Chemical Safety Guidance

February 2020

Carbon Nanotubes and Other Synthetic Insoluble Fibrous Nanoparticles

Occupational Health and Safety Service
HSD060C (rev 4)

UNIVERSITY OF CAMBRIDGE

Contents:

1. Introduction

This guidance has been written in light of concerns over the potentially harmful effects on human health from exposure to airborne synthetic insoluble fibrous nanoparticles including carbon nanotubes (CNTs).

Of particular concern is the analogy that has been drawn between the potential effects of inhaling CNTs and the known harmful effects of inhaling asbestos fibres. Exposure to airborne asbestos fibres is the largest single cause of fatal occupational disease in the UK, currently causing over 5000 deaths per year, over 2500 of which were from mesothelioma and the rest from lung fibrosis, lung cancer etc. Asbestos related diseases take anything from 15 to 50 years to develop from initial exposure, with half of the deaths being in persons over 75 years old.

Whilst the guidance refers to CNTs as a specific example, it should be taken as applying to any other airborne synthetic insoluble fibrous nanoparticles whose physical form is also analogous to asbestos fibres.

For the purpose of this guidance synthetic fibrous nanoparticles are defined as having two dimensions in the order of 100 nm or less that have been produced as a result of, or used in, an activity at work. To fulfil the physical requirements for asbestos-like toxicity it is generally agreed that the fibres should be 'rigid', with a length in excess of 5000 nm. Theoretically, fibres shorter than 5000 nm could be ingested by macrophage cells as part of the body's natural defences and thereby be safely removed from the lungs. Very long fibres should be entrapped in the mucosal defence mechanism of the bronchial tract and be subsequently deposited in the intestinal tract without reaching the lungs.

2. Carbon Nanotubes

Carbon nanotubes are allotropes of carbon having a structure akin to a graphene sheet seamlessly rolled into a tube of potentially 'endless' length. They have been and are the subject of intensive scientific research and are already produced in industrial quantities for incorporation in a range of commercial products.

In their purest form they would only contain carbon atoms, however in practice they may also contain other component substances either inside or attached to the CNTs and/or 'impurities'. Impurities can include residual metal atoms from catalysts used in their synthesis, including iron, nickel, or cobalt, the latter two being carcinogenic in their own right. CNTs are formed as discrete entities, however they tend to agglomerate and aggregate into irregular masses.

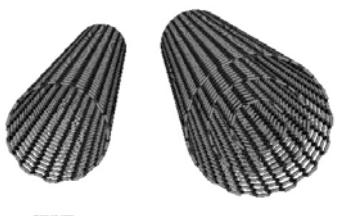
For the purpose of this guidance CNTs can be simply divided into two classes:

Single-Walled Carbon Nanotubes (SWCNTs)

Single-Walled Carbon Nanotubes are a single hollow cylinder of graphene (see below).

Multi-walled Carbon Nanotubes (MWCNTs)

Multi-walled Carbon Nanotubes consist of concentric carbon nanotubes of increasing diameter (see below). MWCNTs are structurally more 'rigid' than SWCNTs.



3. Cause for Concern

A body of experimental evidence has been published implicating **CNTs as potential initiators of inflammation and granulomas in animal models** in a mechanism analogous to asbestos fibre toxicity (see Appendix 1 for more details).

In addition to being physically similar to asbestos fibres, CNTs appear to have a similar ability to persist in the lungs of laboratory animals during the relatively short duration of the studies to date. Recent studies have shown evidence that certain CNTs, under specific in-vitro experimental conditions, may not be bio-persistent in the same way as asbestos. However, even if CNTs were shown not to be bio-persistent in the long term, that would not mean they were inherently safe, merely that they are less likely to cause mesothelioma.
Therefore, until the evidence is clear, the assumption must be that bio-persistence of CNTs could occur in humans and that CNTs have the potential to cause serious adverse health effects if inhaled.

In 2014, the **International Agency for Research on Cancer (IARC)** published an evaluation of the carcinogenic potential of carbon nanotubes. However, because of the variety of carbon nanotube types used in published toxicity studies, the review panel could not draw generalizable conclusions that satisfied the IARC's rigorous standards. The panel concluded that carbon nanotubes "cannot be classified due to a lack of data".
All, that is, apart from Because MWNT-7 had been studied so extensively, there was sufficient evidence for the panel to place this one specific form of carbon nanotubes **into group 2B.**

IARC - "MWNT-7 are possibly carcinogenic to humans"

Therefore, in line with University policy and The Control of Substances Hazardous to Health Regulations (COSHH), a pre-cautionary approach must be adopted when handling fibrous nanoparticles, including CNTs.

