Supplementary Information

Employing nanosafety standards in a nanomaterial research environment: Lessons learned and refinement potential

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Step-by-step information on the Control Banding procedure

1 Core shell / PMMA@PMAA

1.1 Hazard Banding

Concerning poly(methyl methacrylate), (bulk PMMA, the core) and based on the European Chemicals Agency (ECHA) Classification and Labelling (C&L) database inventory, it was highlighted that 519 reported cases have not classified this substance. Rest cases report the following more common hazard statements (Table S1):

Table S1 – ECHA most reported hazard statements for bulk PMMA

Eye Irrit. 2	H319
Skin Irrit. 2	H315
STOT SE 3	H335

So, following the precautionary approach and based on ISO/TS 12901-2 standard ranking, this leads to the categorization of the substance based on ISO/TS 12901-2. It is also worth to be noted that PMMA is a well-known and widely used biomaterial, known for its high biocompatibility.

Concerning poly(methacrylic acid), (bulk PMAA, the shell), it was not registered in the ECHA C&L database inventory. To overcome this issue, we reviewed several Safety Data Sheets (SDS) and certificate of analysis for PMAA from several manufacturers. The SDSs indicate the following hazard statement by the Globally Harmonized System (GHS) for this substance: H315 (Skin Irrit. 2).

Thus, considering the hazards of the constituent materials, the hazard group allocation table from ISO/TS 12901-2, as well as the recommendation to increment by one the specified hazard band of the bulk material (in this case PMMA and PMAA), it is concluded that the corresponding co-polymer nanoparticles should be allocated to Hazard Band C.

1.2 Exposure Banding

The production method for these materials was described as a radical polymerisation production method where the nanoparticles are formed within a solution (Physical form: nanoparticles in suspension), based on Goulis et al. All the processes that take place can be considered as low energy processes (pipetting, dissolving, mixing) and based on ISO/TS 12901-2 Exposure Banding about synthesis, production and manufacturing processes, the radical polymerisation production method can be considered a wet chemistry method. Taking this information into consideration and according to ISO/TS 12901-2, this process can be allocated to the EB1 Exposure Band.

1.3 Risk / Control Banding & Recommendations

Allocation of the hazard band to hazard band C, as well as the exposure band to EB1, for the radical polymerisation production method, leads to the classification of the process in control band CB2, based on the ISO/TS 12901-2 control band matrix (Table S2). The controls proposed to assist in preventing exposure to nanoparticles, are therefore:

CB 2: Local ventilation: extractor hood, slot hood, arm hood, table hood, etc.

It should be noted here, that due to the suspension state of the material during the primary particle synthesis, the overall levels of risk are reasonably low. Local extraction ventilation via an extractor, arm or canopy hood can be acceptable for the wet chemistry synthesis technique.

Table S2 – CB allocation for core-sell PMMA@PMAA nanoparticles

Hazard Band	Exposure potential Band			
	EB1	EB2	EB3	EB4
A	CB1	CB1	CB1	CB2
B	CB1	CB1	CB2	CB3
C	CB2	CB3	CB3	CB4
D	CB3	CB4	CB4	CB5
E	CB4	CB5	CB5	CB5

1.4 Hazard assessment of process reagents

Upon identifying the hazard statements present in the nanoprocess reagents, a general conclusion extracted is that the monomers alone possess a more hazardous profile than that of the produced polymers. Moreover, some of the precursors / reagents used possess significant hazards, allocated even in Hazard Band E. Hazard statements of increased severity are indicated with bold lettering. Reagents have been classified through the Health and Safety Executive (HSE) Control of Substances Hazardous to Health (e-COSHH) tool and based on ISO/TS 12901-2 as well.

Monomers and reagents (methacrylic acid, methyl methacrylate, ethylene glycol dimethacrylate, potassium persulphate) for this radical polymerization, indicate for each one the following hazard statements by GHS, leading to their allocation in the respective hazard band (Table S3):

Table S3 – Hazard assessment of process reagents for core-sell PMMA@PMAA nanoparticles synthesis

MAA (Methacrylic acid)		Hazard Band C
H302	‘Harmful if swallowed – Acute Tox. 4 Oral’ with hazard pictogram GHS07 Exclamation mark and signal word ‘Warning’.	
H311	**‘Toxic in contact with skin – Acute Tox. 3 Dermal’ with hazard pictogram GHS06 Skull and crossbones and signal word ‘Danger’.**	
H314	**‘Causes severe skin burns and eye damage – Skin Cor. 1A’ with hazard pictogram GHS05 Corrosion and signal word ‘Danger’.**	
H318	**‘Causes serious eye damage – Eye Dam. 1’ with hazard pictogram GHS05 Corrosion and signal word ‘Danger’.**	
H332	‘Harmful if inhaled – Acute Tox. 4 Inhalation’ with hazard pictogram GHS07 Exclamation mark and signal word ‘Warning’.	

H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'
MMA (Methyl methacrylate)	**Hazard Band C**
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H317	'**May cause an allergic skin reaction – Skin Sens. 1**' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'
EGDMA (Ethylene glycol dimethacrylate) (crosslinker)	**Hazard Band C**
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'
KPS (Potassium persulfate) (initiator)	**Hazard Band E**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H317	'**May cause an allergic skin reaction – Skin Sens. 1**' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H334	'May cause allergy or asthma symptoms or breathing difficulties if inhaled–Resp. Sens. 1' with hazard pictogram GHS08 Health hazard and signal word 'Danger'.
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'

1.5 Dermal penetration potential

Based on the classification scheme presented by Larese Filon et al, in terms of dermal hazards, excessive dermal penetration hazards from these nanomaterials are not expected, due to their size (160-210 nm). Thus, penetration of the skin is unlikely for these particles.

