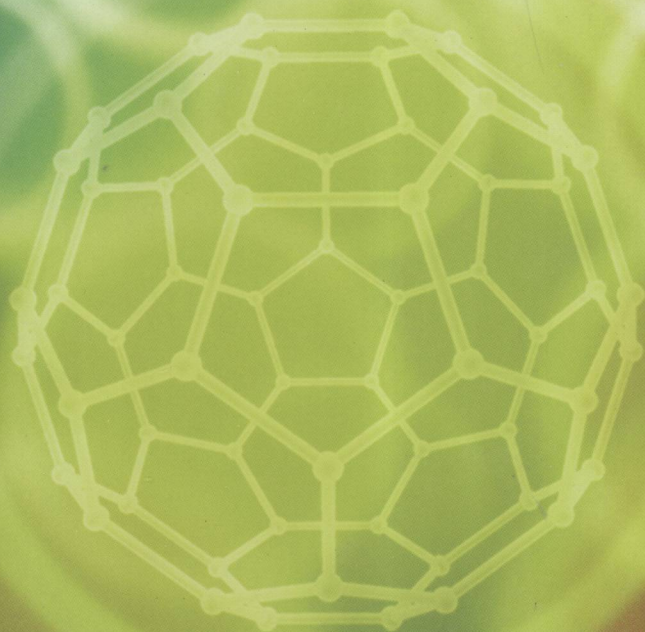
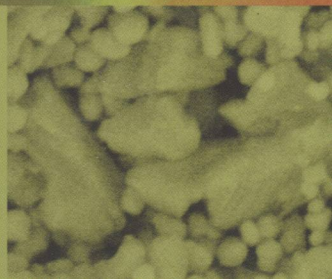


Editors Jamie R. Lead and Emma Smith

Environmental and Human Health Impacts of Nanotechnology



 WILEY



TB383-05
E61

Environmental and Human Health Impacts of Nanotechnology

Edited by

JAMIE R. LEAD

*School of Geography, Earth and Environmental Sciences,
University of Birmingham, UK*

EMMA SMITH

*Department of Biological and Chemical Sciences,
The University of the West Indies, Barbados*



E2010002288

 **WILEY**

A John Wiley and Sons, Ltd., Publication

This edition first published 2009
© 2009 Blackwell Publishing Ltd

Registered office

John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom.
For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com.

The right of the author to be identified as the author of this work has been asserted in accordance with the Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book.

This publication is designed to provide accurate and authoritative information in regard to the subject matter covered. It is sold on the understanding that the publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

The publisher and the author make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of fitness for a particular purpose. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for every situation. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. The fact that an organization or Website is referred to in this work as a citation and/or a potential source of further information does not mean that the author or the publisher endorses the information the organization or Website may provide or recommendations it may make. Further, readers should be aware that Internet Websites listed in this work may have changed or disappeared between when this work was written and when it is read. No warranty may be created or extended by any promotional statements for this work. Neither the publisher nor the author shall be liable for any damages arising herefrom.

Cover photo courtesy of Dr. Ralf Kaegi, Swiss Federal Institute of Aquatic Science and Technology (Eawag)

Library of Congress Cataloging-in-Publication Data

Environmental and human health impacts of nanotechnology / edited by Jamie R. Lead and Emma Smith.
p. cm.

Includes bibliographical references and index.

ISBN 978-1-4051-7634-7

1. Nanoparticles—Environmental aspects. 2. Nanoparticles—Toxicology. 3. Nanostructured materials—Environmental aspects. 4. Nanostructured materials—Health aspects. 5. Nanotechnology—Environmental aspects. 6. Nanotechnology—Health aspects. I. Lead, Jamie R. II. Smith, Emma (Emma L.)

TD196.N36E58 2009

620'.5—dc22

2009009688

ISBN 978-1-4051-7634-7 (H/B)

A catalogue record for this book is available from the British Library.