4. Potential Hazards

Fibrous nanoparticles can, at least theoretically, consist of any number of substances or mixtures of substances. The conventional toxicology of many, but by no means all, of these substances may be documented in Material Safety Data Sheets (MSDSs.

However, **fibrous nanoparticles may exhibit elevated toxicological properties** as a result of their physical and chemical nature. These effects may arise from their:

4.1 Fibrous shape

Rigid insoluble bio-persistent fibres with a high aspect ratio (long and thin) have been implicated in asbestos like toxicology (Appendix 1).

4.2 Small size

The small size of fibrous nanoparticles, may enable them to penetrate into locations in the body that larger insoluble particles could not reach. In addition the smaller the particles are, the more particles there are per unit mass, the so called quantum effect.

Nanoparticles can display many novel properties as a result of their very small size.

For example traditionally inert material can become chemically reactive (i.e. gold), electrical conductance can increase (i.e. CNTs).

Nanoparticles in general are capable of remaining airborne for extended periods of time, during which larger particles would settle out of the air as 'dust' on surfaces. It should however be noted that the inhalation studies of CNT toxicity have in the past been thwarted by problems encountered in trying to generate a stable and uniform dispersion of CNTs in air. This might imply that in the case of CNTs they may not remain airborne in the same way as other nanoparticles. If so, this it is probably as result of the propensity of CNTs to agglomerate and aggregate, into larger particles.

Small nanoparticles can behave almost like a 'gas' and display Brownian motion, furthermore their small size may make nanoparticles more prone to leak through any faults in the seals of filtering devices such as face masks and HEPA filters.

4.3 Potential ability to penetrate natural human defences

Nanoparticles are small enough to reach deep into the lungs and into the alveoli, the site of oxygen uptake. Once in the lungs a proportion of the nanoparticles may become lodged there. MWCNTs have been shown to be capable of passing from the alveoli into the pleural lining of the lung in animal models, this is known to be a prerequisite for the initiation of asbestos associated mesothelioma.

Some nanoparticles may be small enough to cross membrane barriers. Animal studies have shown 30nm particles capable of reaching the brain along the olfactory nerve directly from the nose, however it seems unlikely that CNTs could do this.

Nanoparticles have been shown to accumulate inside the lining of the gut following ingestion. It should be remembered that involuntary ingestion occurs continuously because the lungs natural defence mechanism results in mucous expelled from the windpipe being 'automatically' swallowed.

There is contradictory evidence as to whether some nanoparticles are small enough to cross through the barrier of the skin as some chemicals are known to do. Whilst it seems unlikely that CNTs could do this, they may be small enough to enter pores and hair follicles in the skin.

In animal studies it has been shown that once inside the body nanoparticles can be transported around the body, cross membranes, enter the organs and be excreted in urine. Medical applications are being developed for using CNTs as a drug delivery system that rely on some of these abilities.

Nanoparticles can enter individual cells and could potentially interact with DNA.

4.4 Oxygen radicals

Nanoparticles because of their comparative increased reactivity are believed to have an increased ability to generate oxygen radicals, which are implicated in cancer.

4.5 Increased surface area and availability of bio-reactive sites

It has been demonstrated that the toxicity of some insoluble nanoparticles is proportional to their **surface area not their mass**. In relative terms the smaller the particles are, the larger the surface area, and the more potentially toxic / bio-reactive sites there are on the particle's surface for any given weight of a substance. A 1000 nm particle has only 0.0006% of its molecules on the surface, whilst a 1 nm particle has nearly 50% of its molecules on the surface.

5. Risk of Exposure

The principle exposure routes include those for conventional particles ie: most significantly via **inhalation**, but also ingestion, injection and potentially even absorption. It is reasonable to assume that the toxicology of soluble fibrous nanoparticles would be the same as soluble conventional particles of the same substance. There is evidence to suggest that some nanoparticles are more soluble than their conventional counterparts and by virtue of their unique properties they may have uniquely delivered a chemical to tissues upon which we do not fully understand its toxicological effects.

Inhalation: The shape and very small size of fibrous nanoparticles means that the natural defensive mechanism in the lungs may fail to prevent them penetrating deeply to the alveoli, where insoluble particles may become entrapped and soluble ones dissolve.