1.6 Multiple - path particle dosimetry model (MPPD)

The main component of the core-shell nanostructured co-polymers that will be used as input to the multiple – path particle dosimetry (MPPD) model (ARA Inc., Huntsville, AL, USA) is the core since the airborne behaviour of the particles is reasonably expected to be dominated by the core's mass characteristics. Therefore, properties of PMMA (the core) will be used as input. Characterisation data have revealed that PMMA@PMAA nanostructured co-polymer fall within the range of approximately 160 - 210 nm in diameter. Human airway morphometry and Oronasal-Normal Augmenter breathing scenario were used. Relative density was set at 0.94 g/cm^3 for PMMA core.

A normal distribution between 160 nm and 210 nm was assumed, and a number of scenarios were used as input into the MPPD software, to reveal which fraction of potentially released nanoparticles could present the potential for respiratory deposition, and thus the display of negative effects. The sizes of these particles lead to the display of a generally low respiratory deposition potential (160 nm, 190 nm & 210 nm). Engineered design towards the larger sizes (210 nm) can further diminish this low potential leading to a Safer-by-Design primary particle. It can be observed that the alveolar deposition can be reduced from 12% to 10% for a change in size from 160 to 210 nm, accompanied by a small decrease in tracheobronchial deposition (-1%). Total deposition does not exceed 25%, even for the worst-case scenarios. The recommendation that is derived from the present results is that engineering design for larger particles, close to 210 nm in diameter can lead to inherently safer materials, if this modification does not display any impact on their functional properties. Results are summarized in Figure S1.

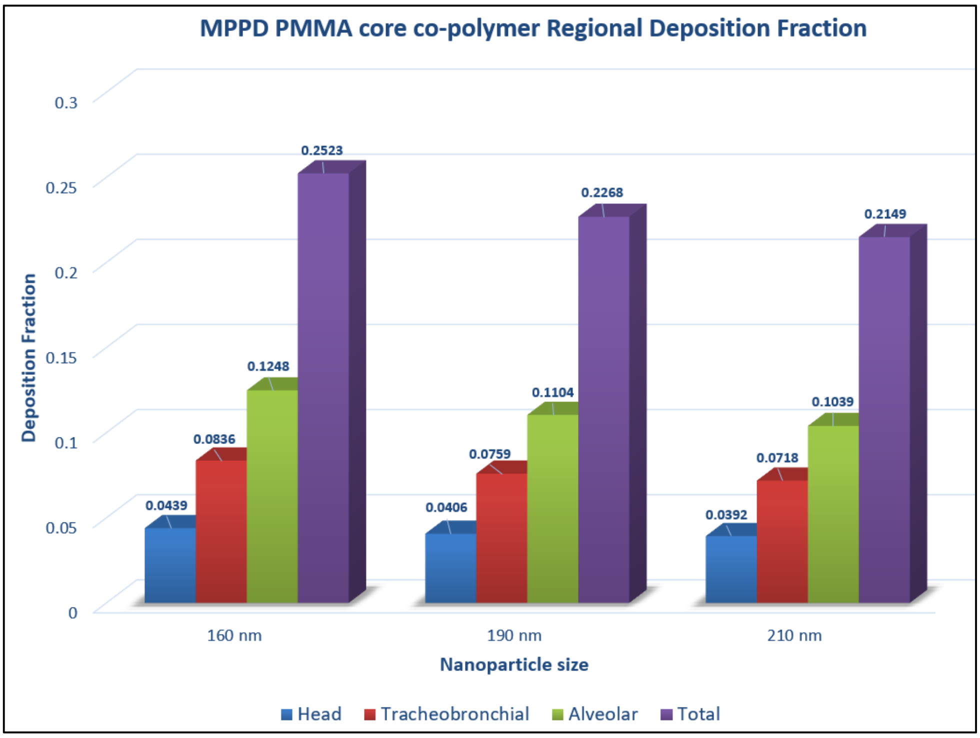

Figure S1 – Total deposition fraction results for core-shell PMMA@PMAA nanoparticles

2 Super Absorbent Polymers (SAPs) / PMAA@SiO_2

2.1 Hazard Banding

Concerning poly(methacrylic acid), (bulk PMAA, the organic core), it was not registered in the ECHA C&L database inventory. To overcome this issue, we reviewed several Safety data sheets (SDS) and Certificate of analysis for PMAA from several manufacturers. The aforementioned SDSs indicates the following hazard statement by GHS for this substance: H315 (Skin Irrit. 2).

In the case of SiO_2 nanoparticles, there is a reasonable knowledge base on hazard indications since this is one of the most extensively studied nanomaterial. The hazard banding decision tree can conclude at hazard tiered question 1, which investigates if nanoparticles have already been classified and labelled according to national, regional legislation or GHS. Exposure limits for nanoscale silica (amorphous) have already been proposed, with the World Health Organization (WHO) suggesting an occupational exposure limit (OEL) at 0.3 mg/m^3. Therefore, hazard statements can be identified for the material in its nanophase and the approach can utilize this as opposed to being based on the bulk material solely, which has been implemented for the previously assessed materials.

In addition, based on the ECHA C&L database inventory, it was highlighted that 235 reported cases had already classified this substance, with common hazard statements being the following (Table S4):

Table S4 – ECHA most reported hazard statements for SiO_2 nanoparticles

Eye Irrit. 2	H319
Skin Irrit. 2	H315
STOT SE 3	H335

So, based on the precautionary approach and on ISO/TS 12901-2 standard ranking, this leads to the categorization of the substance based on ISO/TS 12901-2.

