationships were different, however, at higher pH values. Scarpino et al. (26) found that poliovirus type 1 is about 40 times more resistant than *E. coli* at pH 6. However, Berman and Hoff (3) found that preparations of free simian rotavirus SA11 virions are completely inactivated in times comparable to those for *E. coli*.

Bacterial spores and protozoan cysts. The *B. subtilis* spores and the *A. castellanii* cysts were the most UV-resistant organisms (Fig. 3). The spores were about 9 times more resistant than the vegetative bacteria, and the cysts were about 15 times more resistant (Fig. 4). Although the UV sensitivity of spores prepared and plated on a particular medium was reproducible, the medium on which irradiated *B. subtilis* spores are plated can have an effect on the apparent survival. Spores produced with Schaeffer medium exhibited a different apparent survival when plated on Difco Thermoacidurans agar (21) than when plated on nutrient agar (see above).

Little data are available for UV disinfection of amoebic cysts. Rice and Hoff (23) found that cysts of *Giardia lamblia* are about 68 times more UV resistant than *E. coli* at the −0.6 log survival level. The *A. castellani* cysts used in this study were considerably more sensitive (Fig. 4). Although the UV dose necessary to cause a 99% inactivation of the cysts used in this study was within the operating range of many UV disinfection systems, it was beyond the usual operating dose.

Morris (14) calculated that bacterial spores were approximately 400 times more resistant to chlorine than enteric bacteria. McGrath and Johnson (11), with the same preparation of *Bacillus* spores used in this study, found a resistance to HOCl that was similar to that summarized by Morris (14), considering temperature differences. The data for protozoan cysts are generally difficult to compare with data for *E. coli* because experiments with cysts are generally performed at higher temperatures. From data summarized by the National Research Council (18), the $C \cdot t$ product at 30°C for *Entamoeba histolytica* is approximately 500 times that of *E. coli* at 5°C, so at similar temperatures the difference would be far greater. For *Naegleria gruberi*, a species morphologically similar to *A. castellani* the $C \cdot t$ product at 25°C (25) was about 200 times that for *E. coli* at 5°C. The experiments of Rice et al. (24) with *G. lamblia* were performed at 5°C. Estimating the $C \cdot t$ product from the most sensitive strain used in their experiments, they found that the *G. lamblia* cysts appeared to be 1,000 to 2,000 times more resistant than *E. coli*.

Implications for disinfection. The vegetative bacteria we studied exhibited similar resistance to UV disinfection. The viruses were 3 to 4 times more resistant, the spores were about 9 times more resistant, and the cysts were 15 times more resistant than the vegetative bacteria. Given problems in relating relative resistances to chlorine of *E. coli* and other organisms, probably only large differences should be considered significant in comparing ranges of dose necessary for UV and chlorine disinfection. Still, the difference in dose necessary to inactivate *E. coli*, bacterial spores, and protozoan cysts seem to be much greater for chlorine disinfection than for UV disinfection. With some exceptions, this also appears to be true for enteric viruses, especially for poliovirus, for which the most data are available. The UV resistance of viruses and *E. coli* are not as comparable as implied by Yip and Konasewich (30).

The range of UV dose necessary to disinfect pathogens is narrower than it is for chlorine disinfection. While the minimum dose to effectively eliminate all pathogens is the limiting factor for ideal disinfection, the economic comparisons and routine evaluations of different disinfectants have been made based on coliform or *E. coli* disinfection data. Neither *E. coli* nor coliforms can serve as a quantitative model for disinfection of viruses, spores, or cysts. Also, as Rice et al. (23) point out, the extreme resistance of cysts like *G. lamblia* makes it unlikely that either normal chlorination or UV irradiation procedures would be sufficient to destroy the cysts. It has been noted that organisms used to indicate fecal contamination of water supplies should be distin-

guished from organisms used as models of disinfection (18). As is the case with chlorine disinfection, the survival of coliform bacteria in UV-irradiated effluents does not directly indicate the same level of disinfection for the more resistant organisms.

ACKNOWLEDGMENTS

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Oxidative Mechanisms of Toxicity of Low-Intensity Near-UV Light in *Salmonella typhimurium*

GEORGE F. KRAMER AND BRUCE N. AMES*

Department of Biochemistry, University of California, Berkeley, California 94720

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The exposure of *Salmonella typhimurium* to environmentally relevant near-UV light stress has been studied by the use of a low-intensity, broad-band light source. The exposure of cells to such a light source rapidly induced a growth delay; after continuous exposure for 3 to 4 h, cells began to die at a rapid rate. The oxidative defense regulon controlled by the *oxyR* gene was involved in protecting cells from being killed by near-UV light. This killing may be potentiated by the overexpression of near-UV-absorbing proteins. These results are consistent with near-UV toxicity involving the absorption of light by endogenous photosensitizers, leading to the production of active oxygen species. We have shown, however, that one such species, H_2O_2, is not a major photoproduct involved in killing by near-UV light. Strains lacking alkyl hydroperoxide reductase were more sensitive to near-UV light, indicating that such hydroperoxides may be photoproducts. Near-UV exposure induced sensitivity to high salt levels, indicating that membranes may be a target of near-UV toxicity and a possible source of alkyl hydroperoxides. The demonstration of the inactivation of the heme-containing protein catalase indicates that direct destruction of UV-absorbing macromolecules could be another factor in near-UV toxicity. Cells which have been exposed to near-UV light for long, but sublethal, periods of time (up to 4 h) can recover and resume growth if the UV exposure is stopped but become progressively more sensitive to further stresses, such as H_2O_2. This result indicates that cells gradually accumulated damage during near-UV exposure until toxic levels were reached.

Solar radiation has been shown to be a major deleterious factor in the ability of coliform bacteria to survive outside the animal body (3, 10, 11, 23). Experiments measuring die-off of coliforms in natural waters over extended periods of time (typically between 10 h and several days) showed that the near-UV light (NUV) component of the solar spectrum (300 to 400 nm) appears to be responsible for most of the bacteriocidal effects of solar radiation (3, 10). The ability of coliforms to survive in the external environment is significant not only in terms of sanitation (12), but also in the survival of bacterial species (in the case of pathogens), since transmission between hosts involves such exposure. The importance of NUV on the survival of coliforms in general has also been found to be true for the common experimental organisms *Salmonella typhimurium* and *Escherichia coli* (3).

Many studies have been undertaken to characterize the response of *E. coli* to NUV. When exposed to low levels of broad-band NUV, the cells exhibit a growth delay (8, 18, 19). After the exposure ceases, the cells remain in a growth lag before resuming growth. The mechanism behind the NUV growth lag involves the absorbance of light by the tRNA-modified base 4-thiouridine (S^4U) (8, 18, 19, 36). S^4U undergoes a specific photoreaction which inactivates the tRNA molecule, thus inducing the stringent response which slows cell growth (8, 19, 36). Through this mechanism, one response of *E. coli* to physiologically relevant levels of NUV light is to rapidly cease growth.

The exposure of *E. coli* to NUV has also been shown to have lethal effects at higher fluences (19, 28, 31, 32, 39, 46) and to be strongly dependent on the presence of oxygen (19, 28, 31, 32). NUV toxicity thus may involve the absorption of light by photosensitizers which can transfer the energy to O_2, leading to the production of active oxygen species (3). Evidence has been presented which indicates that one such activated oxygen species, H_2O_2, is formed by NUV exposure (38, 39, 46). Catalase added to the medium was shown to protect against killing by NUV (38), and pretreatment with low doses of H_2O_2 was shown to induce resistance to killing by NUV (39). NUV has also been reported to induce the synthesis of catalase (7). These observations are consistent with the suggestion that the effects of NUV and H_2O_2 are mostly interchangeable (7).

Our experiments have been designed to study the effects of physiologically relevant fluences of NUV on *S. typhimurium*. Much of the previous work on the toxic effects of NUV was performed with high-intensity (100 to 1,000 W/m^2), monochromatic light sources (25, 31, 32, 35, 44–46). The fluence rate has been found to be important in the observed effects of NUV on *E. coli*; i.e., the effect of a short exposure to a high-intensity source does not have the same biological effect as a longer exposure to a lower-intensity light source (22, 30). It is thus unclear whether the effects of high-intensity monochromatic light are the same as physiologically relevant exposures or whether specific photoreactions occur at high intensities which are unimportant at lower fluence rates. The physiological relevance of monochromatic NUV is questionable since all the energy is being directed at a small number of chromophores rather than being spread over a broad range, as in solar NUV. The fluence rate of NUV in the solar spectrum is 35 to 50 W/m^2 (35, 42). Our exposures were designed to more closely approximate environmental conditions, i.e., exposure to a broad-band source with a low fluence rate (approximately 35 W/m^2) for several hours. These experiments were also designed so that the only alteration in the growth conditions of the cells was the imposition of the NUV-light stress. We found that under these conditions, cells begin to die after 3 to 4 h of exposure. The mechanism of toxicity appears to be consistent with the involvement of oxidative damage. However, our results indicate that H_2O_2 itself is not an important

* Corresponding author.

