Toxicological profile for

Activated carbon

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Carbon (PubChem)

1.2. Synonyms

AG 3 (Adsorbent); AG 5; AG 5 (Adsorbent); AK (Adsorbent); AR 3; ART 2; AU 3; Acticarbone; Activated carbon; Activated charcoal; Adsorbit; Amoco PX 21; Anthrasorb; Aqua nuchar; BAU; BG 6080; Black 140; Black pearls; CF 8; CF 8 (Carbon); CLF II; CMB 200; CMB 50; CUZ 3; CWN 2; Calcotone Black; Canesorb; Carbolac; Carbon, colloidal; Carbon-12; Carbopol Extra; Carbopol M; Carbopol Z 4; Carbopol Z Extra; Carbosieve; Carbosorbit R; Caswell No. 161; Cecarbon; Coke powder; Colgon BPL; Colgon PCB-D; Columbia LCK; Conductex, Darco; Filtrasorb; Filtrasorb 200; Filtrasorb 400; Grosafe; Hydrodarco; Irgalite 1104; Jado; K 257; MA 100 (Carbon); Norit; Nuchar; OU-B; Pelikan C 11/1431a; SKG; SKT; SKT (adsorbent); SU 2000; Suchar 681; Supersorbon IV; Supersorbon S 1; U 02; Watercarb; Witcarb 940; XE 340; XF 4175L; Graphite synthetic; CCRIS 8681; EINECS 231-153-3; EPA Pesticide Chemical Code 016001; HSDB 5037 (ChemIDplus); FCM Substance 984 (EFSA, 2012a); Decolourizing carbon (FAO/JECFA, 2010)

1.3. Molecular formula

C

1.4. Structural Formula

C

1.5. Molecular weight (g/mol)

12.011

1.6. CAS registration number

7440-44-0

1.7. Properties

1.7.1. Melting point

4440ºC (12.4 GPa) as diamond, 4489ºC triple point (10.3 Mpa) as graphite; 3650ºC, 3652ºC, 3727ºC (ChemSpider); -182.56ºC (EPISuite, 2017)

1.7.2. Boiling point

3825ºC sublimation point graphite; sublimes at 3642ºC; triple point (graphite-liquid-gas), 4492ºC at a pressure of 101.325 kPa (HSDB, 2009); 5000ºC, 4200ºC (ChemSpider); -161.5ºC (EPISuite, 2017)

1.7.3. Solubility

“Insoluble” (ChemSpider); 22 mg/L at 25ºC (EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

>500ºC (PMCC)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

900ºC (layer)

1.7.8. Decomposition temperature

No data available to us at this time.

1.7.9. Stability

Stable, in the form of powder reacts vigorously with a wide variety of materials; in the rod form is relatively inert; incompatible with strong oxidizing agents; highly flammable in powdered form, Combustible (ChemSpider); Dust explosions are possible when the powder is mixed with air; wet, activated carbon can remove oxygen from a confined space (Haz-Map, 2017)

1.7.10. Vapor pressure

1 mm Hg at 3586ºC (PubChem); 0 mmHg (ChemSpider); $4.66x10^5$ mmHg at 25ºC (EPISuite, 2017)

1.7.11. log Kow

1.09 (EPISuite, 2017)

2. General information

2.1. Exposure

Natural Pollution Sources:

Abundance in earth's crust: approx 0.027%. Cosmic abundance: 6 atoms/atom Si. Occurs in 3 forms: (1) diamond; (2) graphite or black lead; (3) amorphous carbon such as coal, lampblack, and the various forms of artificial carbon. [O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 2006.., p. 293] **PEER REVIEWED** (2009)

... very widely distributed in nature. It is found in abundance in the sun, stars, comets, and atmospheres of most planets. ... Without carbon, the basis for life would be impossible. [Lide, D.R. CRC Handbook of Chemistry and Physics 86TH Edition 2005-2006. CRC Press, Taylor & Francis, Boca Raton, FL 2005, p. 4-8] **PEER REVIEWED**

(14)C Isotope, continuously formed in earth's atmosphere by bombardment of nitrogen with cosmic neutrons. /(14)C/ [O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 2006.., p. 293] **PEER REVIEWED** (2009)