Ingestion: It is unlikely that a significant quantity of a non-toxic substance would be ingested accidently in a laboratory. However, even small amounts of highly toxic or carcinogenic fibrous nanoparticles could be significant because of their potential ability to cross mucosal barriers.

Injection: Accidental injection of fibrous nanoparticles is unlikely in a laboratory. However the effects of fibrous nanoparticles in the human circulatory system are largely unknown, so care must be taken to minimise the risk by avoiding the use of sharps wherever possible.

Absorption: The absorption of fibrous nanoparticles is theoretically possible via the eyes, the mucosal linings of the gastro-intestinal tract, nose, mouth, trachea, bronchioles or alveoli.

6. Legal Requirements / Exposure Limits

Nanoparticles are within the scope of the Control of Substances Hazardous to Health Regulations (**COSHH**) which include requirements to:

- carry out a **risk assessment** and record it in writing,
- **control** exposure
- **monitor** exposure and review assessment

Carry out a risk assessment for the **specific task and the substance used**, see the University's Hazardous Substance Risk Assessment Form on the HSO website at http://www.admin.cam.ac.uk/cam-only/offices/safety/publications/hsd030c/index.html

Completion of the form and the **implementation of the control measures identified** with reference to the guidance below should enable compliance with COSHH and DSEAR (Dangerous Substances Explosive Atmospheres Regulations).

Since the toxicological properties of fibrous nano-particles are not fully known a **precautionary approach should be adopted,** handling them all as if they are highly toxic until scientific evidence is produced that shows otherwise. In particular fibrous nanoparticles of carcinogenic, allergenic, mutagenic or very toxic substances represent a particularly significant hazard and must be very strictly controlled.

Consideration must be given to ALL those who might foreseeably be exposed, not merely those handling them.

There are currently **no** legal Workplace Exposure Limits (WELs) specifically for any nanoparticles, therefore in compliance with COSHH the potential for exposure should be eliminated or strictly controlled to as low a level as is reasonably practicable.
It should be noted that the UK WEL for airborne 'Carbon Black' of 3.5mg / m^3 (3500µg/ m^3) is <u>not</u> considered appropriate for CNTs.

Pauluhn[9] in his 2010 paper proposed a limit of 50µg/m^3 for Bayer's commercial MWCNTs (Baytubes®) based on extrapolation from a rat inhalation study.

In the USA the National Institute of Occupational Safety and Health (NIOSH) has proposed:

"**A Recommended Exposure Limit (REL) of 7 micrograms of carbon nanotubes or carbon nanofibres per cubic meter of air (7µg/ m^3)** as an eight-hour, time-weighted average, respirable mass concentration. This is the concentration that can most reliably be measured with current instrumentation". Furthermore "NIOSH recognizes **that the REL may not be completely health protective** but its use should help lower the risk of developing [work-related] lung disease and assist employers in establishing an occupational health surveillance program that includes elements of hazard and medical surveillance."
NIOSH goes on to recommend that airborne concentrations should be reduced as low as possible below the REL by making optimal use of sampling and analysis.

Whilst the American REL currently has no legal basis in the UK, this guidance supports it use in the University, with the caveats above, in lieu of a proven alternative.

However the measurement of airborne CNTs is not a simple, quick or straightforward task and therefore the preferred / practical option **in most research environments** is to prevent potential exposure through the use of rigorous containment via engineering controls rather than an extensive airborne CNT monitoring regime.

The COSHH 'hierarchy of control' gives precedence to using engineering controls over other methods of control, i.e. containment and local exhaust ventilation to prevent exposure to all individuals before even considering using less rigorous methods with Personal Protective Equipment used only as a last resort.

In March 2009 the Government's Health and Safety Executive (HSE) were concerned enough to issue specific guidance on the 'Risk management of Carbon Nanotubes' **which has since been revised** in 2011 and is available on the HSE website at http://www.hse.gov.uk/pubns/web38.pdf .

7. Controlling Exposure

Wherever possible prevent or minimise the likelihood of releasing airborne carbon nanotubes or other fibrous nanoparticles by the use of appropriate processes, practices, systems and engineering controls.

Where possible keep the material wet or damp to reduce the risk of it becoming airborne and avoid energetic processes that might generate airborne dusts or aerosols. Keep all bottles/vessels containing fibrous nanoparticles sealed when not in immediate use. It must be remembered that just opening a bottle/vessel containing dry CNTs will in itself cause a measureable proportion of the CNTs to become airborne. Where there is a risk of fibrous nanoparticles becoming airborne, the following measures should be used to control and prevent exposure.