TABLE 1. Strains used

Strain	Genotype	Reference
TA4187	*zaj-1034*::Tn*10*	This paper
TA4100	*oxyR1*	4
TA4108	*oxyΔ2* [*oxyR Δ(oxyR argH)2*]	4
TA4113	*katΔ2* [*kat Δ(metB ppc katG argH)2*]	4
TA4265	*katΔ3* [*kat Δ(metB ppc katG argH)3*] *oxyR1*	4
TA4268	*ahp Δ(ahp)3*	26
TA4269	*ahp Δ(ahp)3 zii-614*::Tn*10 oxyR1*	26
TA831	*hisF645*	14
TA4186	*hisF645 gsh-1*	S. Kustu (unpublished data)

agent in the effects of physiologically relevant NUV exposures.

MATERIALS AND METHODS

Bacterial strains are shown in Table 1. H_2O_2 was purchased as a 30% solution from Mallinckrodt, Inc., microperoxidase (MP-9) and isoluminol were purchased from Sigma Chemical Co., and bovine liver catalase was purchased from Sigma as a 17,600-U/mg solid.

Unless otherwise noted, liquid bacterial cultures were grown in minimal VBC salts (47) containing 0.4% glucose. Overnight cultures were grown without shaking at 37°C in minimal VBC salts containing 0.08% glucose. Viable cells were determined by plating dilutions of cells onto nutrient broth plates (0.8% Difco nutrient broth, 0.5% NaCl, and 1.5% agar) and counting colonies after 14 to 20 h of incubation at 37°C. Catalase (140 U/ml) was added to the plating media of all strains in experiments involving Δ*oxyR* to increase the plating efficiency of this mutant (M. Christman and B. Ames, unpublished data).

For irradiation of cells, a foil-lined box was built which contained three 15-W General Electric F15T88LB black light bulbs mounted on the inside of the lid. The ends of the box were open to allow ventilation. Cultures (100 ml) were grown in large flat-bottomed Pyrex petri plates with the lids on. The light bulbs were suspended over the petri plates at a distance of 2.5 cm from the surface of the culture. The flux rate was determined to be approximately 35 W/m^2 by the use of a Spectroline DM-365H detector. To provide proper aeration, the cultures were stirred with a magnetic stir bar while bubbling with humidified air. Cultures were maintained at 37°C. Once the optical density (650 nm) reached 0.15, irradiation was started. Viability was determined at various times by removing samples and performing viable cell counts. Killing curves were generated by averaging the results of two to six separate experiments.

H_2O_2 assays were performed by a method similar to that of Yamamoto et al. (48). The assay mixture contained 0.1 mg of microperoxidase per ml, 2 mM isoluminol, and 10 μM EDTA in a 10 mM borate buffer, pH 8.6. The assay mixture (100 μl) was added to a 5-μl sample. Light emission was measured in a Turner luminometer. The amount of light emitted was found to be proportional to the H_2O_2 concentration, and the limit of sensitivity was well below 1 μM. The H_2O_2 concentration in the medium was determined by filtering cells through a Gelman Acrodisc (pore size, 0.2 μm) and assaying the filtrate. The concentration of H_2O_2 in the medium equals that in the cells, as H_2O_2 is freely diffusible through the cellular membrane (41).

Catalase activity was measured spectrophotometrically by the method of Beers and Sizer (2). Killing caused by H_2O_2 was performed by the method of Imlay and Linn (17), except that top agar was not used in plating cells. Killing caused by high salt levels was performed by placing 50-μl samples of cells in 1.0 ml of NaCl solution. The cells were then shaken at 37°C for 30 min before viability was determined.

RESULTS

Effect of NUV on cell growth. *S. typhimurium* was exposed to NUV during exponential growth in minimal glucose medium. Short exposures (15 min) induced a small delay in growth, after which exponential growth resumed (Fig. 1). This result was consistent with previous reports of an NUV-induced delay in growth (8, 19). When cells were exposed to NUV for 4 h, cell growth was delayed during the exposure (Fig. 1). After exposure, cell growth was delayed for an average of 2 to 3 h before growth was resumed.

Killing of cells by long NUV exposures. Viable cell counts were done to measure the killing of *S. typhimurium* LT2 during NUV exposures of up to 8 h in minimal glucose medium. After 3 to 4 h of exposure, cells began to die rapidly (Fig. 2). The rate of killing was greatest between 5 and 6 h and decreased to a much slower rate between 6 and 8 h (data not shown). This slower rate of killing at longer exposures may represent the emergence of a resistant subpopulation which has successfully adapted to the stress. The killing of *S. typhimurium* thus appears to be a biphasic phenomenon. Changes in sensitivity to NUV could affect either the onset of rapid cell death or the number of cells killed.

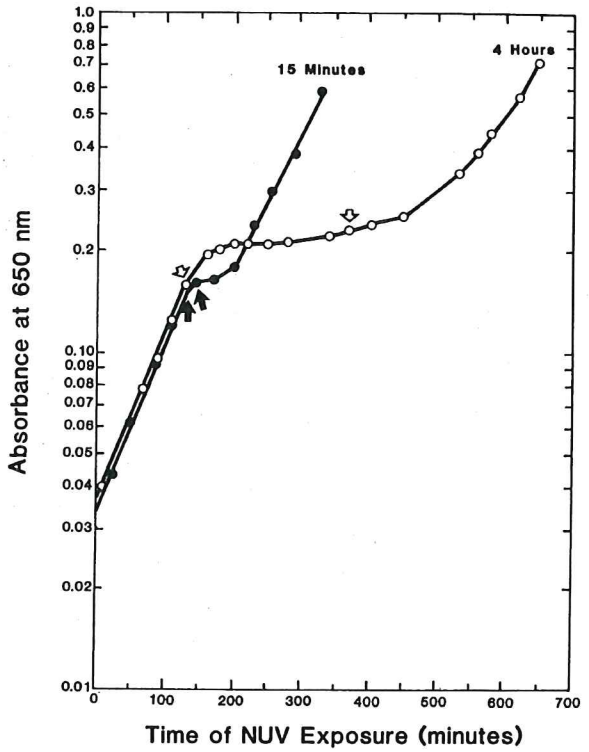

FIG. 1. Effect of NUV on growth of cells. TA4187 was grown in minimal glucose medium and exposed to NUV for 15 min or 4 h as described in the text. The arrows indicate the beginning and the end of the exposure.

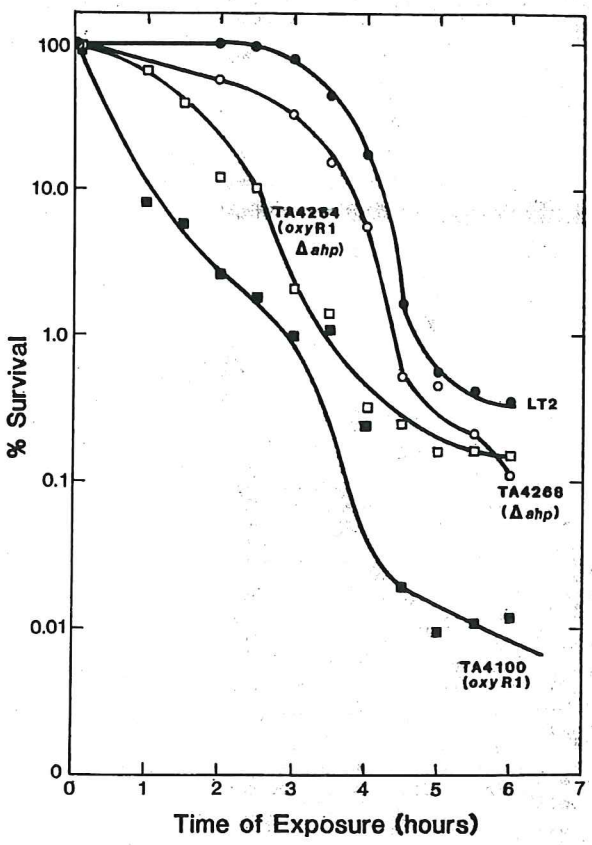

FIG. 2. Effect of Δ*ahp* mutation on NUV sensitivity of LT2 and *oxyR1* cells. Cells were grown in minimal glucose medium. Viability was determined throughout a 6-h exposure as described in the text.

The killing of *S. typhimurium* was enhanced by exposure in the supplemented minimal medium used in the experiments for Fig. 2 and 3. The reason for this difference is unclear, but it may involve the increased production of NUV-absorbing macromolecules (see below). Glutamate may increase flux through the tricarboxylic acid cycle via conversion to α-ketoglutarate, which could lead to increased levels of respiration-associated chromophores.