Set in 10 on 12 pt Times by SNP Best-set Typesetter Ltd., Hong Kong
Printed and bound in Great Britain by CPI Antony Rowe, Chippenham, Wiltshire

Environmental and Human Health Impacts of Nanotechnology

Preface

Manufactured nanoparticles (NPs) are usually defined as materials purposefully produced by human activity and which have at least one dimension between 1 and 100 nm. It is important to distinguish NPs by source; the main other NPs are incidental: that is produced indirectly by human activities including fossil fuel combustion, and natural: that is produced by processes such as chemical hydrolysis, weathering and microbial action. Other size-based definitions of NPs exist and there are a wide variety of material types which fall within this definition. Nanoscience, which is the science dealing with nanoscale materials, can be seen as simply a subset of traditional colloids science. Nevertheless, a large number of novel processes occur below this size due to effects such as exponential increases in specific surface area and surface energy, quantum effects such as quantum confinement (where wave functions are constrained by the small particle size) and under-coordination of bonds at the particle surface. Processes which occur in this size range are thus different in many ways to traditional colloid chemistry and, in general, the differences become more pronounced at smaller sizes.

The current interest in nanotechnology is due to these novel properties and their exploitation in industrial processes and consumer products. Huge and exponentially growing research and development funding from government and private sources has been spent to better develop and exploit these potential uses and NPs are now used widely. Silver NPs are currently used as bacteriocides in cosmetics, fabrics, medical and health-related products and elsewhere. Titanium dioxide NPs are used in sunscreens (along with zinc oxide) and self cleaning surfaces, where they have a photocatalytic effect on organic matter due to the production of reactive oxygen species (ROS) and because of this titania is also used as a bactericide. Cerium dioxide is widely used as an additive to diesel to improve fuel efficiency. A wide range of other materials such as carbon nanotubes, fullerenes, gold, iron, iron oxide and more exotic species are being developed and used.

The extent of the applications and the possibility of unusual and unknown 'nano' effects has led to concern about their environmental and human health effects in the scientific community and equal concern in industry and from regulators and policy makers. A major driver for this in some quarters is undoubtedly the example of genetically modified organisms. The extensive public backlash has made the future of that technology quite uncertain and there has been a different approach in nanotechnology to openness and acknowledgement of the risks and a commitment to reducing these risks. Public response to nanoscience and

nanotechnology is currently limited by a lack of knowledge and wider impact but is generally positive with benefits expected in health, energy and the environment to name a few. Nevertheless, it is quite feasible that this attitude will change, particularly in view of developments in next generation nanomaterials, including self-organisation and self-assembly and the increasingly researched interface between 'bio' and 'nano'.

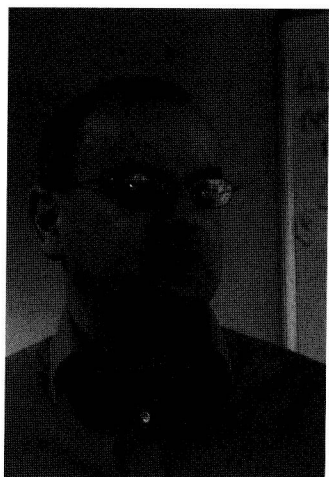
There are considerable benefits to be gained from the exploitation of nanoscience but current research tells us that there are indeed potential hazards in this area. It is incumbent on the relevant communities to ensure that NPs and other nanomaterials are used appropriately and designed and tested to be of minimal hazard and that exposure is not widespread; risk needs to be minimised and seen to be minimised to allow the full benefits of nanoscience and nanotechnology to be derived.

Understanding the behaviour and impacts of nanotechnology in the environment and in human health is a daunting task and many questions remain to be answered: how do we measure concentrations of NPs in complex biological and environmental media?; what are the concentrations in environmental media and in organisms?; what are the correct metrics of measurement (mass or number concentrations for instance)?; what are the sources to the environment and humans?; what are the environmental transport pathways and ultimate sinks of NPs?; are NPs bioavailable and are they subject to bioaccumulation and biomagnification?; how do NPs distribute in the sub-cellular, organ and body environments?; how are transport, bioavailability and effects related to NP physico-chemical structure? Although a substantial amount of research is being performed, the research spending on the risks of nanotechnology and the health and safety and environmental implications is still tiny in comparison to its development and exploitation. This balance is unlikely to change enormously but there are good arguments to say that this should happen and change should come quickly. The questions above and related questions remain unanswered in the main and the purpose of this volume is to collate and discuss our current knowledge and point to future areas of research which are required.

We would like to acknowledge and thank a number of people and institutions which made this book possible. The UK Natural Environment Research Council (NERC) provided funding via a Knowledge Transfer Network entitled Engineered nanoparticles in the natural aquatic environment (Nanonet), which enabled all authors and editors to convene for a two-day workshop to discuss the issues and finalise the chapters. We would like to thank the chapter authors for their efforts and their timely submissions, and the patience and help of the publishing team which was essential to the editors.