Thus, considering the hazards of the constituent materials, the hazard group allocation table from ISO/TS 12901-2, as well as the recommendation to increment by one the specified hazard band of the bulk material (in this case PMAA, because studied SiO_2 is already in nanoparticle form), it is concluded that the corresponding super absorbent polymers (SAPs) should be allocated to Hazard Band C.

2.2 Exposure Banding

The production method for the SAP materials was described as a radical polymerisation production method where the nanoparticles are formed within a solution (Physical form: nanoparticles in suspension), based on Kartsonakis et al. All the processes that take place can be considered as low energy processes (pipetting, dissolving, mixing) and based on ISO/TS 12901-2 Exposure Banding about synthesis, production and manufacturing processes, the radical polymerisation production method can be considered a wet chemistry method. Taking this information into consideration and according to ISO/TS 12901-2, this process can be allocated to the EB1 Exposure Band.

2.3 Risk / Control Banding & Recommendations

Allocation of the hazard band to hazard band C, as well as the exposure band to EB1, for the radical polymerisation production method, leads to the classification of the process in control band CB2, based on the ISO/TS 12901-2 control band matrix (Table S5). The controls proposed to assist in preventing exposure to nanoparticles, are therefore:

CB 2: Local ventilation: extractor hood, slot hood, arm hood, table hood, etc.

It should be noted here, that due to the suspension state of the material during the primary particle synthesis, the overall levels of risk are reasonably low. Local extraction ventilation via an extractor, arm or canopy hood can be acceptable for the wet chemistry synthesis technique.

Table S5 – CB allocation for SAPs nanoparticles

Hazard Band	Exposure potential Band			
	EB1	**EB2**	**EB3**	**EB4**
A	CB1	CB1	CB1	CB2
B	CB1	CB1	CB2	CB3
C	CB2	CB3	CB3	CB4
D	CB3	CB4	CB4	CB5
E	CB4	CB5	CB5	CB5

2.4 Hazard assessment of process reagents

Upon identifying the hazard statements present in the nano process reagents, a general conclusion extracted is that the monomers alone possess a more hazardous profile than that of the produced polymers, as it was highlighted in the case of core-shell nanoparticles as well. Moreover, some of the precursors / reagents used possess significant hazards, allocated even in Hazard Band E. The Hazard statements of increased severity are indicated with bold lettering. Reagents have been classified through HSE e-COSHH tool and based on ISO/TS 12901-2 as well.

Monomers and reagents (methacrylic acid, acetonitrile, ammonium hydroxide, tetraethyl orthosilicate, ethylene glycol dimethacrylate, potassium persulphate) for this radical polymerization, indicate for each one the following hazard statements by GHS, leading to their allocation in the respective hazard band (Table S6):

Table S6 – Hazard assessment of process reagents for SAPs nanoparticles synthesis

MAA (Methacrylic acid)		**Hazard Band C**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H311	**'Toxic in contact with skin – Acute Tox. 3 Dermal' with hazard pictogram GHS06 Skull and crossbones and signal word 'Danger'.**	
H314	**'Causes severe skin burns and eye damage – Skin Cor. 1A' with hazard pictogram GHS05 Corrosion and signal word 'Danger'.**	
H318	**'Causes serious eye damage – Eye Dam. 1' with hazard pictogram GHS05 Corrosion and signal word 'Danger'.**	
H332	'Harmful if inhaled – Acute Tox. 4 Inhalation' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'	
EGDMA (Ethylene glycol dimethacrylate) (crosslinker)		**Hazard Band C**
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	

H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'	
KPS (Potassium persulfate) (initiator)		**Hazard Band E**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H317	'**May cause an allergic skin reaction – Skin Sens. 1**' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H334	'May cause allergy or asthma symptoms or breathing difficulties if inhaled–Resp. Sens. 1' with hazard pictogram GHS08 Health hazard and signal word 'Danger'.	
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'	
TEOS (Tetraethyl orthosilicate)		**Hazard Band B**
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H332	'Harmful if inhaled – Acute Tox. 4 Inhalation' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'	
ACN (Acetonitrile)		**Hazard Band B**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H312	'Harmful in contact with skin – Acute Tox. 4 Dermal' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'	
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H332	'Harmful if inhaled – Acute Tox. 4 Inhalation' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
NH_4OH (Ammonium hydroxide)		**Hazard Band C**
H314	'**Causes severe skin burns and eye damage – Skin Cor. 1A**' with hazard pictogram GHS05 Corrosion and signal word 'Danger'.	
H318	'**Causes serious eye damage – Eye Dam. 1**' with hazard pictogram GHS05 Corrosion and signal word 'Danger'.	

2.5 Dermal penetration potential

In terms of dermal hazards, excessive dermal penetration hazards from these nanomaterials are not expected, due to their size (170-360 nm). Thus, penetration of the skin is unlikely for these particles.

2.6 Multiple - path particle dosimetry model (MPPD)

Similarly to the core-shell particles, the main component of the Super absorbent polymer nanoparticles that will be used as input to the model is the core. The PMAA core is selected, due to its high influence in the mass characteristics of the particle as a whole. Characterization data have revealed that PMAA@SiO_2 SAPs nanoparticles fall within the range of approximately 170 - 360 nm in diameter. Moreover, Human airway morphometry and Oronasal-Normal Augmenter breathing scenario were used. Relative density was set at 1.02 g/cm^3 for PMAA core.

Thus, a number of scenarios that fall within the aforementioned range were input into the MPPD software, to reveal which fraction of potentially released nanoparticles could present the potential for respiratory deposition, and thus the display of negative effects.