Sensitization to NUV killing by overexpression of NUV-absorbing proteins. To investigate the mechanism of toxicity of low-intensity NUV, we assayed the NUV sensitivity of several mutants defective in oxidative defense pathways. The *oxyR* regulon codes for at least nine proteins involved in defense against oxidizing agents (4). These proteins include catalase HP-I (product of the *katG* gene) and an alkyl hydroperoxide reductase (product of the *ahp* gene) (27). The *oxyR* protein is a positive regulatory element which is necessary for inducing the system in response to stress (4). The *oxyR1* mutant constitutively overexpresses the regulon (e.g., a 50-fold increase in catalase and a 20-fold increase in alkyl hydroperoxide reductase) and is thus resistant to a variety of oxidants including H_2O_2. We have assayed the sensitivity of the *oxyR1* mutant to NUV and found it to be much more sensitive to killing by NUV than the wild type, LT2, was (Fig. 2). This sensitization affected both phases of killing by NUV. The cells began to die earlier in the exposure (within 1 h), and more were killed. The sensitivity of the *oxyR1* mutant to NUV may be a result of the overproduction of NUV-absorbing proteins. Catalase HP-I, which contains two heme groups per tetramer (5) and alkyl hydroperoxide reductase, which contains a flavin (4), absorb significantly in the NUV range. The overexpression of all *oxyR* proteins, except catalase (*oxyR1* Δ*katG*) or alkyl hydroperoxide reductase (*oxyR1* Δ*ahp*) (27), resulted in less NUV sensitivity compared with *oxyR1* (Fig. 2 and 3A). The overexpression of alkyl hydroperoxide reductase appeared to mainly affect the onset of rapid killing (*oxyR1* Δ*ahp* versus *oxyR1* in Fig. 2) while the overexpression of catalase appeared to mainly affect the number of cells killed (*oxyR1* Δ*katG* versus *oxyR1* in Fig. 3A). The involvement of catalase HP-I in the photosensitization of *oxyR1* was further demonstrated by our finding that the overproduction of catalase from a multicopy clone of *katG* (B. L. Triggs-Raine and P. C. Loewen, Gene, in press) also resulted in increased sensitivity to killing by NUV (data not shown). The sum total of these effects would not appear to account for all the photosensitivity of *oxyR1*. The overexpression of other *oxyR* proteins could be involved, or the overexpression of both catalase and alkyl hydroperoxide reductase may act synergistically to photosensitize *oxyR1*.

Involvement of the *oxyR* regulon in protection from killing by NUV. The overexpression of the *oxyR* regulon leads to photosensitization, but the induction of *oxyR* proteins to levels lower than those found in *oxyR1* may still be important in protecting against killing by NUV since this killing is believed to be mediated by oxidative mechanisms. We have tested this hypothesis using a Δ*oxyR* strain which is unable to induce the *oxyR* regulon in response to H_2O_2 (4). The Δ*oxyR* mutant was found to be hypersensitive to killing by NUV (Fig. 3B). The onset of cell death was much earlier, and more mutant cells were killed, compared with wild-type cells. Since the deletion in the Δ*oxyR* strain includes genes other than *oxyR* (4), it is possible that this increased sensitivity may be a result of the deletion of a gene other than *oxyR*. This possibility has been investigated by the use of a plasmid into which an insert containing only the *oxyR* gene has been cloned (M. Christman and B. Ames, unpublished data). The insertion of this plasmid into the Δ*oxyR* strain greatly increased its resistance to NUV killing, indicating that the deletion of the *oxyR* gene was responsible for the photosensitivity of the Δ*oxyR* mutant. The importance of several other oxidative stress-inducible enzymes in sensitivity to killing by NUV has been investigated. The involvement of catalase HP-I was measured by comparing the killing by NUV of LT2 with that of a Δ*katG* strain, which has been found to be sensitive to H_2O_2 killing (27) (Fig. 3B). The Δ*katG* had the same sensitivity to NUV as LT2, indicating that catalase HP-I induction was not involved in protection from killing by NUV. Others have also reported that *katG* mutations do not affect NUV sensitivity in *E. coli* (40; A. Eisenstark and G. Perrot, Mol. Gen. Genet., in press). The importance of the *oxyR* alkyl hydroperoxide reductase was determined by comparing LT2 with a Δ*ahp* strain (Fig. 2). The Δ*ahp* strain appeared to be more sensitive to killing by NUV than the wild type was. The Δ*ahp* cells began to die much earlier (killing was measurable by 2 h), and more cells appeared to be killed. Glutathione reductase is another *oxyR*-regulated protein involved in oxidative defense (4). The involvement of the glutathione-glutathione reductase system in defense against NUV toxicity has been determined by measuring the NUV sensitivity of a *gsh* mutant which is defective in glutathione synthesis. Measurements of intracellular thiol levels indicated that this strain was a *ghsA*

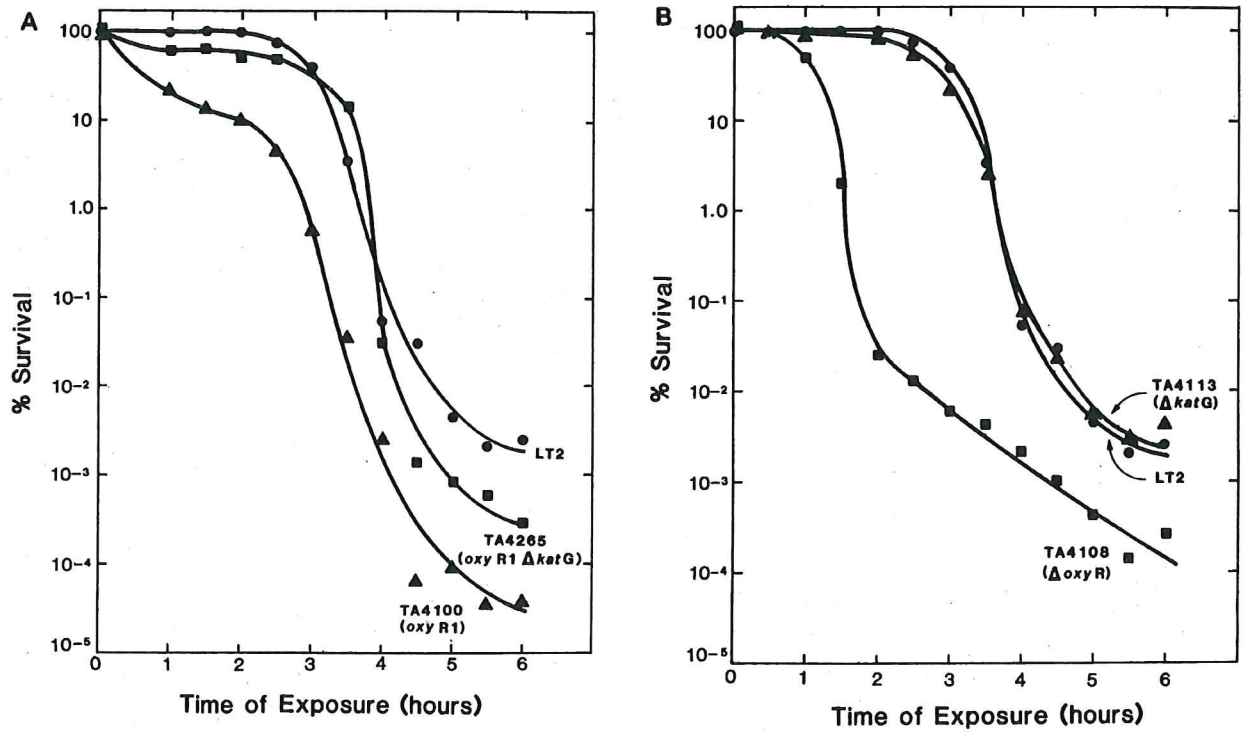

FIG. 3. Killing of mutants in oxidative defense pathways by NUV. Cells were grown in minimal glucose medium supplemented with 15 mM glutamate, 1 mM arginine, and 1 mM methionine. Viability was determined throughout a 6-h exposure. (A) Killing of *oxyR1* strains; (B) Killing of Δ*oxyR* and Δ*katG* mutants.

mutant (data not shown). This mutant has been found to be more sensitive to NUV killing (data not shown). This increased sensitivity was demonstrated by the onset of the rapid killing phase beginning almost 1 h earlier than with the parent strain. The shapes of the killing curves are otherwise the same.

Induction of sensitivity to H_2O_2 by NUV exposure. Pretreatment of cells with a low dose of H_2O_2 induces resistance to killing by higher doses (4, 6). Thus, the induction of resistance to killing by H_2O_2 after NUV exposure (46) is consistent with the reports of H_2O_2 being an important photoproduct. We have attempted to reproduce these results using physiologically relevant NUV exposures. H_2O_2 sensitivity was measured at various times during a 4-h exposure. Cells were removed from irradiation and immediately subjected to several different concentrations of H_2O_2. Viability was determined after 15 min of exposure to H_2O_2. Surprisingly, our results demonstrate that NUV induced sensitivity to H_2O_2 (Fig. 4). The shape of the H_2O_2 killing curve of non-NUV-treated cells was typical of the H_2O_2 killing curves reported for *E. coli* (17). The degree of H_2O_2 sensitivity increased with longer exposure; cells which have been exposed to NUV for 3 to 4 h were much more sensitive than those which were exposed for 1 to 2 h (data not shown). This sensitization to H_2O_2 was also quite rapid. After 15 min of NUV exposure, the cells were 40-fold more sensitive to killing by exposure to 10 mM H_2O_2 for 60 min than were non-NUV-treated cells (data not shown). In other experiments, cells were allowed to resume growth after a 15-min NUV exposure before their H_2O_2 sensitivity was measured. By 25 min after the exposure ended, the cells were no longer hypersensitive to H_2O_2, and no significant resistance to H_2O_2 was seen up to 45 min after the NUV exposure (data not shown).