7.1 Principal Engineering Control Measures

As far as reasonably practical all 'synthetic' airborne nano-fibres including carbon nanotubes should be captured by local exhaust ventilation (LEV) and the exhaust air HEPA filtered to remove the nano-fibres before venting to a 'safe place outside', in compliance with the 'precautionary good practice approach' in the HSE guidance i.e.

Carbon nanotubes and other insoluble fibrous nanoparticles that have the potential to become airborne should be handled under HEPA‡ filtered local exhaust ventilation (LEV).

‡ High Efficiency Particulate Air (HEPA) filters are designed to remove at least 99.97% of airborne particles with a diameter of 300 nm, which is regarded as the Most Penetrating Particle Size (MPPS). Larger and **smaller** particles are filtered with even higher efficiency.

A HEPA filtered microbiological safety cabinet (MSC) or a bespoke containment facility, all venting to a safe place outside. Using double HEPA filtered cabinets increases the level of protection and can also provide a safe means of carrying out filter changes (note: double HEPA filtered cabinets are available for handling asbestos fibres and by implication these should be suitable for CNTs).

In the event that other volatile chemicals are used in conjunction with the fibrous nanoparticles careful consideration should be given to the point of discharge and the composition/type of the HEPA filter itself. For instance a glass fibre or a PTFE based HEPA filter would be the filter media of choice where other chemicals were likely to react with the cellulose matrix in a standard filter, i.e. where nitric acid is used to wash CNTs.

7.1.1 Recirculating Cabinets

The use of recirculating fume cupboards or recirculating HEPA cabinets to control any hazardous substance must be subject to rigorous risk assessment and should only be considered where external venting to a 'safe place' is not reasonably practicable. Recirculating HEPA filtered cabinets are designed to capture 'dusts' and can filter nanoparticles >2nm, but not volatile chemicals such as solvents and acids.

The HSE guidance and the International Standards Office Technical Report (ISO/TR 122885) on nano-technologies advice on the use of recirculating HEPA filtered cabinets for nanoparticles **qualifies their use**. In particular it limits the quantities of nanoparticles considered as appropriate to handle in such units (see Appendix 2).

NB: Recirculating '*fume cupboards'* rely on an absorbent filter to remove volatile chemicals, however when the filter saturates the volatile chemicals will be released into the room, they therefore require regular maintenance / filter changes.

7.2 Exceptional Control Measures

If, and only if, it is not reasonably practicable to prevent all airborne exposure to carbon nanotubes and other fibrous nanoparticles using HEPA filtered LEV, then in addition to the use of the LEV, anyone who could potentially still be exposed must wear **suitable Respiratory Protective Equipment** (RPE) to prevent that exposure.

In these circumstances, gloves and overalls must also be worn that are suitable for the task, see section 7.3 below. Provision must be made to allow clean overalls and gloves to be put on and dirty ones removed in a manner that does not contaminate the individuals or the general workplace i.e. using a contained changing area/booth.

It must be emphasised that the use of RPE as a means of preventing exposure should only be **a last resort** (COSHH) and must not be undertaken lightly nor without full consideration of the practicality of using engineering controls.
Furthermore the HSE have recommended that operations with CNTs necessitating the use of RPE to protect individuals should be conducted in HEPA filtered 'clean rooms' to prevent the spread of contamination.

Remember that all RPE, including disposable masks, must be suitable for the task and face fitted for the individual by a competent face fit tester.

Disposable masks (no less than FFP3 standard) are only suitable as a secondary precautionary measure against accidental 'spillage' not as a first line of protection.

Full face P3 particulate respirators that protect the eyes and lungs are required for any work in an atmosphere containing airborne 'synthetic' fibrous nanoparticles.

For further information on the selection, use, maintenance and face fit testing of RPE see the University Guidance on the Occupational Health and Safety Office website.

7.3 Additional Control Measures

- Use suitable eye protection when handling any chemicals including nanoparticles (a minimum of close fitting safety glasses).

- Use appropriate gloves.
    - The latest research suggests that nanoparticles do not penetrate through intact disposable gloves, unlike many chemicals.
    - Currently the only criteria that can be readily accessed to 'judge' potential nanoparticle penetration of gloves is virus penetration testing to ASTM F1671-97b / ISO 16604, which uses a 28nm bacteriophage (see manufacturers specifications.
    - Using light coloured gloves will allow potential contamination from CNTs to be clearly visible and therefore facilitates containment.