Jamie Lead
Emma Smith
March 2009

Biographies



Jamie Lead is Professor of Environmental Nanoscience in the School of Geography, Earth and Environmental Sciences, University of Birmingham, UK. Professor Lead completed his PhD at Lancaster University, UK, in 1994 after investigating lanthanide and actinide speciation in natural waters and soils. At the same institution he later undertook postdoctoral research on the impact of size of natural aquatic colloids on transition metal chemistry. In 1998, he undertook further postdoctoral work at Geneva University, Switzerland, developing and using fluorescence correlation spectroscopy to quantify diffusion coefficients of natural organic macromolecules. In 2000, he became a Lecturer at the University of Birmingham and became full Professor at Birmingham in 2008. Professor Lead is Director of the Facility for Environmental Nanoparticle

Analysis and Characterisation (FENAC), which is a national UK centre collaborating with the biological community investigating nanoparticle fate and effects. He has been a visiting researcher at CSIRO, Australia, and is a Fellow of the Royal Society of Chemistry, the International Union of Pure and Applied Chemistry and the Institute of Nanotechnology.

Professor Lead's main research interests, where he has published widely, relate to the relationships between chemistry, transport and bio-uptake of pollutants, especially in relation to the nanoscale in the environment. In particular, he is interested in the structure of natural 'nanocolloids' and the role this has in metal and manufactured nanoparticle chemistry, fate and behaviour. He is currently collaborating extensively with the ecotoxicological community by synthesising nanoparticles of silver, cerium, iron oxide and other materials and ensuring their full characterisation. These collaborations are particularly focussed on investigating mechanisms of nanoparticle biological uptake and effects



Dr Emma Smith is currently Lecturer in Environmental Chemistry at the University of the West Indies. She received a degree in Oceanography and Chemistry from the University of Liverpool and a Masters in Marine Resource Development and Protection with distinction from Heriot Watt University. Her PhD thesis, *Unresolved Complex Mixtures of Aromatic Hydrocarbons in the Marine Environment: Solubility, Toxicity and Photodegradation Studies*, was carried out at Plymouth University in conjunction with Plymouth Marine Laboratory and won the SETAC Young

Scientist Award in 2000 at World Congress in Brighton. Dr Smith then worked at Plymouth University on the characterisation of bioaccumulated and unidentified agent(s) causing reduced scope for growth in mussels and the potential ecological effects of chemically dispersed and biodegraded crude oils. She then worked at the University of Toronto within the Environmental NMR Centre, evaluating climatic controls on soil organic carbon composition and potential responses to global warming. Following this Dr Smith worked with Professor Lead at the University of Birmingham implementing the Nanonet project, a Knowledge Transfer (KT) Network in the area of manufactured nanomaterials (MNs) in the natural aquatic environment.

In her current position she is responsible for teaching environmental chemistry, oceanography and ecotoxicology at UWI and is working with the Caribbean Ecohealth Programme and an EU Outreach project on assessing the potential environmental and human health effects of pollution.

Contributors

Robert J. Aitken Institute of Occupational Medicine, Edinburgh, UK

Simon C. Apte Centre for Environmental Contaminants Research, CSIRO Land and Water, Bangor, Australia

Deborah M. Aruguete Department of Geosciences, Virginia Polytechnic Institute and State University, Blacksburg, USA

Mohamed Baalousha School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham, UK

Graeme E. Batley Centre for Environmental Contaminants Research, CSIRO Land and Water, Bangor, Australia

Aurélié Charron Transport and Environment Laboratory, INRETS – French National Institute for Transport and Safety Research, Bron, France

John W. Cherrie Institute of Occupational Medicine, Edinburgh, UK

Paul Christian School of Chemistry, University of Manchester, Manchester, UK

Martin J. D. Clift Institute for Anatomy, Division of Histology, University of Bern, Bern, Switzerland

Robert A. Dorey Microsystems & Nanotechnology Centre, Cranfield University, Cranfield, UK

Karen S. Galea Institute of Occupational Medicine, Edinburgh, UK

John F. Garrod Department for Environment, Food and Rural Affairs, London, UK

Richard D. Handy School of Biological Sciences, University of Plymouth, Plymouth, UK