The MPPD simulations show a consistent reduction trend in alveolar and tracheobronchial deposition potential as the particle size increases, and a generally low deposition potential, similarly to the core-shell nanoparticles. The lowest deposition values can be obtained for the 360 nm particles, at 7.9% for alveolar deposition, minimized from a 12% max shown for 170 nm sized particles. Total deposition reaches a low of 18% for the 360 nm particles and does not exceed 25%, even for the worst-case scenarios. Engineering design for larger particles is a viable Safer-by-Material design strategy in this case as well. Results are summarized and can be seen in Figure S2.

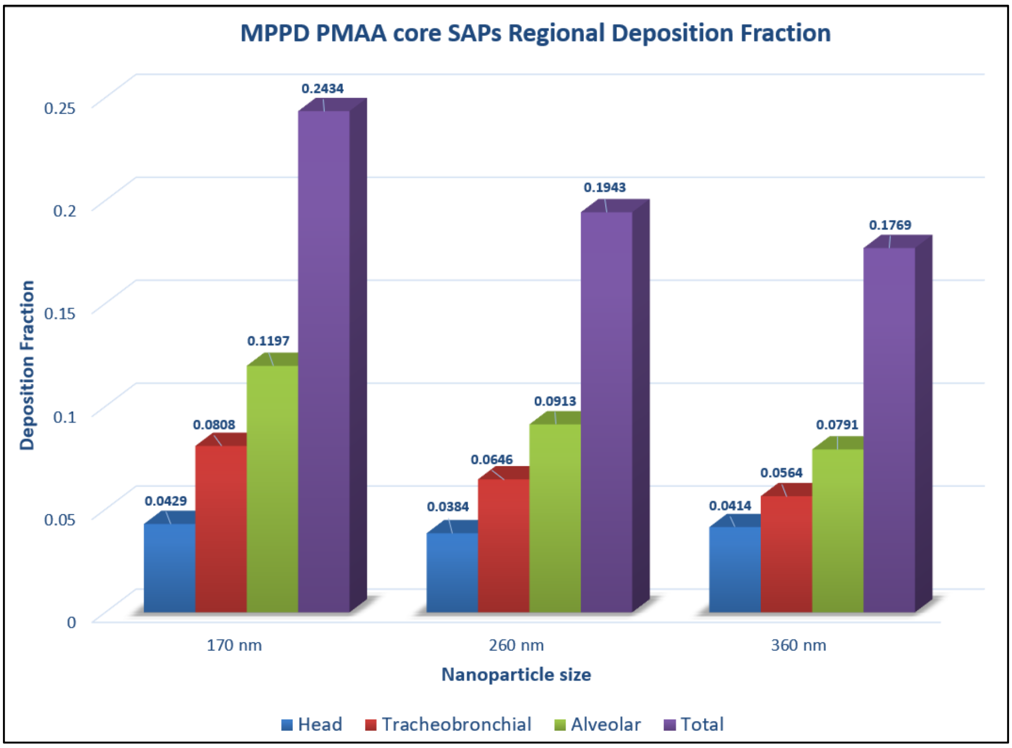

Figure S2 – Total deposition fraction results for SAPs nanoparticles

3 Magnetite (Fe_3O_4) nanoparticles

3.1 Hazard Banding

For the hazard banding of magnetic nanoparticles, there have been adequate hazard data produced in the nanosafety literature, to enable concluding at hazard tiered question 1 of the ISO/TS 12901-2, which investigates if nanoparticles have already been classified and labeled according to national or regional legislation or GHS. Therefore, hazard statements can be identified for the material in its nanophase. For Fe_3O_4 nanoparticles, there is an adequate level of hazard information to support banding and they have been classified by GHS.

In addition, based on the ECHA C&L database inventory, it was highlighted that most reported cases had already classified this substance, with common hazard statements being the following (Table S7):

Table S7 – ECHA most reported hazard statements for magnetite nanoparticles

Fe_3O_4 nanoparticles (5 nm – 50 nm sizes)		**Hazard Band D**
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'	
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H373	'**May cause damage to organs through prolonged or repeated exposure – STOT RE 2'** with hazard pictogram GHS08 Health hazard and signal word 'Warning'.	

So, based on the precautionary approach and on ISO/TS 12901-2 standard ranking, this leads to the categorization of the substance based on ISO/TS 12901-2.

Moreover, characterisation data from Yazdani et al., have revealed that the diameter of produced magnetite nanoparticles (Fe_3O_4) is below 50 nm and falls within the range of approximately 5 - 50 nm. It should be noted that the size of magnetic nanoparticles is a very important factor for the evaluation of possible health risks and negative effects. Inhalation and dermal hazards are directly connected to the size of released nanoparticles. It is considered that 50 nm is a threshold below which increased hazardous properties are displayed, and other nanosafety control banding approaches have adopted this threshold.

According to the hazard group allocation table from ISO/TS 12901-2 and given the severity of the above hazard statements, particularly the aforementioned hazard statement H373 (Specific target organ toxicity-repeated exposure) which is considered quite impactful, it is concluded that the produced magnetite nanoparticles (Fe_3O_4) should be allocated to Hazard Band D. This information, based both on size characteristics of the produced magnetite particles and on the hazard statements from the ECHA C&L database inventory, is adequate to classify the magnetic nanoparticles in hazard band D.

3.2 Exposure Banding

Precursors ($FeCl_24H_2O$, $FeSO_47H_2O$, $FeCl_36H_2O$, $Fe_2(SO_4)_3$, $Fe(NO_3)_39H_2O$ and NaOH) were used through the co-precipitation method to produce magnetic nanoparticles. The production method for these nanomaterials was described as a co-precipitation production method where the precipitated nanoparticles are formed within a solution (Physical form: nanoparticles in suspension), based on

Yazdani et al. All the processes that take place can be considered as low energy processes (pipetting, dissolving, washing, mixing) and based on ISO/TS 12901-2 Exposure Banding about synthesis, production and manufacturing processes, the co-precipitation production method can be considered a wet chemistry method. Taking this information into consideration and according to ISO/TS 12901-2, this process can be allocated to the EB1 Exposure Band.