- Wear: lab coats or where appropriate disposable overalls.
    - The European NANOSH project reported in 2008 that nanoparticles can permeate through some intact disposable overall materials. The report recommended the use of non-woven Tyvek / Tychem polyethylene overalls for nanoparticles rather than paper or cotton.
    - The light (white) colour of overalls will allow potential contamination from CNTs to be clearly visible and therefore facilitate containment.

- Consider using work surfaces that are of a light colour and therefore easily show CNT contamination. This can also be achieved through the use of 'benchkote' type surface coverings which also aid decontamination (as hazardous waste).

- Thoroughly clean the work area and all equipment immediately after use or following a spillage by **wet-wipe cleaning**.
    - Do not use vacuum cleaners unless fitted with a HEPA filter.
    - Do not brush or dry sweep when cleaning
    - Do NOT use compressed air for cleaning.

- Transport fibrous nanoparticles in sealed robust labelled containers **inside secondary containment** capable of withstanding foreseeable impacts. i.e. bottles inside robust plastic outer containers or similar.

- Dispose of fibrous nanoparticles and contaminated personal protective equipment etc as hazardous waste via the University's hazardous waste contractor for incineration, in clearly labelled sealed double plastic bags as a minimum. The requirement to incinerate CNT waste is the policy of both the University and the UK's Environment Agency.

- All control equipment must be subject to regular inspection and testing in accordance with Control of Substances Hazardous to Health Regulations and the Provision and Use of Personal Protective Equipment Regulations.

A summary of Carbon Nanotube control measures may be found in the flow chart of Appendix 4.

8. Effectiveness of Control Measures

The effectiveness of control measures can not be automatically assumed when handling nanoparticles. Respirators, HEPA filtered cabinets and most importantly fume cupboards were not specifically designed for this task, so evidence should be sought as to their effectiveness before use. Recent studies indicate that the filter media in respirators (FFP2 / FFP3) and HEPA filters are reasonably efficient at capturing the range of nanoparticles tested to date. However, there is evidence that, for some nanoparticles, the maximum penetrating particle size (MPPS) is smaller than the 'classic' 300 nm salt particles used to test HEPA filters.

Written records of annual Local Exhaust Ventilation testing (COSHH Regs) and monthly RPE checks of reusable face masks must be kept (PPE Regs).

9. Monitoring Exposure

Monitoring airborne nanoparticles is still a developing area of metrology.
Using specialist equipment it is currently possible to count the total number of airborne nanoparticles, record their size distribution and measure their surface area. However having done this it is currently impossible to know what the particles counted / measured actually consisted of. In a normal laboratory environment these measurements would be taken against a variable nanoparticle 'background' of anything between 5,000 and 60,000 nanoparticles per cubic centimetre, much of which is probably derived from traffic pollution.

A number of strategies are being developed, notably by the UK's HSE/HSL and USA's NIOSH, to make meaningful nanoparticle measurements against a potentially variable background. These techniques use multiple particle counters, in different locations, surface area measurement instruments and Transmission Electron Microscopy (TEM). See Appendix 3 for summary of the HSL method for monitoring airborne nanoparticles.

It should be noted that this background problem does not apply to operations undertaken in 'ISO clean rooms' where it has been shown that the continuous operation of HEPA filtration of the air in the room effectively reduces the background level to zero.

Whilst electron microscopy can be used to identify nanoparticles collected on filters this is qualitative technique and it is not reasonably practicable to use it to carry out quantitative monitoring. It may however be a useful technique for establishing if control measures are failing to fully contain a nanoparticle, assuming it is identifiable under electron microscopy.

10. Health Surveillance

It is University policy that all those working with nanoparticles should complete a University COSHH Health Record Form, available on the Safety Office website at:

http://www.admin.cam.ac.uk/cam-only/offices/safety/publications/hsd033c/index.html

In compliance with University policy, Departments are expected to keep this form for 40 years, after the person has left University employment.

11. Additional Risks

The risk of fire and explosion from nanoparticle dust may be elevated due to increased surface area. Substances that are normally regarded as non-flammable solids may become flammable in the nanoparticle state. Where applicable, potential risks should be assessed as part of the Hazardous substance risk assessment under DSEAR

12. Environmental Considerations

All CNTs should be treated as hazardous chemical waste and sent for incineration in line with UK Environment Agency policy and guidance.
In time airborne synthetic nanoparticles, including CNTs, tend to agglomerate or attach themselves to ambient particles in a similar way to those from air pollution. Little is known about nanoparticles in the aquatic environment, although some adverse effects have been indicated. The University's policy is to treat all material containing nano-particles as hazardous waste. Therefore NO free nanoparticles should enter any non-hazardous waste stream or be disposed of via the drains.