Paul T. C. Harrison Institute of Environment and Health, Cranfield Health, Cranfield University, Cranfield, UK

Roy M. Harrison School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham, UK

Martin Hassellöv Department of Chemistry, University of Gothenburg, Gothenburg, Sweden

Michael F. Hochella, Jr Department of Geosciences, Virginia Polytechnic Institute and State University, Blacksburg, USA

Thilo Hofmann Department of Environmental Geosciences, Vienna University, Austria

Helinor Johnston Applied Research Centre for Health, Environment and Society, Edinburgh Napier University, Edinburgh, UK

Ralf Kaegi Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland

Jamie R. Lead School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham, UK

Leonard S. Levy Institute of Environment and Health, Cranfield Health, Cranfield University, Cranfield, UK

Juan Liu Chemical and Material Sciences Division, Pacific Northwest National Laboratory, Rickland, USA

Richard Owen School of Biosciences, University of Westminster, London, UK

Simon J. Pollard Collaborative Centre of Excellence in Understanding and Managing Natural and Environmental Risks, School of Applied Sciences, Cranfield University, Cranfield, UK

Sophie A. Rocks Collaborative Centre of Excellence in Understanding and Managing Natural and Environmental Risks, School of Applied Sciences, Cranfield University, Cranfield, UK

Nicola J. Rogers Centre for Environmental Contaminants Research, CSIRO Land and Water, Bangor, Australia

Vicki Stone Applied Research Centre for Health, Environment and Society,
Edinburgh Napier University, Edinburgh, UK

C. Lang Tran Institute of Occupational Medicine, Edinburgh, UK

Frank von der Kammer Department of Environmental Geosciences, Vienna
University, Austria

Contents

<i>Preface</i>	xiii
<i>Biographies</i>	xv
<i>Contributors</i>	xvii
1. Overview of Nanoscience in the Environment	1
<i>Mohamed Baalousha and Jamie R. Lead</i>	
1.1 Introduction	1
1.2 History	2
1.3 Definitions	3
1.4 Investment and International Efforts	6
1.5 Development: Four Anticipated Generations	6
1.6 Applications of Nanotechnology	7
1.7 Potential Benefits of Nanotechnology	8
1.7.1 Environmental	8
1.7.2 Human Health	9
1.8 Potential Adverse Effects of Nanomaterials	10
1.8.1 Environmental	10
1.8.2 Human Health	12
1.9 Classification	12
1.9.1 Chemistry	12
1.9.2 Origin	13
1.9.3 Size	13
1.9.4 State	14
1.10 Sources of Nanomaterials in the Environment	14
1.11 Properties of Nanomaterials	14
1.12 Nanomaterial Structure–Toxicity Relationship	15
1.13 Environmental Fate and Behaviour of Nanomaterials	16
1.13.1 Fate in Air	17
1.13.2 Fate in Water	17
1.13.3 Fate in Soil	19
1.14 Potential for Human Exposure	20
1.15 Detection and Characterization of Nanomaterials	21
1.16 Issues to be Addressed	21
1.16.1 Nomenclature	21

1.16.2	Future Development and Risk	23
1.16.3	Dosimetry	23
1.16.4	Methods of Detection and Characterization	23
1.16.5	Environmental Fate of Nanomaterials and their (Eco)Toxicology	23
1.17	Conclusion	24
1.18	References	24
2.	Nanomaterials: Properties, Preparation and Applications	31
	<i>Paul Christian</i>	
2.1	Overview	31
2.2	Introduction	32
2.3	Nanoparticle Architecture	35
2.3.1	Nanoparticle Surface	38
2.3.2	Charge Stabilisation	41
2.3.3	Steric Stabilisation	42
2.4	Particle Properties	45
2.4.1	Surface Plasmon Resonance	45
2.4.2	Catalysis	46
2.4.3	Quantum Confinement	47
2.4.4	Mechanical Performance	48
2.4.5	Magnetic Properties	49
2.4.6	Interfacial Properties	49
2.4.7	Other Properties	50
2.5	Nanoparticle Preparation	53
2.5.1	The Challenges of Nanoparticle Synthesis: Scale Up	53
2.5.2	Reactivity	53
2.5.3	Dispersability	53
2.5.4	Cost	54
2.5.5	Methods: Natural Sources	54
2.5.6	Top Down	55
2.5.7	Bottom Up	55
2.5.8	Metal Nanoparticles	59
2.5.9	Carbon	60
2.5.10	Graphene	62
2.5.11	Carbon Black	62
2.5.12	Inorganic Compounds	63
2.5.13	Polymers	64
2.6	Applications of Nanoparticles and Nanotechnology	65
2.6.1	The Past	65
2.6.2	The Present and Near Future	67
2.7	Implication for Environmental Issues	72
2.8	Conclusions	73
2.9	References	73