Effect of NUV on catalase. The sensitization by NUV to H_2O_2 seen in our experiments was quite rapid (15 min) compared with the length of exposure before toxic effects were seen (3 to 4 h). It thus seemed possible that sensitization to H_2O_2 might involve damage of a specific target involved in H_2O_2 sensitivity rather than general cellular damage, which would make cells less resistant to further stress. The ability of catalase to absorb NUV in these conditions is implied by the photosensitization resulting from its overproduction. The effect of NUV of low fluence rate on catalase activity was determined by exposing a solution of bovine liver catalase to NUV and measuring its activity spectrophotometrically. NUV exposure rapidly inactivated the catalase (Fig. 5). After 30 min of exposure, only 15% of the initial catalase activity remained. This effect of NUV on catalase was also demonstrated in vivo by measuring catalase activity in the *oxyR1* strain during NUV exposure (data not shown). For a control, the effect of NUV on a non-NUV-absorbing enzyme was determined. Exposure of bacterial alkaline phosphatase to NUV under the same conditions used to expose catalase resulted in no decrease in activity (data not shown), indicating that the effect of NUV on catalase was a result of the ability of this enzyme to absorb NUV. These results were consistent with reports of the inactivation of catalase by sunlight (26).

Is H_2O_2 a photoproduct of NUV? To determine directly whether H_2O_2 was produced by NUV, we measured the H_2O_2 concentrations in cells throughout a 4-h NUV exposure. H_2O_2 levels were measured by using a microperoxidase-luminol assay which has a sensitivity limit of >0.5

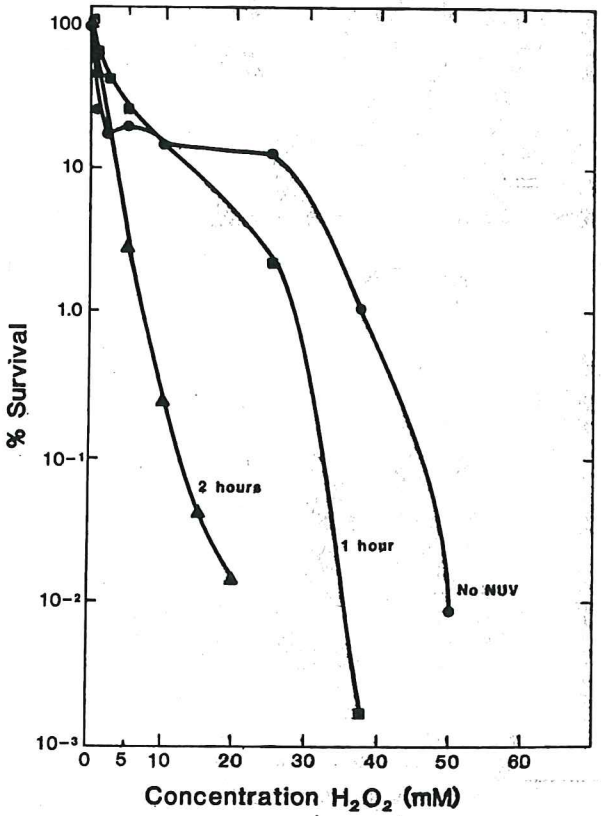

FIG. 4. Sensitization to H_2O_2 killing by NUV exposure. After TA4187 was irradiated with NUV for 0, 1, or 2 h, cells were exposed to several different concentrations of H_2O_2 for 15 min.

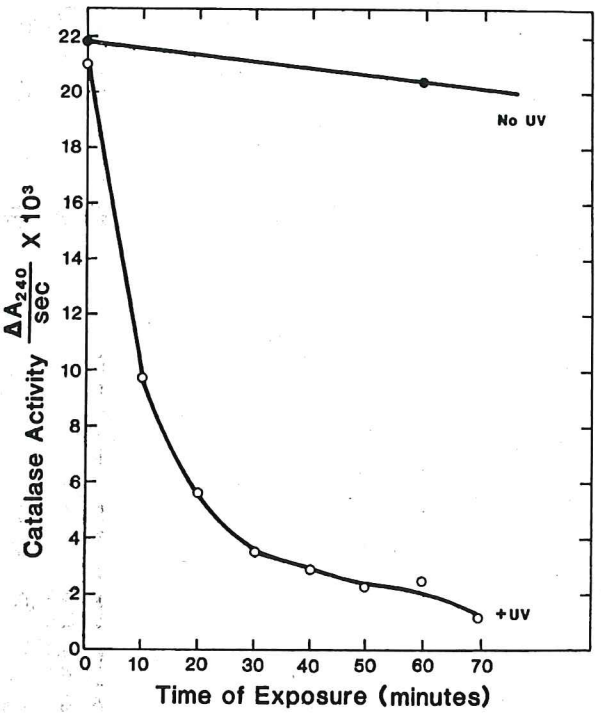

FIG. 5. Inactivation of catalase by NUV exposure. An 8-μg/ml (140 U/ml) solution of bovine liver catalase was exposed to NUV for 70 min. Activity was determined spectrophotometrically by measuring H_2O_2 destruction (decrease in A_{240}).

μM (48). We found that H_2O_2 concentrations in the cells remained below 1 μM throughout a 4-h exposure (Table 2). Reconstruction experiments demonstrated that H_2O_2 could be detected under these conditions (data not shown).

Sensitization to high salt concentrations by NUV. Membrane damage has been reported to be involved in the toxic effects of NUV (20, 21, 28). This conclusion was based on the observation that cells are sensitive to high salt levels after NUV exposure (20, 28) and on the influence of membrane composition on killing by NUV (21). We attempted to reproduce these results in our system by NaCl treatment of cells after NUV exposure. After treatment with NUV for 4 h, cells were treated with high levels of salt for 30 min. The NUV-treated cells were more sensitive to killing caused by salt than were untreated cells (Fig. 6). This result is consistent with reports of NUV toxicity involving membrane damage.

DISCUSSION

Exposure to solar radiation, especially the NUV region, affects the survival of bacteria in the natural environment (3, 10). We have studied the effect of a broad-band NUV light source with a fluence rate similar to that of the sun on the survival of *S. typhimurium*. Cells were resistant to short exposures but began to die rapidly after 3 to 4 h of exposure. Our results were consistent with reports of light-dependent die-off of bacteria in the environment (3, 10, 11, 23).

NUV toxicity has been reported to be oxygen dependent (19, 28, 31, 32) and may involve photosensitization by endogenous NUV-absorbing molecules, such as flavin adenine dinucleotide and heme (3, 18, 19). Such a mechanism would involve the absorption of light by chromophores resulting in their excitation, followed by reaction with O_2 to form active oxygen species which would be the primary damaging agents (3). This oxygen dependence has been demonstrated with both monochromatic (31, 32) and broad-band NUV sources (28). We have shown that the increased synthesis of the NUV-absorbing proteins catalase (contains heme) and alkyl hydroperoxide reductase (contains flavin adenine dinucleotide) leads to increased sensitivity to NUV killing. These proteins appeared to be acting as endogenous photosensitizers. These results were consistent with recently published work showing that cytochromes may also be acting in this manner (43). Further evidence of the involvement of oxidative damage in NUV toxicity came from our demonstration of the involvement of the oxidative

TABLE 2. Formation of H_2O_2 in cells by NUV exposure

Sample[a]	Luminometer counts	$[H_2O_2]^b$ (μM)
Blank	0.5	0
1 μM H_2O_2	19	
10 μM H_2O_2	170	
Cells (0)	3.2	<0.2
Cells (2 h)	2.5	<0.2
Cells (4 h)	0.8	<0.2

[a] Blank sample consisted of medium alone; H_2O_2 standard samples consisted of the indicated concentration of H_2O_2 in medium, and the samples with cells consisted of the culture medium from TA4187 which had been exposed to NUV for the indicated time.

[b] Calculated from a standard curve constructed over a range of 1 to 100 μM H_2O_2. The correlation coefficient was 0.991.

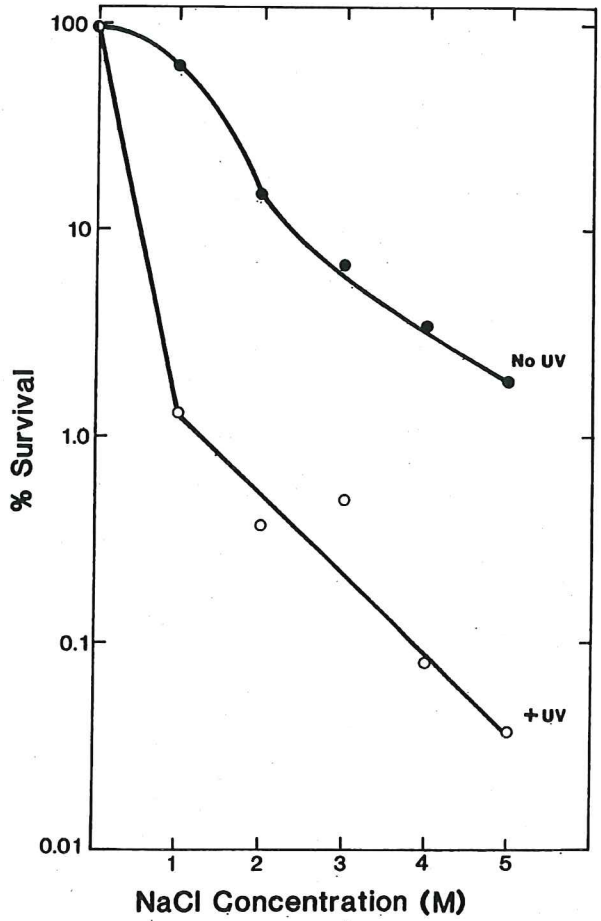

FIG. 6. Sensitization to killing by high salt after NUV exposure. TA4187 was irradiated with NUV for 4 h and then exposed to the indicated concentration of NaCl. Viability was corrected for killing by NUV.

defense regulon *oxyR* in protection from this toxicity. Strains which are unable to induce the *oxyR* regulon (Δ*oxyR*) are hypersensitive to oxidative stress (4). We have shown that Δ*oxyR* is extremely hypersensitive to NUV killing; the cells are killed much more rapidly and to a greater extent than wild-type cells are. Oxidative defense pathways are thus crucial in protecting against NUV damage.