3.3 Risk / Control Banding & Recommendations

Allocation of the hazard band to hazard band D, as well as the exposure band to EB1, for the co-precipitation production method, leads to the classification of the process in control band CB3, based on the ISO/TS 12901-2 control band matrix (Table S8). The controls proposed in order to assist in preventing exposure to nanoparticles, are therefore:

CB 3: Enclosed ventilation: ventilated booth, fume hood, closed reactor with regular opening.

A variable air volume (VAV) fume hood with bypass openings or a partially enclosed ventilated setup is also applicable from a cost-effectiveness perspective. Overall, if the cost is too high, a constant air volume (CAV) fume hood with bypass openings can be applicable. Preserving fume hood controls is recommended, however local extraction via e.g., an extractor arm can be acceptable for the wet chemistry production method.

Table S8 – CB allocation for magnetite nanoparticles

Hazard Band	Exposure potential Band			
	EB1	**EB2**	**EB3**	**EB4**
A	CB1	CB1	CB1	CB2
B	CB1	CB1	CB2	CB3
C	CB2	CB3	CB3	CB4
D	CB3	CB4	CB4	CB5
E	CB4	CB5	CB5	CB5

3.4 Hazard assessment of process reagents

Safety data sheets (SDS) and certificate of analysis, for Fe_3O_4 nanoparticles reagents ($FeCl_2$, $FeCl_3$, $FeSO_4$, $Fe_2(SO_4)_3$, $Fe(NO_3)_3$ & NaOH), indicate for each one the following hazard statements by GHS, leading to their allocation in the respective hazard band (Table S9). The Hazard statements of increased severity are indicated with bold lettering. Reagents have been classified through HSE e-COSHH tool and based on ISO/TS 12901-2 as well.

Table S9 – Hazard assessment of process reagents for magnetite nanoparticles synthesis

$FeCl_2$ (Iron II Chloride)		**Hazard Band C**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H314	'**Causes severe skin burns and eye damage – Skin Cor. 1B**' with hazard pictogram GHS05 Corrosion and signal word 'Danger'.	
H318	'**Causes serious eye damage – Eye Dam. 1**' with hazard pictogram GHS05 Corrosion and signal word 'Danger'.	
$FeCl_3$ (Iron III Chloride)		**Hazard Band C**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H314	'**Causes severe skin burns and eye damage – Skin Cor. 1B**' with hazard pictogram GHS05 Corrosion and signal word 'Danger'.	
H318	'**Causes serious eye damage – Eye Dam. 1**' with hazard pictogram GHS05 Corrosion and signal word 'Danger'.	
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	

NaOH (Sodium hydroxide)	Hazard Band C
H314	**'Causes severe skin burns and eye damage – Skin Cor. 1B'** with hazard pictogram GHS05 Corrosion and signal word 'Danger'.
H318	**'Causes serious eye damage – Eye Dam. 1'** with hazard pictogram GHS05 Corrosion and signal word 'Danger'.
$FeSO_4$ (Iron II Sulfate)	**Hazard Band C**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
$Fe_2(SO_4)_3$ (Iron III Sulfate)	**Hazard Band C**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H318	**'Causes serious eye damage – Eye Dam. 1'** with hazard pictogram GHS05 Corrosion and signal word 'Danger'.
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
$Fe(NO_3)_3$ (Iron III Nitrate)	**Hazard Band C**
H314	**'Causes severe skin burns and eye damage – Skin Cor. 1B'** with hazard pictogram GHS05 Corrosion and signal word 'Danger'.
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.

3.5 Dermal penetration potential

Based on the classification scheme presented by Larese Filon et al., conversely to what has been assessed for the previous nanomaterials, dermal penetration hazards from Fe_3O_4 nanoparticles are expected, mostly for particles that fall within the range of 5-20 nm of the total range (5-50 nm). Moreover, if the skin is injured, absorption is also possible for particles between 20-45 nm. Overall, penetration of the skin is possible for these particles, therefore, increased precautions for dermal exposure are recommended.

3.6 Multiple - path particle dosimetry model (MPPD)

Characterization data for Fe_3O_4 nanoparticles based on Yazdani et al., fall within the range of approximately 5-50 nm in diameter. Moreover, Human airway morphometry and Oronasal-Normal Augmenter breathing scenario were used. Relative density was set at 5.2 g/cm^3 for Fe_3O_4 nanoparticles. Thus, several scenarios that fall within the range were input into the MPPD software, to reveal which fraction of potentially released nanoparticles could present the potential for respiratory deposition, and thus the display of negative effects.

The magnetic nanoparticles display the highest alveolar deposition potential of the nanomaterials studied so far. Modifications in size do not have a consistent trend, as it has been demonstrated for all previous particles. Lowest deposition in alveoli is displayed from the smallest nanoparticles (5 nm), at 1.3%, however accompanied by very high total deposition at 90%. The highest alveolar deposition potential is displayed from the 30 nm sized particles and diminishes for larger sizes. Additionally, the lower size is reasonably expected to lead to higher specific surface area and reactivity, potentially causing increased hazard effect potential. 50 nm particles display the lowest total deposition (51%), however this is conflicting with a high 27% alveolar deposition potential. Thus, lowest hazard effects may be displayed by the 50 nm particles, however it is questionable if the modification of size within the 5-50 nm range can lead to actual Safer-by-Material-Design nanoparticles. Additionally, it has been acknowledged that small size is a determinant property for the final display of application-appropriate magnetic properties for these particles. This leads to inapplicability for recommending an increase in size over 50 nm for the magnetic nanoparticles from the viewpoint of safety, as the final material properties would be severely impacted. Results are summarized in Figure S3.