Diamond, an allotropic form of carbon, crystallizes isometrically, consists of carbon atoms covalently bound by single bonds only in a predominantly octahedral structure. The purest diamonds used for gems are mined in South Africa, lower grades in Brazil, Venezuela, India, Borneo, Arkansas. /Diamond/ [Lewis, R.J. Sr.; Hawley's Condensed Chemical Dictionary 15th Edition. John Wiley & Sons, Inc. New York, NY 2007., p. 386] **PEER REVIEWED**

Artificial Pollution Sources:

A fourth form, known as "white" carbon**,** is now thought to exist. [Lide, D.R. CRC Handbook of Chemistry and Physics 86TH Edition 2005-2006. CRC Press, Taylor & Francis, Boca Raton, FL 2005, p. 4-8] **PEER REVIEWED**

Environmental Abiotic Degradation:

... is rapidly oxidized to carbon dioxide ... /which enters/ into animals and plants by photosynthesis and metabolism. /(14)C/ [O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 2006.., p. 293] **PEER REVIEWED**

Major Uses:

Use in fuel industry ... /and in/ paints, lacquers and varnishes industry ... Use as absorbents and adsorbents. [European Chemicals Bureau; IUCLID Dataset, Carbon (7440-44-0) p.4 (2000 CD-ROM edition). Available from, as of July 18, 2008: http://esis.jrc.ec.europa.eu/ **PEER REVIEWED**

Decolorizing sugar, water and air purification, solvent recovery, waste treatment, removal of sulfur dioxide from stack gasses and "clean" rooms, deodorant, removal of jet fumes from airports, catalyst for natural gas purification, brewing, chromium electroplating, air conditioning. /Carbon, activated/ [Lewis, R.J. Sr.; Hawley's Condensed Chemical Dictionary 15th Edition. John Wiley & Sons, Inc. New York, NY 2007., p. 232] **PEER REVIEWED**

As strong reducing agent and is used as such in purifying metals; in electrodes, electrical devices ... and steel [Lewis, R.J. Sr.; Hawley's Condensed Chemical Dictionary 15th Edition. John Wiley & Sons, Inc. New York, NY 2007., p. 231] **PEER REVIEWED**

For carbon (USEPA/OPP Pesticide Code: 016001) ACTIVE products with label matches. /SRP: Registered for use in the U.S. but approved pesticide uses may change periodically and so federal, state and local authorities must be consulted for currently approved uses./ [National Pesticide Information Retrieval System's USEPA/OPP Chemical Ingredients Database on Carbon (7440-44-0). Available from, as of July 1, 2008: http://ppis.ceris.purdue.edu/htbin/epachem.com **PEER REVIEWED**

... One form of carbon, activated charcoal, is given orally as an adsorbent for treatment of accidental drug poisoning.

Human exposure is expected to be negligible for carbon when it is used as one component in gas-producing cartridges placed in animal burrows. Ignited cartridges are to be quickly placed into burrows which are then covered to entrap the generated fumes. Improperly covered burrows could result in inhalation exposure to the fumes if the applicator remains in close proximity to the burrow. [USEPA/Office of Pesticide Programs; Reregistration Eligibility Decision Document - Carbon and Carbon Dioxide p.6 (September 1991). Available from, as of July 19, 2008: http://www.epa.gov/pesticides/reregistration/status.htm] **PEER REVIEWED**

As taken from HSDB, 2009

Activated carbon is listed as an ingredient of arts and crafts (1-5%), auto (1-10%), inside the home, pesticide (9.3%) and pet care (10-30%) products by the US Department of Health and Human Services (2017).

“CNT (carbon nanotubes) and CNF (carbon nanofibres) are currently used in many industrial and biomedical applications, including electronics, lithium-ion batteries, solar cells, super capacitors, thermoplastics, polymer composites, coatings, adhesives, biosensors, enhanced electron-scanning microscopy imaging techniques, inks, and in pharmaceutical/biomedical devices. CNT and CNF can be encountered in facilities ranging from research laboratories and production plants to operations where CNT and CNF are processed, used, disposed, or recycled. The data on worker personal exposures to CNT and CNF are extremely limited, but reported workplace airborne concentrations for CNT [Maynard et al. 2004; Han et al. 2008a; Bello et al. 2009, 2010; Tsai et al. 2009; Lee et al. 2010; Cena and Peters 2011; Dahm et al. 2011] and CNF [Methner et al. 2007; Evans et al. 2010; Birch 2011a; Birch et al. 2011b] indicate the potential for worker exposures in many tasks or processes and the reduction or elimination of exposures when measures to control exposure are used.