3. Size/Shape–Property Relationships of Non-Carbonaceous Inorganic Nanoparticles and Their Environmental Implications 79

Deborah M. Aruguete, Juan Liu and Michael F. Hochella, Jr

3.1	Introduction	79
3.2	Inorganic Nanoparticle Anatomy	80
3.3	Redox Chemistry of Nanoparticles	81
3.3.1	Photoredox Chemistry in Semiconductor Nanoparticles	81
3.3.2	Redox Chemistry in Other Nanoparticle Systems	84
3.4	Size Effects in Nanoparticle Sorption Processes	87
3.5	Nanoparticle Fate: Dissolution and Solid State Cation Movement	89
3.5.1	Basic Energetic and Kinetic Considerations of Nanoparticle Dissolution	89
3.5.2	Effects of Nanoparticle Morphology	91
3.5.3	Effects of Nanoparticle Coatings and External Substances	92
3.5.4	Case Study: The Dissolution of Lead Sulfide Nanoparticles	94
3.5.5	Solid State Cation Movement in Nanoparticles	96
3.6	Effect of Nanoparticle Aggregation on Physical and Chemical Properties	98
3.7	Environmental Implications: General Discussion, Recommendations and Outlook	99
3.8	References	101

4. Natural Colloids and Nanoparticles in Aquatic and Terrestrial Environments 109

Mohamed Baalousha, Jamie R. Lead, Frank von der Kammer and Thilo Hofmann

4.1	Introduction	109
4.2	Definition	112
4.3	Major Types of Environmental Colloids	112
4.3.1	Inorganic Colloids	114
4.3.2	Organic Macromolecules	117
4.4	Intrinsic Properties of Environmental Colloidal Particles	121
4.4.1	Size	121
4.4.2	Surface Charge	121
4.4.3	Surface Coating by Natural Organic Matter	123
4.4.4	Fractal Dimension	124
4.5	Interaction Forces Between Colloidal Particles	126
4.5.1	DLVO Theory	127
4.5.2	Stability Criteria	129
4.5.3	Aggregation Kinetics	129
4.5.4	Non-DLVO Interactions	130
4.6	Fate and Behaviour of Colloids in Aquatic Systems	136
4.6.1	Aggregation	136

4.6.2	Disaggregation: Effect of Natural Organic Matter	140
4.6.3	Sedimentation Behaviour	141
4.7	Fate and Behaviour of Colloids and Nanoparticles in Porous Media	141
4.7.1	Saturated Porous Media	143
4.7.2	Unsaturated Porous Media	146
4.8	Conclusion	147
4.9	References	147
5.	Atmospheric Nanoparticles	163
	<i>Aur�lie Charron and Roy M. Harrison</i>	
5.1	Introduction	163
5.2	Sources of Atmospheric Nanoparticles	164
5.2.1	Sources of Primary Nanoparticles	164
5.2.2	Secondary Sources	175
5.3	Chemical Composition of Atmospheric Nanoparticles	178
5.4	Fate and Behaviour of Atmospheric Nanoparticles	181
5.5	Atmospheric Concentrations	182
5.5.1	Spatial Variations	182
5.5.2	Temporal Variations	185
5.6	Measurement Methods for Atmospheric Nanoparticles	187
5.6.1	Particle Number Concentration	189
5.6.2	Surface Area	193
5.6.3	Mass Concentration	194
5.6.4	Chemical Composition	196
5.7	Conclusions	200
5.8	References	201
6.	Analysis and Characterization of Manufactured Nanoparticles in Aquatic Environments	211
	<i>Martin Hassell�v and Ralf Kaegi</i>	
6.1	Introduction	211
6.1.1	Nanoparticles in the Aquatic Environment	212
6.1.2	Concepts and Definitions Relating to Analysis and Characterization	212
6.2	Nanoparticle Analysis and Characterization Methods	214
6.2.1	Important Nanoparticle Characteristics	214
6.2.2	Sampling, NP Extraction, Sample Preparations	224
6.2.3	Light Scattering Methods	224
6.2.4	Other Electromagnetic Scattering Methods	229
6.2.5	Fractionation and Separation Methods	230
6.2.6	Microscopic Methods	237
6.2.7	Spectroscopic Methods	249
6.2.8	Surface Area Measurements with Nitrogen Gas Adsorption	251