Appendix 1

The Body of Evidence Causing Concern:

Asbestos fibres are capable of initiating mesothelioma, a specific cancer of the mesothelial lining of the chest (the pleura). Mesothelioma is currently almost exclusively associated with exposure to airborne asbestos fibres, with symptoms taking between 15 and 50 years to develop in humans.

Over a decade ago it was suggested that CNTs might mimic the toxicological effects of asbestos, however experiments have been handicapped by the difficulties in reliably generating known levels of airborne CNTs for inhalation studies.

To date there are no reported cases of adverse health effects arising as a result of humans inhaling CNTs. However there have been several animal studies showing such adverse effects.

The two highly publicised studies published in early 2008 were performed on the mesothelial lining of the **abdominal cavity of mice by direct injection**, **not inhalation**, of 'long' Multi-Walled CNTs [1, 2]. In both studies this resulted in asbestos like, length dependent pathogenic behaviour, including development of inflammation and granulomas. Long straight MWCNTs gave similar results to asbestos controls which are known to cause mesothelioma by inhalation in humans.

An **inhalation** study of single walled CNTs in mice, using a new aerosolisation technique, has since shown the development of inflammation, fibrosis, mutagenesis, and oxidative stress[3],confirming this groups earlier work using aspirated CNTs [4]. However the work of Bonner et al[10], indicates that the consequences of asbestos and CNTs inhalation may be divergent in animal models.

In March 2009 the US government's NIOSH website released a pre-publication statement that their research has "demonstrated the ability of MWCNTs to migrate from the lungs to the pleura" [5]. NIOSH also published the draft of a Current Intelligence Bulletin: *Occupational Exposure to Carbon nanotubes and Nanofibres*[6].

In summation <u>rodent studies</u> have shown:

1. CNTs having an adverse effect at least equal to other hazardous substances such as carbon black, crystalline silica and asbestos.
2. Early onset & persistence of pulmonary fibrosis following exposure to CNTs.
3. The ability of CNTs to migrate from the lungs to the pleural lining of the lung.

Whilst the experimental evidence in laboratory animals is becoming stronger, it has not gone unchallenged [7,8] and questions remain including:

a. *Are CNTs biopersistant in humans long enough to cause mesothelioma?*

b. *If inhaled by humans would carbon nanotubes reach the alveoli and migrate from the lungs to the pleura?*

c. *If carbon nanotubes migrated to the pleura from the alveoli would they cause mesothelioma in humans?*

However it must be assumed the answers to these questions could be yes!

Therefore a precautionary approach must be followed and exposure eliminated or reduced to as low as reasonably practicable.

Appendix 2

The International Standards Office Technical Report (ISO/TR 12885) with respect to the use HEPA filtered cabinets for nanoparticles.

Comparison of US Microbiological Safety Cabinet Characteristics and applicability for nanoparticles

			Applications	
BSC Class	**Face Velocity m/s**	**Airflow Pattern**	**Nonvolatile Toxic Chemicals**	**Volatile Toxic Chemicals**
I *	0.4	In at front then through HEPA to the outside or recirculate into the room through HEPA	Yes	When exhausted outdoors [1,2]
II, A1	0.4	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit	Yes (minute amounts)	No
II, B1	0.5	30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter	Yes	Yes (minute amounts)[1,2]
II, B2	0.5	No recirculation; total exhaust to the outside through a HEPA filter	Yes	Yes (small amounts) [1,2]
II, A2	0.5	Similar to II, A1, but has 100 lfpm intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to outside through a canopy unit	Yes	When exhausted outdoors (Formerly "B3") (minute amounts) [1,2]
III	N/A	Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection	Yes	Yes (small amounts) [1,2]

1. Installation may require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

2. In no instance should the chemical concentration approach the lower explosion limits of the compounds

(Taken from Appendix A of ISO/TR 12885, Technical Report: Nanotechnologies – Health and safety practices in occupational settings relevant to nanotechnologies. Which in turn cites its source as the US Department of Health and Human Services publication 'Biosafety in Microbiological and Biomedical Laboratories, 2007')

* A Class I microbiological safety cabinet is similar in operation to a HEPA filtered fume cupboard or HEPA filtered cabinet, drawing in air through the front opening before HEPA filtering the exhaust.