Other endogenous photosensitizers likely to be important in NUV toxicity include cytochromes (3). Cytochromes are part of the membrane-bound electron transport chain and may thus serve to target NUV damage to membranes (43). Membrane damage has been reported to be involved in NUV toxicity (20, 21, 28). We have also observed that cells became sensitive to killing by high salt levels after NUV treatment, which is consistent with this involvement of membrane damage in NUV toxicity. The formation of active oxygen species in close proximity to membranes could lead to the formation of lipid hydroperoxides (21). *S. typhimurium* contains an enzyme, alkyl hydroperoxide reductase, which can break down such lipid hydroperoxides in vitro (4; F. S. Jacobson, R. W. Morgan, M. F. Christman, and B. N. Ames, manuscript in preparation). We have shown that a strain which lacks this enzyme is more sensitive to NUV killing. Thus, lipid hydroperoxides may be important photoproducts.

Glutathione has been reported to be involved in protecting cells from oxidative stress (33), which is consistent with the observation that glutathione reductase is an *oxyR*-regulated protein (4). However, glutathione does not appear to be necessary to protect cells against general oxidative stress such as H_2O_2 (13) (23a), although mutants lacking glutathione are hypersensitive to the sulfhydryl reagent *N*-ethylmaleimide (13). The glutathione-glutathione reductase system may function specifically to protect sulfhydryl groups from oxidation. We have shown that a *gsh* mutant is more sensitive to NUV stress, indicating that another target of NUV toxicity may be the oxidation of sulfhydryl groups.

Alkyl hydroperoxide reductase and glutathione reductase may not be the only *oxyR* proteins involved in protection from NUV damage, as the Δ*ahp* and *gsh* mutants are not nearly as photosensitive as is the Δ*oxyR* mutant. Other *oxyR* proteins are probably involved in protecting other potential damage sites. This damage may fall into two general categories: direct damage to NUV-absorbing macromolecules, such as NAD, flavins, and heme-containing proteins, and secondary damage to non-NUV-absorbing molecules mediated by active oxygen species. The formation of single-strand breaks has been implicated in NUV toxicity (37). Since DNA does not absorb NUV, such damage may be a result of radical formation. We have shown that catalase is rapidly inactivated by NUV, and inactivation of ribonucleotide reductase by NUV has also been reported (34). The NUV toxicity seen in our experiments may thus be a result of accumulation of damage at a variety of sites. The progressive sensitization of cells to H_2O_2 killing by NUV exposure is consistent with this hypothesis. Cells which have been exposed to NUV for 3 h were not dying, but were more sensitive to H_2O_2 killing (20 mM, 10-min exposure) by 3 orders of magnitude compared with untreated cells. The NUV-treated cells appeared to have accumulated damage which is not toxic itself but which sensitized them to further stress. Killing caused by a low fluence rate of NUV thus may be a complex process which involves accumulation of damage by both direct and indirect mechanisms until a point is reached where enough damage has accumulated to kill the cells.

H_2O_2 has been reported to be an important photoproduct in NUV toxicity (7, 38–40, 46). Our data, however, show that H_2O_2 itself does not mediate the toxic effects of broadband NUV at a low fluence rate, on cells. This conclusion is based on the following observations. (i) NUV treatment did not induce H_2O_2 resistance. (ii) Treatment of cells with catalase after NUV irradiation had no effect on killing by NUV (data not shown). (iii) We have measured H_2O_2 levels in cells during irradiation and have seen no H_2O_2 accumulation above a concentration of 1 μM, even at times when cell death was occurring. (iv) A strain with a deletion in the *katG* gene was not sensitive to killing by NUV. The catalase encoded by the *katG* gene is the one induced by H_2O_2 (24) and would thus be important in protecting the cells from NUV damage if H_2O_2 were a toxic agent. There are several possible reasons for the differences between our results and those reported previously. Tyrrell used a high-intensity monochromatic light in his experiments (46). Other differences could be due to experimental design. In the experiments of Sammartano et al., the cells were placed on ice (37, 38, 40) and transferred from rich to minimal media (37) before beginning NUV exposure. Thus, the effects measured

were the result of the simultaneous subjection of cells to two or three different stresses: nutrient downshift (stringent response), cold shock, and NUV irradiation. These procedures led to results (40) which are also at variance with the work of others (17) on the H_2O_2 sensitivity of *recA* mutants. It is thus unclear whether the results of such experiments are relevant to the physiological response of cells to NUV since both cold shock (P. G. Jones, R. A. VanBogelen, and F. C. Neidhardt. Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, H-47, p. 135) and nutrient downshift (29) would involve global changes in cellular regulation.

Our results show that H_2O_2 itself is not a damaging agent in NUV toxicity, but do not rule out its involvement in the formation of more toxic oxygen species. The addition of sublethal amounts of H_2O_2 during NUV irradiation has been shown to increase NUV killing (15, 16). This synergism is thought to be the result of superoxide anion formation by NUV irradiation of H_2O_2 (1). The superoxide anion can exert deleterious effects in biological systems and could further react with H_2O_2 to form the more reactive hydroxyl radical (9). Thus, if small amounts of H_2O_2 were formed during NUV exposure, the H_2O_2 could be harmful to the cell through superoxide anion and hydroxyl radical production rather than being toxic itself. Such a process could contribute to the NUV toxicity measured in our experiments, but may be of minor importance since H_2O_2 levels remain very low.

Our results demonstrate the importance of using environmentally relevant exposure conditions for studying the physiological effects of NUV. Many studies on NUV toxicity have been done using a high fluence rate of monochromatic light. The use of such light sources may promote photoreactions which are not physiologically relevant, especially since monochromatic light can focus all of the energy present on one specific chromophore. Such effects may be at least partially responsible for the report that H_2O_2 is directly involved in NUV toxicity, whereas with a more physiologically relevant exposure, we see no H_2O_2 production. We have also found that S^4U, which has been found to sensitize cells to killing by monochromatic light (32, 42), actually functions to protect cells from killing by a low fluence rate of NUV (G. Kramer and B. Ames, unpublished results). This result is an example of an important physiological response to NUV which can be overlooked by the use of nonphysiological exposures. The study of environmentally relevant cellular responses to NUV should therefore involve experiments in which the light source is as close to physiological conditions as possible and in which NUV is the only stress involved.

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Review

Existing and potential applications of ultraviolet light in the food industry – a critical review

Thomas Bintsis,[1] Evanthia Litopoulou-Tzanetaki[2] and Richard K Robinson[1]*
[1]Department of Food Science and Technology, The University of Reading, Reading, Berkshire, UK
[2]Laboratory of Food Microbiology and Hygiene, Aristotelian University of Thessaloniki, 540 06 Thessaloniki, Greece

Abstract: Short-wave ultraviolet light (UVC, 254 nm) can reduce dramatically the microbial load in air or on hard surfaces free from food residues, and can eliminate pathogens from potable water filtered to remove organic residues and 'clumps' of bacteria. More recently, approval of the Food and Drug Administration (USA) has been sought for a system for the destruction of pathogenic bacteria in fruit juices using UVC, and the same approach could perhaps be applied to remove spoilage organisms from cider or wines. In contrast, long-wave UV light (UVA, >320 nm) has limited microbiocidal properties, and for practical applications its effectiveness has to be enhanced by the presence of photosensitive compounds (eg furocoumarins) that will diffuse into a microbial cell prior to irradiation. The penetration of UVA into water is better than that of UVC, and its bacteriocidal action in the presence of photosensitisers can be rapid. However, pure furocoumarins are expensive and their addition to foodstuffs might be questioned on safety grounds.

Keywords: ultraviolet light; microbiocidal action; furocoumarins; water and fruit juice treatments

INTRODUCTION

Food safety is one of the most important issues facing the food manufacturing and service industries, for as consumers demand an increasing variety of ready-to-eat meals or dishes on a menu, so the risk of microbial contamination of an ingredient or the finished meal increases. The application of HACCP (hazard analysis critical control point) systems, heat treatments and efficient cold chains helps to reduce the opportunities for pathogenic micro-organisms to gain access to a food and/or grow to levels that will pose a risk from infection or toxin production, but, even so, the number of incidents of food-borne disease continues to rise in most industrialised countries.