6.2.9	Method Validation	251
6.3	Analytical Test Strategy in NP Exposure Assessment	252
6.3.1	Initial Material Characterization	252
6.3.2	Fate and Behaviour Assessment	252
6.3.3	Exposure Characterization in Effect Assessment Experiments	253
6.3.4	Monitoring Nanopollution	254
6.4	Conclusions	255
6.5	Acknowledgements	256
6.6	References	256
7.	Ecotoxicology of Manufactured Nanoparticles	267
	<i>Simon C. Apte, Nicola J. Rogers and Graeme E. Bailey</i>	
7.1	Introduction	267
7.2	Physico-Chemical Transformation of Nanoparticles	269
7.2.1	Particle Dispersion and Aggregation	270
7.2.2	Nanoparticle Dissolution	272
7.2.3	Oxidation	273
7.2.4	Adsorption Reactions	275
7.3	Mechanisms of Nanoparticle Toxicity in the Environment	275
7.3.1	Exposure Routes	275
7.3.2	Nanoparticle Interactions with Cells: Cellular Uptake	277
7.3.3	Toxicity Mechanisms	280
7.3.4	Bioaccumulation	283
7.4	Development of Valid/Realistic Toxicity Testing Protocols	284
7.5	Review of Ecotoxicity Studies	285
7.5.1	Overview	285
7.5.2	Carbon-Based Nanoparticles	289
7.5.3	Metal Oxides	293
7.5.4	Silver	296
7.5.5	Copper	298
7.5.6	Quantum Dots	298
7.5.7	Iron	299
7.6	General Conclusions and Future Directions	300
7.7	References	301
8.	Exposure to Nanoparticles	307
	<i>Robert J. Aitken, Karen S. Galea, C. Lang Tran and John W. Cherrie</i>	
8.1	Introduction	307
8.2	Physical Characteristics and Properties of Nanoparticles	309
8.2.1	Terminology and Definitions	309
8.2.2	Nanoparticle Types	311

8.2.3	Nanoparticle Production Processes	314
8.2.4	Nanoparticle Behaviour	316
8.3	Nanoparticle Exposure	319
8.3.1	Exposure Scenarios	319
8.3.2	Exposure Metrics	327
8.3.3	Methods of Measuring and Characterising Exposure to Nanoparticles	330
8.3.4	Studies Investigating Nanoparticle Exposure	338
8.3.5	Numbers of People Potentially Exposed	344
8.4	Control of Exposure	346
8.4.1	Introduction	346
8.4.2	Inhalation Exposure	347
8.4.3	Dermal Exposure	349
8.4.4	Ingestion Exposure	350
8.5	Discussion	350
8.6	References	353
9.	Human Toxicology and Effects of Nanoparticles	357
	<i>Vicki Stone, Martin J.D. Clift and Helinor Johnston</i>	
9.1	Introduction	357
9.1.1	Toxicology – What Is It?	357
9.1.2	Particle Toxicology	357
9.1.3	Risk Assessment	358
9.2	Ultrafine Particle Toxicology	360
9.2.1	Air Pollution	360
9.2.2	Testing the Ultrafine Particle Hypothesis	361
9.2.3	Reactive Oxygen Species and Oxidative Stress	363
9.2.4	Uptake of Nanoparticles into Cells	365
9.2.5	Interaction of Nanoparticles with Defence Mechanisms	366
9.2.6	Nanoparticle Interactions with Other Pollutants and Molecules	367
9.3	Engineered Nanoparticles	368
9.3.1	Fullerenes	369
9.3.2	Nanotubes and Other Fibre-Like Nanostructures	370
9.3.3	Metals	373
9.3.4	Metal Oxides	374
9.3.5	Quantum Dots	377
9.4	Relating Physico-Chemical Properties to Toxicity: Structure-Activity Relationships	379
9.5	Suggestions for Future Study Designs	381
9.6	Conclusions	381
9.7	Abbreviations	382
9.8	References	382