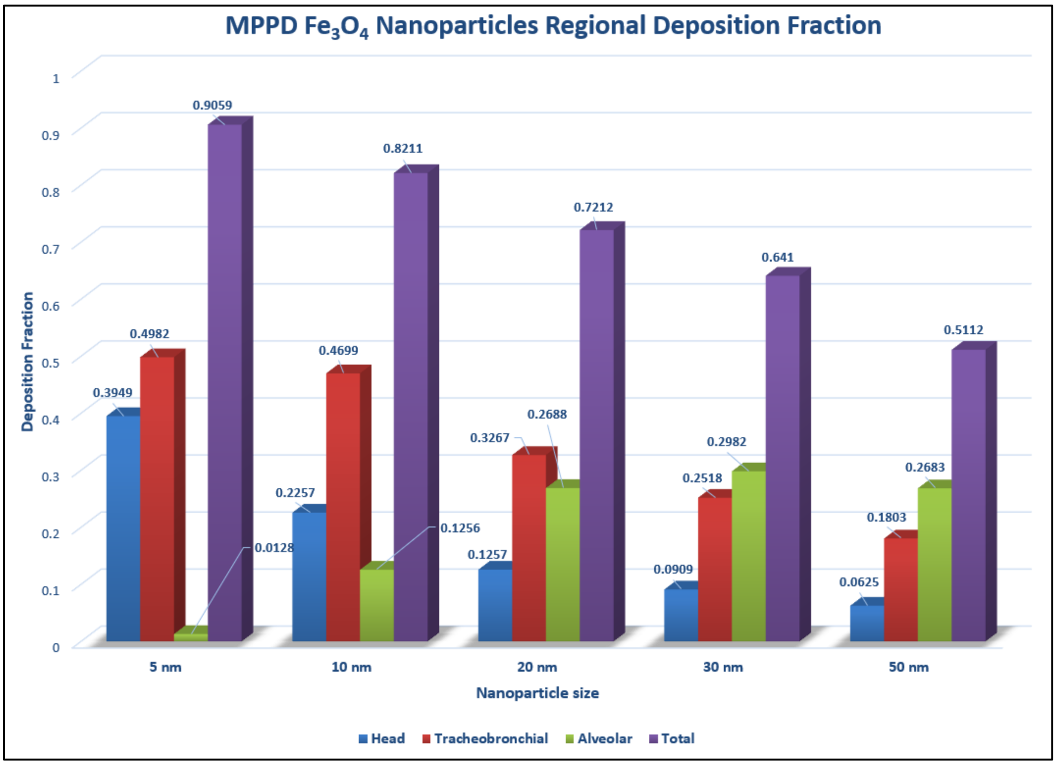

Figure S3 – Total deposition fraction results for magnetite nanoparticles

As a conclusion, magnetic nanoparticles display a moderately high hazard potential, primarily because of their small size. This low size range also results to higher dermal exposure potential, as well as significantly higher respiratory deposition compared to other materials. Within the 5 – 50 nm size range, the MPPD simulations have shown that modifications in size do not produce a consistent inherent safety increase effect. A primary particle size of 30 nm is expected to have the highest potential for adverse effects.

4 Hybrid Carbon Nanotubes (SiO_2@CNTs)

4.1 Hazard Banding

For the hazard banding of Carbon Nanotubes, the assessment concludes at tiered question 3, which investigates the possibility of nanoobjects to contain biopersistent fibers or fiber–like structures. For the purpose of this part of ISO/TS 12901-2, the definition of a long biopersistent fiber relates to the fact that some respirable, biopersistent, long and rigid fibers can penetrate mesothelium such as the pleura, inducing a sustainable inflammatory response as a consequence of macrophage mediated frustrated phagocytosis, which can ultimately result in mesotheliomas. This physio-pathological mechanism is commonly named as the Fibre paradigm by the WHO. Therefore, any nanoobject that falls within the definition of a rigid fiber (fiber with length > 5 μm, diameter < 3 μm) should be considered as a material whose toxicity is driven by the Fibre paradigm and should be allocated to the highest hazard band, unless inhalation toxicology data provide evidence that it is not the case.

Based on the characterization data of Kainourgios et al., the produced carbon nanotubes (CNTs) are defined as 'rigid fibers'. The length of the produced CNTs fall within the range of approximately 5-10 μm and their diameter is about 50 nm. Thus, according to the ISO/TS 12901-2 decision tree, the produced CNTs are nanoobjects with biopersistent fibre-like structure and their toxicity is driven by the fibre paradigm. Consequently, produced CNTs should be allocated to the highest possible hazard band that is in Hazard Band E.

In addition, based on the ECHA C&L database inventory, it was highlighted that most reported cases had already classified multi-walled carbon nanotubes (MWCNTs), with common hazard statements by GHS being the following and confirming the classification to Hazard Band E (Table S10):

Table S10 – ECHA most reported hazard statements for CNTs

Fibre-like CNTs		**Hazard Band E**
H315	'Causes skin irritation – Skin Irrit. 2' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.	
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'	
H340	**'May cause genetic defects – Germ cell Muta. 1B'** with hazard pictogram GHS08 Health hazard and signal word 'Danger'.	
H351	**'Suspected of causing cancer – Carc. 2'** with hazard pictogram GHS08 Health hazard and signal word 'Warning'.	