Reversing this trend will not be easy, and yet many restaurants serve hundreds of meals per day without incident, and many food factories have equally commendable records with respect to hygiene. Obviously there may be many reasons why a particular company exposes the consumer, on occasions, to microbiologically unsafe products, but any procedure that could help to improve the situation must be welcome. One such procedure could involve the irradiation of food contact surfaces, rinsing water for food or process plant or air over a food preparation area with short-wave ultraviolet (UV) light, for the equipment is relatively inexpensive, the technique is, subject to certain safety precautions, easy to use and the radiation is lethal to most types of micro-organism. Whether the technique could or should be more widely applied in food preparation or production areas is a matter for speculation, as are the possible beneficial roles of long-wave UV light. Consequently, the aim of this present review is to consider some of the current applications of UV radiation in the food industry, and attempt to assess whether the microbiocidal effects of UV should be exploited further.

NATURE OF UV RADIATION

Ultraviolet (UV) light occupies a wide band of wavelengths in the non-ionising region of the electromagnetic spectrum between X-rays (200 nm) and visible light (400 nm). For practical purposes the UV spectrum can be subdivided into three regions:

- short-wave UV (UVC) with wavelengths from 200 to 280 nm;
- medium-wave UV (UVB) with wavelengths from 280 to 320 nm;
- long-wave UV (UVA) with wavelengths from 320 to 400 nm.

The intensity of UV radiation is expressed as irradiance or intensity flux ($W m^{-2}$), while the dose,

* Correspondence to: Richard K Robinson, Department of Food Science and Technology, The University of Reading, Reading, Berkshire, UK
E-mail: r.k.robinson@afnovell.reading.ac.uk
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which is a function of the intensity and time of exposure, is expressed as radiant exposure (Jm^{-2}).[1]

SOURCES OF UV RADIATION

Solar radiation

The sun emits radiation across a wide range of wavelengths, but the relative intensities of ultraviolet radiation reaching the earth's surface depend, to a considerable extent, on attenuation by the atmosphere through absorption and scattering. UVC is completely absorbed in the upper and middle atmospheres by ozone and molecular oxygen, but, while UVB is similarly attenuated, some UVB does reach the surface—much to the delight of sunbathers! However, UVA is barely affected, and hence the terrestrial environment is exposed mainly to ultraviolet radiation between 290 and 400 nm.[2] The intensity flux of UVA is about 35–50 Wm^{-2} at sea level,[3] and under these conditions a dose of 200 kJm^{-2} will be delivered over about 1 h of exposure. As a consequence, potentially lethal photoproducts can be formed at a considerable rate, and life in the open air would not be possible without the action of repair processes that ensure a drastic reduction in the damage caused by UVA.[4]

Artificial sources

Long-wave UV lamps

The light from mercury vapour lamps can be filtered to remove the visible spectrum and give an emission that is primarily UVA.[5]

Medium-wave UV lamps

Mercury vapour lamps are sometimes designed with pressures that produce maximum radiation in the UVB region, and using glass bulbs that freely transmit this energy.

Short-wave UV lamps

Mercury lamps designed to produce energy in the germicidal region (254 nm) are electrically identical to fluorescent lamps, but they lack the phosphor coating, and the use of glass allows the transmission of UVC. It should be noted that radiation below 260 nm will produce ozone which has to be monitored to prevent a hazard to health; a working atmosphere should not contain more than 0.2 mgl^{-1} of air.

SHORT-WAVE UV RADIATION (UVC)

Impact on living cells

UV radiation in the range of 250–260 nm is lethal to most micro-organisms, including bacteria, viruses, protozoa, mycelial fungi, yeasts and algae. The relationship between germicidal effect and wavelength is illustrated in Fig 1, which shows the maximum effect at 254 nm and a fall to practically zero at 320 nm; in fact, the effectiveness at 320 nm is 0.4% of the peak value.

The damage inflicted by UVC probably involves

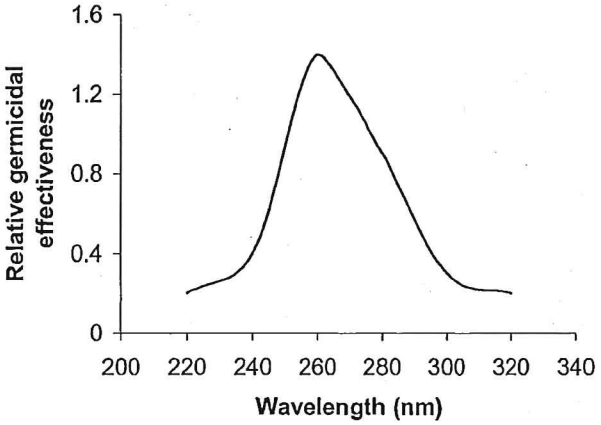

Figure 1. Direct lethality of UV wavelengths (after Ref 6).

specific target molecules,[7] and a dose in the range from 0.5 to 20 Jm^{-2} leads to lethality by directly altering microbial DNA through dimer formation. The main types of photoproduct in UV-irradiated DNA are cyclobutyl-type dimers (pyrimidine dimers), pyrimidine adducts and DNA–protein cross-links.[4] Purines are approximately 10-fold more resistant to photochemical alteration than are the pyrimidines, and because of this difference in sensitivity it has been implied that the photochemistry of the purines is not important biologically; by the time a significant amount of purine damage has occurred, the cells would have been inactivated by pyrimidine damage anyway.[8]

Once the DNA has been damaged, the micro-organisms can no longer reproduce and the risk of disease arising from them is eliminated. Temperatures between 5 and 37 °C have little, if any, influence on the microbiocidal action of radiation,[9] but moisture exerts a very marked effect. Where bacteria are suspended in air, an increase in relative humidity results in a greatly reduced death rate, especially at humidities greater than about 50%. Similarly, bacteria suspended in a liquid medium are much more resistant than those suspended in air, even after making allowance for the absorption of the medium.

Practical applications

The applications of the germicidal effects of UV fall into three broad categories: (a) inhibition of micro-organisms on surfaces; (b) destruction of micro-organisms in air; (c) sterilisation of liquids.

Disinfection of surfaces

The first category under this heading includes the sterilisation of packaging materials, eg containers, wrappers or bottle caps, by arranging appropriate lamps over conveyors. The success of this application depends on the material surfaces being clean and free from any dirt which would absorb the radiation and hence protect the bacteria.

During the manufacture of aseptically filled UHT

dairy products, for example, UV sterilisation has been applied to the foil caps of HDPE bottles[10] and to cartons for liquid products.[11] Similarly, the Hamba BK10010/10 has been used for aseptic yoghurt filling, and all the packaging materials, eg plastic cups and aluminium foil lids, are sterilised using UVC lamps working at 100–200 mW cm^{-2};[12] the shelf-life of fruit yoghurt packaged in containers sterilised by UVC lamps was extended by about 2 weeks at 5–7 °C. The disinfection of working surfaces in food preparation areas could merit serious assessment as well, but the limiting factor could be the presence of irregularities which would protect bacteria from incident UV.

Short-wave UV can also be employed to treat the surface of an actual food. For example, it has been used to control food spoilage micro-organisms such as *Bacillus stearothermophilus* in thin layers of sugar[13] or *Pseudomonas* spp on the surface of meat.[14] However, meat that has been exposed directly to UV light sometimes develops off-flavours, and a similar problem has been encountered with milk. It has been suggested that these undesirable flavours arise owing to absorption of ozone and oxides of nitrogen, as well as to direct photochemical effects on the lipid fractions of milk or meat. These latter effects can be reduced by filtering-out the shorter wavelengths or covering the product with a layer of inert gas prior to irradiation,[9] but in any event there appears to be no evidence that any of the photoproducts are harmful to humans.

Fresh fish is another product with a superficial flora of *Pseudomonas* spp, and Huang and Toledo[15] demonstrated the effectiveness of reducing initial bacterial counts, using UVC irradiation, in prolonging the storage life of fish. Kuo *et al*[16] showed that UVC radiation is effective in reducing the total aerobic and mould counts, along with *Salmonella typhimurium*, on the surfaces of egg shells; this latter treatment may be of little practical use, as the more important pathogen with respect to hen's eggs, ie *Salmonella enteritidis*, would be inside the egg and protected by the shell. In the baking industry, contamination of fresh products with mould spores has always been a problem, but, with bread, irradiation of the loaves as they emerge from the oven is reported to extend significantly their shelf-lives.[6]

A combination of UVC radiation and heat has been suggested by Tanaka and Kawaguchi[17] for the production of high-quality raw meat. More specifically, the same authors envisaged that: (a) retail portions of meat could be vacuum-packed using a membrane that transmits UVC; (b) the surface of the meat would be sterilised with UVC; (c) the membrane would then be heat-shrunk using water at temperature sufficient to kill any bacteria that had survived the UV treatment; and (d) the meat would then be cooled rapidly to maintain quality.

Given the growing demand for 'organic' foods, the potential use of UVC as an alternative to fungicides for the control of post-harvest diseases of stored vegetables such as carrots has attracted attention.[18] For example, a pre-storage treatment of carrots with UVC induces the accumulation of the phytoalexin 6-methoxymellein (an isocoumarin), and this change increases tissue resistance to fungal pathogens.

Disinfection of air

In hospitals, UVC lamps have been used to create a curtain or barrier of radiation through which air must pass before reaching patients sensitive to infection, and UV radiation at 254 nm and 0.25 W m^{-2} has been used in the United States since the 1930s to decrease the number of air-borne bacteria in operating theatres.