Occupational exposure to all types of CNT and CNF can be quantified using NIOSH Method 5040. A multi-tiered exposure measurement strategy is recommended for determining worker exposure to CNT and CNF [Section 6.1]. When exposure to other types of EC (e.g., diesel soot, carbon black) are absent or negligible, environmental background EC concentrations are typically < 1 µg/m3 including in facilities where CNT and CNF are produced and used [Evans et al. 2010; Birch 2011a, b; Dahm et al. 2011]. Thus, an elevated airborne EC concentration relative to background (environmental and in non-process areas in the workplace) is a reasonable indicator of CNT or CNF exposure. When exposure to other types of EC is possible, additional analytical techniques may be required to better characterize exposures. For example, analysis of airborne samples by

transmission electron microscopy (TEM) equipped with energy dispersive x-ray spectroscopy (EDS) can help to verify the presence of CNT and CNF.

....NIOSH recommends that exposures to CNT and CNF be kept below the recommended exposure limit (REL) of 1 µg/m3 of respirable elemental carbon as an 8-hr TWA. Because there may be other sources of elemental carbon in the workplace that could interfere in the determination of CNT and CNF exposures, other analytical techniques such as transmission electron microscopy are described that could assist in characterizing exposures. Studies have shown that airborne background (environmental and in non-process areas in the workplace) concentrations to elemental carbon are typically less than 1 µg/m3 and that an elevated exposure to elemental carbon in the workplace is a reasonable indicator of CNT or CNF exposure [Evans et al. 2010; Birch 2011a, b; Dahm et al. 2011]. Studies have also shown in some manufacturing operations that exposures can be controlled below the REL when engineering controls are used [Dahm et al. 2011]. However, NIOSH has not assessed the extent to which exposures can be controlled during the life cycle of CNT/CNF product use, but since airborne CNT/CNF behave as classical aerosols, the control of worker exposures appears feasible with standard exposure control techniques (e.g., source enclosure, local-exhaust ventilation) [NIOSH 2009a]. Previously in a 2010 draft of this CIB for public comment, NIOSH indicated that the risks could occur with exposures less than 1 µg/m3 but that the analytic limit of quantification was 7 µg/m3. Based on subsequent improvements in sampling and analytic methods, NIOSH is now recommending an exposure limit at the current analytical limit of quantification of 1 µg/m3….The recommended exposure limit is in units of mass/unit volume of air, which is how the exposures in the animal studies were quantified and it is the exposure metric that generally is used in the practice of industrial hygiene. In the future, as more data are obtained, a recommended exposure limit might be based on a different exposure metric better correlated with toxicological effects, such as CNT/CNF number concentration [Schulte et al. 2012].

There are many uncertainties in assessing risks to workers exposed to CNT/CNF. These uncertainties, as described and evaluated in this document, do not lessen the concern or diminish the recommendations. Other investigators and organizations have been concerned about the same effects and have recommended occupational exposure limits (OELs) for CNT within the range of 1–50 µg/m3 [Nanocyl 2009; Aschberger et al. 2010; Pauluhn 2010b; Nakanishi (ed) 2011a,b]. The relative consistency in these proposed OELs demonstrates the need to manage CNT/CNF as a new and more active form of carbon. To put this in perspective, since there is no Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for CNT/CNF, the PEL for graphite (5,000 µg/m3) or carbon black (3,500 µg/m3) [NIOSH 2007] might inappropriately be applied as a guide to control worker exposures to CNT/CNF. Based on the information presented in this document, the PELs for graphite or carbon black would not protect workers exposed to CNT/CNF.

In summary, the findings and recommendations in this Current Intelligence Bulletin are intended to minimize the potential health risks associated with occupational exposure to CNT and CNF by recommending a working lifetime exposure limit (1 µg/m3, 8-hr TWA, 45 years), a sampling and analytical method to detect CNT and CNF, medical surveillance and screening and other guidelines. The expanding use of CNT/CNF products in commerce and research warrants these protective actions."