Appendix 3 HSL Nanoparticle Measurement procedure – August 2011

"Three TSI P-Trak CPC particle counters will be used to measure the number concentration of airborne nanoparticles: CPC1 for near-field measurements, CPC2 for far-field measurements and CP3 as a mobile monitor. An optical particle counter (OPC) will also be used to measure the number concentration of particles between 0.5 and 10 µm in size. An Aerotrak 9000 will be used to measure the surface area concentration of airborne nanoparticles.

Near-field monitoring

Measurements using CPC1, OPC and Aerotrak 9000 will be carried out before, during and after the activity under study takes place. They will be positioned close to the worker (within an approximate 1m radius of the worker's head) taking care that they do not hinder or interfere with the workers' normal duties. Short lengths of conductive tubing (< 1m) will be used to help enable sampling within the workers breathing zone. Non-activity periods (before and after the activity period) will be monitored for at least 15 minutes if possible.

Far-field monitoring

Measurements using CPC2 will be carried out before, during and after the activity under study takes place. CPC2 is stationary and will be located at a distance from the activity, such that it measures airborne particle concentrations that are representative of the background concentration near the activity. A distance of at least 2 m is suggested; although a quick measurement (before the activity begins) using one of the CPCs will determine whether the far field concentration is representative of the near field "non-activity" concentration. The non-activity periods (before and after the activity period) will be monitored for at least 15 minutes if possible.

If there is time available, the OPC and Aerotrak 9000 monitors will be used to briefly monitor far-field concentrations before and after the activity takes place.