For the handling of sensitive foodstuffs, a system which combines a laminar flow of air through filters to remove particles of size > 0.1 μm, and the use of UV radiation to kill any live micro-organisms that remain, has been suggested for the provision of clean sterilised air in the workplace.[19] Similarly, the microbiological quality of mechanically peeled fruit and vegetables is improved when UV-treated air is blown through the peeling unit counter-current to the flow of product.[20] The microbiological quality of air in cold stores can also be improved using an air sterilisation unit,[21] and the same technique has been applied to the air in egg hatching cabinets.[22]

Disinfection of liquids

Treatment with UVC is one of the simplest and most environmentally friendly ways of destroying a wide range of micro-organisms in water.[23,24] It has been used to disinfect sewage effluent, drinking water and water for swimming pools, and the combination of UV and ozone has a very powerful oxidising action which can reduce the organic content of water to extremely low levels.[2]

As UVC disinfects without any change in colour, flavour, odour or pH, it is an effective means of ensuring that drinking water is microbiologically safe;[25] the normal performance criterion is based on a 99.999% reduction of micro-organisms with a treatment time of < 1 min. The major limitations on the effectiveness of UVC radiation in this context are the following.

- Lack of penetration: in distilled water, UV radiation at 254 nm will have suffered a 30% loss in intensity 40 cm below the surface, while sea water will cause the same reduction over ~10 cm; a solution of sucrose (10%) or a natural spring water containing high levels of iron will cause the same loss within 5 cm.[25]
- In natural water supplies, any suspended solids must be filtered-out prior to treatment, and occasional 'clumps' of bacteria can pose a similar problem, ie the outer cells protect the more deep-seated ones. However, as small-scale water filtration units have become more efficient, so the ability of UVC systems to generate safe, potable water supplies has improved. In Japan, UVC radiation has been used for disinfection of natural mineral water with

no effect on the mineral content of the water or generation of taints or off-flavours;[26] the eradication of *Enterococcus faecalis* was the essential aim of the latter process.

In some food processing industries the situation may be rather different, and a simple reduction in the microbial load in a water supply may be more than adequate. For example, in the brewing industry a treatment that does not alter the taste or quality of the end-product is essential, and a number of breweries have become major users of UV disinfection systems.[27–29] Dosages in brewery water treatment can be quite high to ensure the absence of any spoilage problems during the early stages of the brewing process, and doses range from 300 to 600 Jm^{-2} compared with 200 to 300 Jm^{-2} for the treatment of potable water.

Similar applications tend to be limited by the lack of penetration of UVC into liquids containing organic matter, but the disinfection of the brine used to salt Mozzarella cheese has been proposed,[30] thus enabling spent brine to be reused rather than replaced. The brine has to be filtered to remove any cheese residues before treatment, and, after replenishing the level of NaCl, it is pumped back to the storage vats containing the cheese.

The treatment of more opaque liquids is clearly a problem, and yet Lodi *et al*[31] used UVC to reduce the total colony counts in samples of caprine milk by 50–60%, along with a specific fall in coliform counts of 80–90%. However, while these latter reductions could be valuable to prevent on-farm spoilage of milks with high bacterial counts, the presence of 10% of the original coliform populations would still render the milk unacceptable for human consumption. Whether or not the process could be made sufficiently reliable to replace pasteurisation for milk is an open question, but it may be relevant that, at one time, milk in Germany and North America was treated with UVC to enhance the concentration of vitamin D.[32] The most successful system was the Lembke plant in which turbulent flow was achieved by pumping milk at high velocity through transparent tubes of 1 cm diameter, and, as 80% of the UV radiation reached the milk, it was found that over 99% of bacteria initially present in the milk could be destroyed.[32] It is worth noting though that the keeping quality of such milk was worse than that of heat-pasteurised milk, even though the latter had a higher microbial count, and this anomaly was ascribed to the selective survival of coliforms.

More recently, it has been reported that the Food and Drug Administration (USA) is considering allowing UVC to be used to eliminate pathogens from fruit juices.[33] The alleged success of the system depends on ensuring that the flow of the juice is turbulent rather than laminar, holding the temperature of the juice below 5 °C and applying a rigorous HACCP programme. It is suggested also that this 'light-processed' juice retains its levels of vitamins A, B, C and E, and other processors of liquid products may well monitor this development with interest. However, as unpasteurised fruit juice has been recorded as a source of infection from *Escherichia coli* O157,[33] the comments of Burton[32] about the ability of coliforms to survive UCV treatments could be pertinent, as could earlier reports on the treatment of cider[34] and maple syrup.[35] In both cases the authors recorded reductions in microbial counts following UVC treatments, but no attempt appears to have been made to identify which genera survived.

One final barrier to the use of UVC for destroying pathogens in liquids appears to be the absence of any test to confirm that a specified treatment has taken place. Thus pasteurised milk can be subjected to the classic alkaline phosphatase test,[36] other heat treatments below 100 °C can be monitored by the acid phosphatase test,[37] but how can a Public Health Authority check a UVC-treated product? The author of Ref 33 suggests that records of product flow rates and UV emissions should be sufficient to ensure product integrity, but then a chart from a temperature recorder does not confirm that raw milk is not contaminating pasteurised product through a damaged gasket.

LONG-WAVE UV RADIATION (UVA)

Impact on living cells

As UVA is poorly absorbed by living cells compared with UVC, little attention has been paid to any potential biocidal role. However, remedies of sunlight and herbs have been used for thousands of years to treat dermatological conditions such as psoriasis, a practice that confirms that some penetration of the skin cells does occur. Similarly, UVA does affect microbial cells, but it is far less effective than UVC. For example, the incident energy required to bring about a 50% reduction in microbial counts was 5 Jm^{-2} using UVA, whereas UVC achieved the same impact with only 10^{-5} Jm^{-2}.[38] Nevertheless, given that UVA is much safer for an operator to use than UVC, eg less risk of damage to the eyes if protective goggles are defective, interest in the sterilising effect of UVA has recently been revived.

The mode of action of UVA within cells is significantly different from that of UVC,[39] and the most likely effect(s) of UVA on micro-organisms are through:

(a) membrane damage—unsaturated fatty acids are readily oxidised to hydroperoxides, thus inducing changes in membrane permeability;[40]
(b) an oxygen-dependent reaction involving endogenous photosensitizing pigments—this mechanism involves the absorption of light by chromophores, resulting in their excitation, followed by reaction with oxygen to form active oxygen species or H_2O_2 which may be the primary agents of cell damage;[3,7,40] the latter compound has been impli-

cated as H_2O_2 pretreatment of cells of *E coli* induced resistance to UVA, probably because a repair system specific to oxidative damage was induced.[41]

A large number of compounds commonly present in micro-organisms have been suggested as possible endogenous target molecules, but the low lethality of UVA against micro-organisms means that it has little practical value unless the rate of kill can be enhanced by means of exogenous photosensitisers absorbed into the cell.[42] One group of compounds that meet this requirement are the tricyclic furocoumarins (see Fig 2), which are formed by the fusion of a furan ring with a coumarin molecule.[44] In general, Gram-negative bacteria are more resistant to hydrophobic antimicrobial substances (eg furocoumarins) than are Gram-positive species, principally because the outer cell membrane of the former contains lipopolysaccharides which can delay, or perhaps prevent, the entry of hydrophobic molecules into the cell.[45,46] Consequently, it may be that the hydrophobic furocoumarins are largely retained in the outer cell membrane of Gram-negative bacteria, and cannot diffuse into the cell to react with the DNA.[47] In addition, since the effectiveness of furocoumarins as antimicrobial agents depends on contact with the DNA, their distribution within a cell and interactions with other components (ie proteins) may also influence their antimicrobial activity.[48]

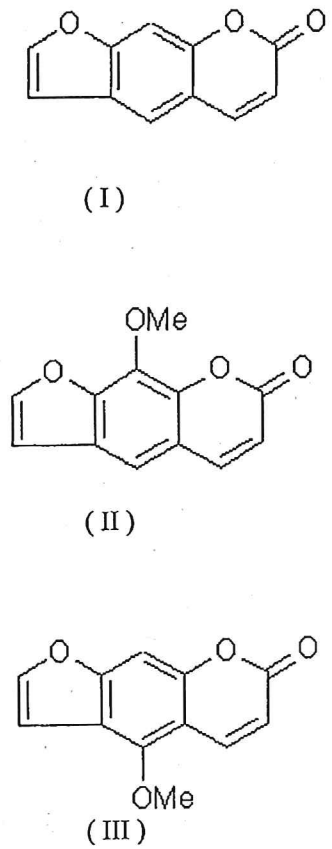

Figure 2. Chemical structure of some linear furocoumarins: (I) psoralen; (II) bergapten; (III) xanthotoxin (after Ref 43).

Potential use of the UVA/furocoumarin system

Antimicrobial activity

The furocoumarins are best known for their use in medicine, and a combination treatment involving 8-methoxypsoralen and sunlight/UVA radiation has found success in the control of psoriasis.[49] In a different context, Lin *et al*[50] employed $5\,\mu g\,ml^{-1}$ of 8-methoxypsoralen with UVA to kill bacteria in human platelet concentrates required for transfusions.