In the case of SiO_2 nanoparticles, their diameter was approximately 350 nm. The hazards are much less severe, allocating them to Hazard band B. Indicatively, a comparison between the proposed OELs for SiO_2 at 0.3 mg/m^3 and for CNTs at 1-50 μg/m^3 show a definitive indication of the high concern that has been expressed for CNTs throughout the nanosafety field. According to the hazard group allocation table from ISO/TS 12901-2 and the hazard statements for CNTs, it is concluded and confirmed that the produced hybrid materials containing CNTs should be allocated to Hazard Band E.

4.2 Exposure Banding

The production method for the hybrid MWCNT-based materials was described as a chemical vapor condensation process (chemical vapor deposition-CVD). Based on Kainourgios et al., the process includes formation of colloidal silica by means of sol gel (Stöber method) and functionalization of the hydroxyl groups of colloidal silica with iron chloride aqueous solution. Then it is placed inside a CVD furnace under oxygen atmosphere and high temperature conditions (700^{o}C) to form iron oxide nanoparticles and a reduction process follows under H_2 flow. Finally, acetylene as a carbon source is inserted to the system to initiate the growth of CNTs on the colloidal silica surface. The step that defines the process is the last one, where CNTs are produced through CVD process with acetylene as a carbon source. According to ISO/TS 12901-2 exposure banding for synthesis, production, and manufacturing processes, CVD exposure potential is considered very high. Thus, exposure band for CVD process is the highest possible and CVD process can be allocated to the EB4 Exposure Band.

4.3 Risk / Control Banding & Recommendations

Allocation of the hazard band to hazard band E, as well as the exposure band to EB4, to produce MWCNTs via CVD leads to the classification of the process in control band CB5, based on the ISO/TS 12901-2 control band matrix (Table S11). The controls proposed to assist in preventing exposure to nanoparticles are:

CB 5: Full containment: glove box/bag, continuously closed systems, and review by a specialist: seek expert advice.

Consequently, it is necessary to perform the whole process within a glove box isolator, an enclosed harvest reactor, or a custom fabricated ventilated enclosure. CVD reactors are enclosed by design, however allocation to this control band also entails the function of an emission removal system for the reactor.

Table S11 – CB allocation for CNTs

Hazard band	Exposure potential band			
	EB 1	**EB 2**	**EB 3**	**EB 4**
A	CB 1	CB 1	CB 1	CB 2
B	CB 1	CB 1	CB 2	CB 3
C	CB 2	CB 3	CB 3	CB 4
D	CB 3	CB 4	CB 4	CB 5
E	CB 4	CB 5	CB 5	CB 5

4.4 Hazard assessment of process reagents

SDSs and certificate of analysis for SiO_2@CNTs reagents ($FeCl_2$, NH_3, TEOS, Ethanol), indicate for each one the following hazard statements by GHS, leading to their allocation in the respective hazard band (Table S12). The Hazard statements of increased severity are indicated with bold lettering. Reagents have been classified through HSE e-COSHH tool and based on ISO/TS 12901-2 as well. CVD process also entails pressurized vessel use for process reagent inflow. While not taken into account for health and exposure risks, these process elements are reasonably taken into account in the ISO methodology as the CVD process is directly forwarded to the highest priority exposure class.

Table S12 – Hazard assessment of process reagents for CNTs synthesis

SiO_2@CNTs	
$FeCl_2$ (Iron II Chloride)	**Hazard Band C**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H314	**'Causes severe skin burns and eye damage – Skin Cor. 1B'** with hazard pictogram GHS05 Corrosion and signal word 'Danger'.
H318	**'Causes serious eye damage – Eye Dam. 1'** with hazard pictogram GHS05 Corrosion and signal word 'Danger'.
Hydrogen Gas Cylinder	**Hazard Bands not applicable**
H220	Extremely flammable gas
H280	Contains gas under pressure; may explode if heated
Ethanol	**Hazard Band A**
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
TEOS (Tetraethyl orthosilicate)	**Hazard Band B**
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H332	'Harmful if inhaled – Acute Tox. 4 Inhalation' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'
NH_3 (Ammonia solution)	**Hazard Band C**
H302	'Harmful if swallowed – Acute Tox. 4 Oral' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H314	**'Causes severe skin burns and eye damage – Skin Cor. 1B'** with hazard pictogram GHS05 Corrosion and signal word 'Danger'.
H318	**'Causes serious eye damage – Eye Dam. 1'** with hazard pictogram GHS05 Corrosion and signal word 'Danger'.
H319	'Causes serious eye irritation – Eye Irrit. 2A' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H335	'May cause respiratory irritation – STOT SE 3' with hazard pictogram GHS07 Exclamation mark and signal word 'Warning'.
H351	**'Suspected of causing cancer – Carc. 2'** with hazard pictogram GHS08 Health hazard and signal word 'Warning'.
Acetylene Gas Cylinder	**Hazard Bands not applicable**
H220	Extremely flammable gas
H280	Contains gas under pressure; may explode if heated
*	May displace oxygen and cause rapid suffocation
*	May form explosive mixtures with air.
* OSHA-defined H-statement	

4.5 Dermal penetration potential

In terms of dermal hazards, excessive dermal penetration hazards from CNTs are not expected, due to their size (5-10 μm in length, 50 nm in diameter). Penetration of the skin is unlikely for these particles. Nevertheless, in view of their fibrous nature, an increased level of precaution should be present for dermal exposure as well. Some of these materials may fit in the low-dermal-hazard band (21-45 nm) proposed by Larese Filon et al., for which skin absorption can be possible only on damaged skin. However, these are fibrous particles of high aspect ratio, so it is highly unlikely that this classification may represent dermal penetration hazards.

4.6 Multiple - path particle dosimetry model (MPPD)

The main and most hazardous component of the SiO_2@CNTs nanoparticles are the CNTs. A Safer-by-Design approach would reasonably have to be based on engineered design of CNTs for diminished inherent hazard. Therefore, CNTs will be used as input to the MPPD model to reveal the potential for respiratory deposition and negative effects.