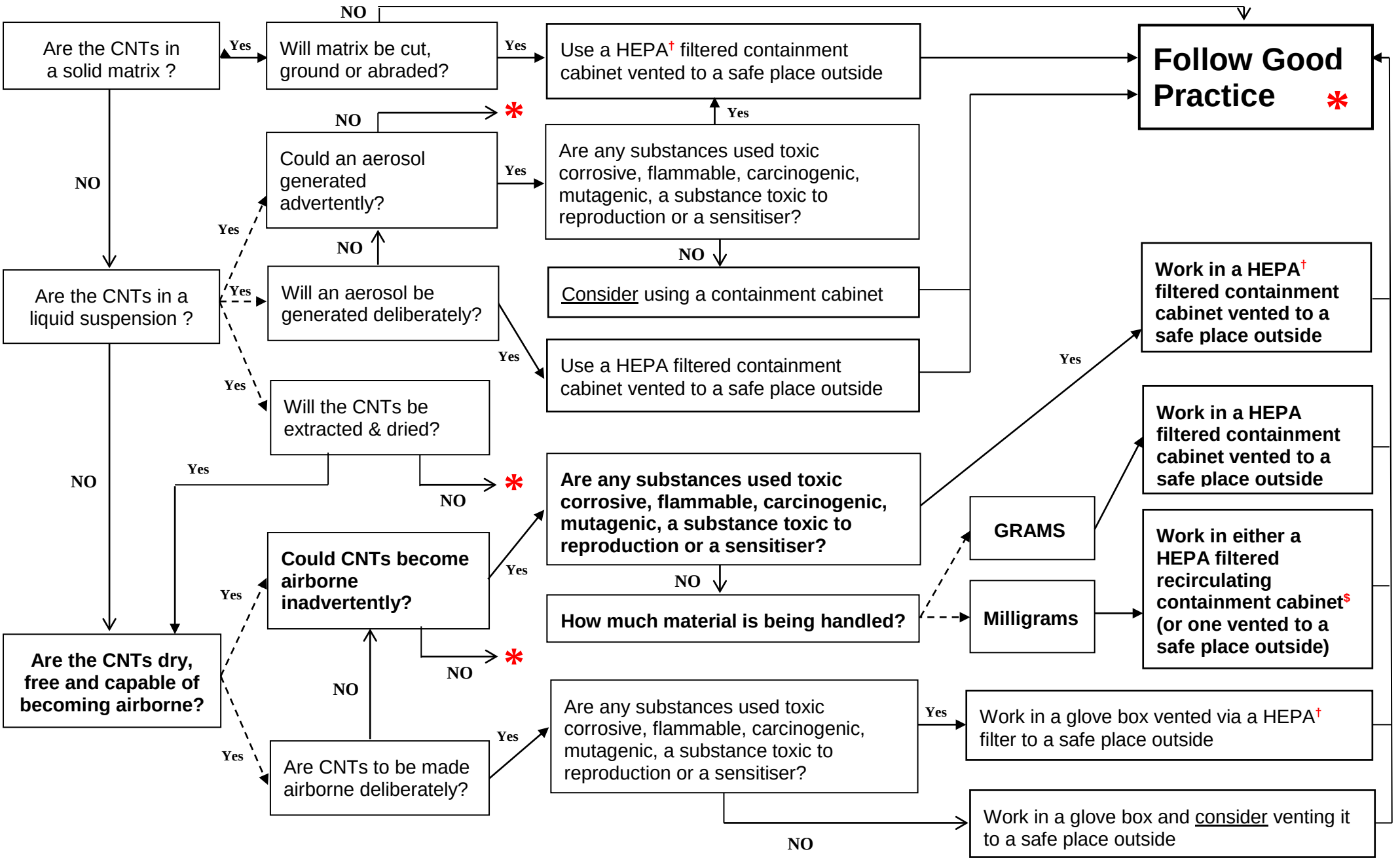
Mobile monitoring

Extraneous sources of nanoparticle such as: passing lorries/fork lift trucks, electric motors, smoke-generating systems, welding/soldering activities, open doors and windows can influence particle concentration readings greatly. Therefore, CPC3 will be used to investigate any other potential sources of nanoparticle and if possible these will be isolated or stopped during the monitoring period. In any event, the times at which these occur will be noted. CPC3 might also be used with the telescopic probe attachment to monitor particle number concentration inside containment/fume cupboards during activity periods.

Collection of samples for TEM analysis

One pumped sampler and one EP will be positioned next to CPC1 and CPC2. Samples collected inside containments/fume-cupboards are also very useful for comparison with samples collected outside containments/fume-cupboards and if possible these will be taken using additional pumped samplers."

Appendix 4 Carbon Nano-Tube Control Measures Flow Chart:



Key to Appendix 4 Flow Chart:

† If corrosive chemicals are also used the HEPA filter must be constructed of glass-fibre and not cellulose.

$ Substances that evolve toxic or flammable vapours such as acids and solvents as well as toxic or flammable gases should NOT be used in a recirculating HEPA cabinet

'CNT' refers to Carbon Nano-Tubes and other Synthetic Insoluble Fibrous Nanoparticles

* Good Practice

1. **When handling carbon nanotubes minimise the potential to make the material airborne**; whatever form it is in.
2. **Wear suitable Personal Protective Equipment**
    a. Overalls or laboratory coats
    b. Eye protection, as a minimum safety glasses
    c. Gloves
        i. Good quality disposable gloves or
        ii. Reusable gloves rubber,/ nitrile etc
    d. If airborne carbon nanotubes are not adequately controlled by the engineering control methods detailed in the flow chart above then respiratory protective equipment, face masks, may be needed‡
    e. Any other PPE, as necessary for the procedure / process being undertaken, such as protective shoes, visors, aprons, hearing protection etc
3. **Regularly clean the work area by wet wiping**
    a. Do Not use compressed air when cleaning
    b. Do NOT use brushes on nanoparticle material
    c. Only HEPA filtered vacuum cleaners may be used with nanoparticles
4. All control equipment must be subject to regular inspection and annual testing, at least every 14 months as required by COSHH.
5. Dispose of all materials containing carbon nanotubes as hazardous waste unless proven to be non-toxic and environmentally safe.

‡ RPE must be suitable for the task and in accordance with COSHH must be **face fitted for the individual**. FFP3 disposable masks are only suitable as a precautionary measure against accidental spillage. Full face P3 particulate respirators would be required for work in an atmosphere containing free airborne nanoparticles.

References

1. Takagi et al: Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-walled carbon nanotube. *J. Toxicol. Sci* Vol 33 No1 105-116, 2008

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Further Reading

General Safe Practices for Working with Engineered Nanomaterials in Research Laboratories – available on the NIOSH website at: http://www.cdc.gov/niosh/docs/2012-147/

Working Safely with Nanomaterials in Research and Development – developed by the UK NanoSafety Group and available on the SAFENANO website at: http://www.safenano.org/uk-nanosafety-group/

Using Nanomaterials at Work – an HSE publication available at: http://www.hse.gov.uk/pubns/books/hsg272.htm

Occupational Exposure to Carbon Nanotubes and Nanofibers – Günter Oberdörster et al. Current Intelligence Bulletin 65: (National Institute for Occupational Safety and Health, 2013).

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HSD060C (rev 4)