Unlike the situation where UVA stimulates endogenous target molecules, the activated furocoumarins form cross-links between complimentary strands of DNA, so preventing the strands from replicating.[43] In addition, UVA plus furcoumarin produces DNA monadducts which damage both eucaryotic and bacterial cells, but the relative lethal impacts of cross-link formation or monoadduct action may vary.[51] The amount of furocoumarin needed to stimulate this reaction is very small, and, in a model food system under UVA illumination, Ulate-Rodriguez *et al*[52] tested the antimicrobial properties of linear furocoumarins at levels of $2–53\,\mu g\,ml^{-1}$ against *Listeria monocytogenes*, *E coli* O157:H7 and *Micrococcus luteus*. *L monocytogenes* was inhibited, but *E coli* O157:H7 and *M luteus* were found to be more resistant; considerable variation in sensitivity has been found even with a single species.[6] More recently, Bintsis (unpublished) found that *L innocua*, *E coli* and *Staphylococcus aureus* suspended in tubes of Maximum Recovery Diluent (MRD Code No CM733, Unipath Ltd, Basingstoke, Hants, UK) (5.0×10^6 colony-forming units (cfu) in 10 ml) were inactivated rapidly by UVA and psoralen (see Table 1), whereas *Yarrowia lipolytica* and *Debaryomyces hansenii* (5.0×10^5 cfu in 10 ml) were slightly more resistant.

These figures confirm that the UVA/furocoumarin system can have a dramatic microbiocidal impact. However, it was recorded in a separate trial (no micro-organisms present) that the loss of irradiance through

Table 1. Inactivation of different types of micro-organism following a 60 s treatment with UVA[a]/psoralen ($5\,\mu g\,ml^{-1}$)

Micro-organism	*% killing*
Listeria innocua	99.8
Escherichia coli O157:H7	99
Staphylococcus aureus	99.9
Debaryomyces hansenii	97.5
Yarrowia lipolytica	82.7

[a]The experiment was performed with a Black-Ray Display Lamp (XX-15BLB) (Ultraviolet Products, Cambridge, UK) with the surface of the liquid at a distance of 10 cm from the lamp. At 10 cm the intensity was $45\,W\,m^{-2}$.

MRD was 34% over 5 cm, while in a simulated cheese brine including casamino acids (1.0%), lactose (0.5%) and NaCl (6.0%) the loss was approximately 95% over 5 cm. As neither tap water nor sucrose solutions (<10%) caused any loss of irradiance under the same conditions, it may be the peptides and amino acids in the MRD and brine that absorbed the radiation. For practical applications this point needs further study, because it has been reported elsewhere that UVA has the advantage over UVC in that amino acids do not absorb UV at wavelengths >300 nm.[4]

Potential applications in food processing

Natural furocoumarins have been isolated from five plant families, namely the *Umbelliferae* (eg celery, parsley and parsnip), *Rutaceae* (eg bergamot fruit and lime), *Moraceae* (eg fig), *Leguminosae* and *Orchidaceae*. Typical concentrations of furocoumarins are shown in Table 2, and, while psoralen is the most photoreactive, it is likely that initial addition rates could be calculated on the basis of total furocoumarins; the wide variation in concentrations within the same species is a reflection of differences between cultivars, season/location of collection and method(s) of analysis. However, it should be borne in mind that if the UVA/furocoumarin system was to be used rather than UVC to sanitise a cheese brine, for example, each litre of cheese brine would have to be dosed with a minimum of 5000 μg of furocoumarin prior to irradiation, so that some of the concentrations in parsley or celery are clearly too low to be of practical or economic value. Furthermore, a number of studies have highlighted the fact that handlers of celery are prone to light-induced dermatitis due to UVA/furocoumarin interactions.[63–65] Consequently, although it might be attractive from a marketing standpoint to employ natural plant materials as a source of furocoumarins, the practical hurdles may prove insurmountable.

Nevertheless, it remains feasible to suggest that a combination of UVA and photosensitisers could be used to increase the shelf-life of perishable products, with the furocoumarins being incorporated, perhaps, into the packaging materials. If these same compounds then diffused into microbial contaminants on the surface of a product, they could be sensitised by natural light.[66] The direct addition of furocoumarins to foods could be a further option, but on the negative side it is important to highlight the facts that: (a) pure furocoumarins such as psoralen are expensive; and (b) although in the treatment of psoriasis the daily dose of 8-methoxypsoralen is about 20 mg, there is a recognised toxicological risk to the patient.[67] In particular, the ingestion of natural furocoumarins has been linked with the onset and/or development of cutaneous carcinomas,[68–70] and hence this risk alone will prevent their commercial exploitation within the food context.

If the phototoxic side-effects could be eliminated, then the UVA/furocoumarin system might be worth further evaluation, and it could be relevant that a number of synthetic furocoumarins are available that have the same therapeutic activity as 8-methoxypsoralen but, at least in mice, induce no toxic or carcinogenic reactions.[68,71,72] At present, the cost of such compounds would prohibit their use as components of any food preservation system.

Practical applications of UVA alone

Detection of chemical residues

The principal application of UVA within the food industry has been in relation to quality control, particularly for the detection of aflatoxins from *Aspergillus flavus* or *Aspergillus parasiticus* on various grains and nuts, eg maize, cottonseeds or peanuts, during storage. The aflatoxins have absorption maxima around 360 nm, and while aflatoxin B fluoresces blue at 425 nm, aflatoxin G produces a green–blue emission at 450 nm; these reactions can be employed to detect low levels of aflatoxin.[73] UVA is also reported to degrade aflatoxin M_1 in milk.[74,75]

In some stores and shops it may be necessary to check for the presence of rodents, and while dry rodent urine (fresh) glows blue–white under UVA, older deposits give a yellow–white glow. Rodent hairs also glow blue–white and are easily identified on sacks or intermixed with food grains.[76]

In the dairy industry, fresh deposits of milk-stone—a long-enduring problem—will fluoresce a strong yellow–white/bright blue–white under UVA.[77]

Detection of micro-organisms

The rapid identification of coliform bacteria in water is essential to ensure that public drinking water is safe,

Table 2. Some reported furocoumarin contents in various plants of the *Umbelliferae* and *Rutaceae;* all figures as $\mu g\,g^{-1}$ on a fresh or dry weight basis (see footnotes)

Plant	*Psoralen*[c]	*Xanthotoxin*[c]	*Bergapten*[c]	*Total linear furocoumarins*	*Reference*
Celery[a]	0.01–4.18	0.08–16.86	0.46–28.51	0.56–49.84	3–57
Lime peel[b]	14±2	42±6	1406±18		52
Parsley[b]	32.3–104.7	5.3–53	56.7–479.2	94.3–541.5	47, 58 52
Parsnip[a]	0.01–10.5	170–682	213–430		58–61
Angelica[a]		427.3	3477.0		59
Heracleum[a]	6.1–6.5	140–150	64–68	220±9	62

[a] Concentration expressed on a fresh weight basis.
[b] Concentration expressed on a dry weight basis.
[c] See Fig 2 for the chemical structure.

and the auto-analysis test is performed in test tubes pre-filled with a powdered, coliform-specific indicator nutrient.[76] After incubation at 35°C for 24h, any indicator-positive tube is illuminated with UVA, and fluorescence of the solution indicates the presence of *E coli* and hence a risk of faecal contamination. The bacterium *Pseudomonas aeruginosa* which causes rots in eggs, meat and fish can also be detected by its yellow–green fluorescence under UVA radiation.[76]

In another application a redox dye, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), has been employed for the direct epifluorescent microscopic enumeration of live bacteria in environmental samples.[78] The CTC competes directly with molecular oxygen as an electron acceptor, and the reducing power generated by the electron transport system converts CTC into its reduced formazan, which accumulates in metabolically active bacteria. When illuminated with long-wave UV (>350nm), the reduced CTC fluoresces bright red and is easily detected. However, the application of this technique to foods needs to be carefully assessed, as some foods may contain significant levels of natural or artificial quenchers.

In order to reduce the risk of microbial contamination from flying insects, much use is made of traps in which a UVA fluorescent lamp is mounted in a unit containing a high-voltage grid. The insect, attracted by the UVA lamp, flies into the unit and is electrocuted in the air gap between the high-voltage grid and a grounded metal screen. Such units are commonly found in areas where food is prepared and/or sold.[2]

CONCLUSIONS

While the UVA/furocoumarin system has a superficial attraction for sanitising solutions, ie better penetration of the radiation, it has yet to find a commercial niche. By contrast, UVC enjoys a good reputation for sanitising the air or food contact surfaces, and it seems likely that its use will expand as the supporting technology improves. For example, safe drinking water can assured by exposure to UVC systems so long as the associated filtration system is capable of removing all particulates, and recent advances in North America suggest that fluids containing suspended solids can be treated as well. The security offered by this latter system remains under scrutiny, for it is not clear at present whether the radiation levels would be effective if a sample of fruit juice, for example, was contaminated with *E coli* or some other pathogen prior to treatment.

Nevertheless, it is evident that the food industry is faced with two conflicting pressures. On the one hand, there is the need to produce microbiologically 'safe' food, while on the other, consumers are seeking foods with more natural flavours and textures. Consequently, a resurgence of interest in UVC could well be appropriate, for it does seem that UV radiation is one of the least exploited antimicrobial treatments for surfaces and, perhaps, foods themselves.

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