Characterization data for CNTs based on Kainourgios et al., have revealed that the length of the produced CNTs fall within the range of approximately 5-10 μm and their diameter is approximately 50 nm. Moreover, Human airway morphometry and Oronasal-Normal Augmenter breathing scenario were used. Relative density for CNTs falls within the range of 1.7 – 2.1 g/cm^3. Thus, a number of scenarios that fall within the aforementioned range were input into the MPPD software, to reveal which fraction of potentially released CNTs could present the potential for respiratory deposition, and thus the display of negative effects. Given that length has been described as the primary control mechanism for Safer-by-Design CNTs approaches, two length scenarios were tested:

- 50 nm in diameter (D), 10 μm in length (L)
- 50 nm in diameter (D), 5 μm in length (L)

For the previous nanomaterial cases, it is consistently reported that larger sizes can lead to lower alveolar deposition. The fundamental similarity of the previously explored materials is their spherical shape (defined within the MPPD model as aspect ratio = 1), which is a primary determinant of particle airborne behavior.

Concerning CNTs, MPPD simulations show that the modification in aspect ratio does have an impact on alveolar deposition (Figure S4). CNTs of a steady 50 nm diameter of 100 and 200 Aspect Ratio (leading to 5 or 10 μm in length respectively) display a 15.49 – 11.57 % alveolar deposition, with longer CNTs (10μm) alveolar deposition being lower. Total deposition for both CNTs lengths is comparable (slightly lower for the shorter CNTs, at 50.77 % as opposed to 51.72 % for the longer CNTs).

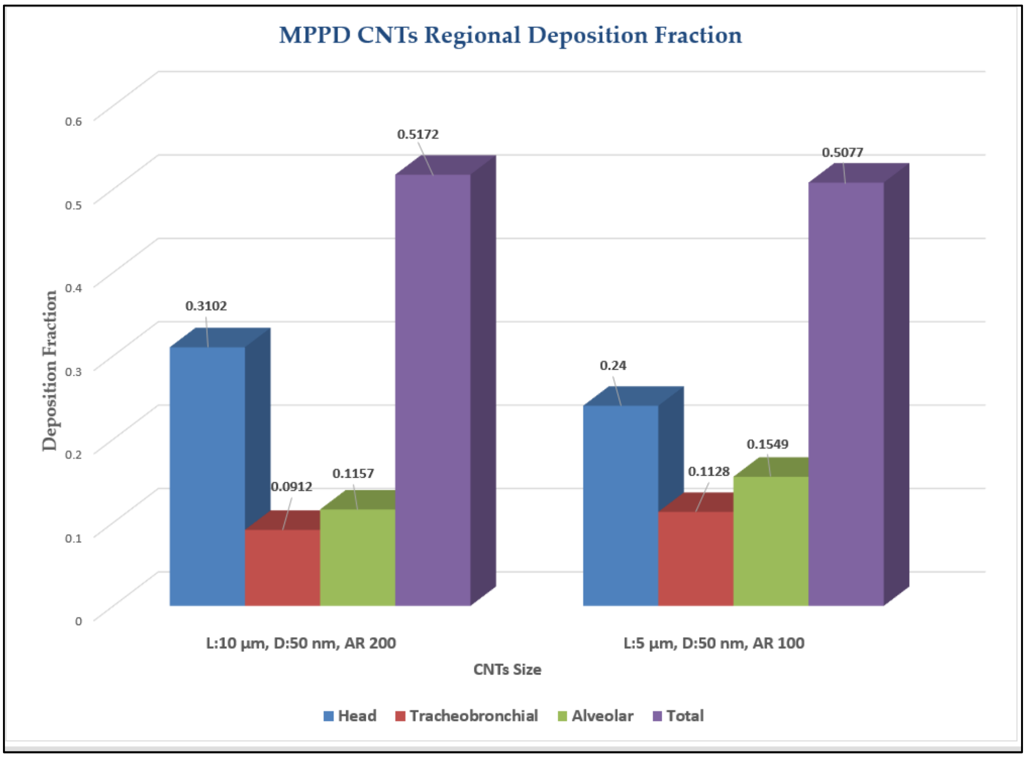

Figure S4 – Total deposition fraction results for SiO_2@CNTs

Seeing that length is an important determinant for controlling CNT hazard, this is an important insight in supporting a Safer-by-Design strategy for CNTs. Long and rigid biopersistent fibers can penetrate mesothelium and induce a sustainable inflammatory response because of macrophage mediated frustrated phagocytosis. This physio-pathological mechanism is commonly named as the fibre paradigm. Here it should be mentioned that, beside the fact that alveolar and tracheobronchial deposition fraction seems to slightly increase as aspect ratio is reduced (or CNTs length is reduced), inflammation and toxicity impacts caused by shorter inhaled and deposited CNTs due to the fibre paradigm are reasonably expected to be much milder. Therefore, in the assessments, CNTs deposition fraction at alveolar & tracheobronchial region ought to be considered in conjunction with CNTs intrinsic properties (e.g., length or aspect ratio) that define their behavior if inhaled. Taking this into account when interpreting the results, a slight increase in alveolar deposition fraction caused by shorter CNTs, is outweighed by the much more benign behavior that shorter CNTs possess. On the other hand, a slight decrease in alveolar deposition fraction that is observed, caused by longer CNTs is counterbalanced by the harmful behavior that longer CNTs possess. In conclusion, shorter CNTs lead to lower hazard potential, while minimally interfering with deposition potential, rendering the control of length as a viable Safer-by-Material-design strategy.