As taken from NIOSH, 2013.

Summarised data on use levels of vegetable carbon in foodstuffs reported from industries.

Foodstuffs	**Data provided by**	**Reported range of typical use levels (lowest-highest) (mg/kg)**	**Maxiumun reported use levels (mg/kg)**
Desserts including flavoured milk products	NATCOL, 2007	100	2500
Confectionery	CIAA, 2009	5 – 2500	8000

	NATCOL, 2007	550	2000
Decorations and coatings	CIAA, 2009	10000	10000
Fine Bakery Wares	CIAA, 2009	37	60
Mustard	CIAA, 2009	200	200
Sauces[1]	CIAA, 2009	60 – 230	540
Margarine	CIAA, 2009	290	410
Edible ices	CIAA, 2009 NATCOL, 2007	135 – 157 500	1185 1250

[1] The levels reported for “sauces” have also been applied to the whole food category “Sauces, seasonings (for example curry powder, tandoori), pickles, relishes, chutney and piccalilli” because the consumption data available do not allow to differentiate sauces from the other foods covered by this food category.

As taken from EFSA, 2012b

Carbon black (CAS RN 1333-86-4 / 7440-44-0) is used as colourant ingredient in cosmetics in the EU, and charcoal powder (CAS RN 16291-96-6; 7440-44-0 (generic)) is used as an abrasive, absorbent and opacifying agent. As taken from CosIng (Cosmetic substances and ingredients database). Accessed February 2018, available at http://ec.europa.eu/growth/tools-databases/cosing/

“Human Health Assessment

……. When used as an additive in plastics, the substance is expected to be manufactured in or imported into Canada encapsulated in a solid polymer matrix. The potential site of exposure to the substance is expected to be within industrial facilities. Therefore, direct exposure of the general population is expected to be low. No significant environmental release is anticipated due to the specialized use under this notification and therefore indirect exposure of the general population from environmental media is also expected to be low. However, if the substance is produced in different forms (e.g. liquid polymer form), applied in different formulations or used in any other potential applications, an increased direct or indirect exposure potential may exist. ……. The use of the substance in consumer products or in products intended for use by or for children may significantly alter the exposure of the general population resulting in the substance becoming harmful to human health. Similarly, the import or manufacture of the substance in quantities greater than 10 000 kg/yr may significantly increase the exposure levels of the general population resulting in the substance becoming harmful to human health. …..”

As taken from Environment Canada, 2015

National Occupational Exposure Survey (1981 - 1983)
Estimated Numbers of Employees Potentially Exposed to Specific Agents by Occupation*

Agent Name	CARBON
CAS #	7440-44-0
RTECS #	FF5250000
Agent Code	80243

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
019	MANAGERS AND ADMINISTRATORS, N.E.C.	1,407	
027	PERSONNEL, TRAINING, AND LABOR RELATIONS SPECIALISTS	50	
045	METALLURGICAL AND MATERIALS ENGINEERS	64	

055	ELECTRICAL AND ELECTRONIC ENGINEERS	5	
056	INDUSTRIAL ENGINEERS	82	
057	MECHANICAL ENGINEERS	543	
059	ENGINEERS, N.E.C.	697	299
073	CHEMISTS, EXCEPT BIOCHEMISTS	930	191
084	PHYSICIANS	201	176
096	PHARMACISTS	738	369
213	ELECTRICAL AND ELECTRONIC TECHNICIANS	40	17
216	ENGINEERING TECHNICIANS, N.E.C.	726	131
224	CHEMICAL TECHNICIANS	2,607	39
235	TECHNICIANS, N.E.C.	572	25
278	NEWS VENDORS	423	
313	SECRETARIES	1,456	1,456
335	FILE CLERKS	413	
356	MAIL CLERKS, EXC. POSTAL SERVICE	5,446	
363	PRODUCTION COORDINATORS	8	
364	TRAFFIC, SHIPPING, AND RECEIVING CLERKS	191	52
365	STOCK AND INVENTORY CLERKS	1,537	16
374	MATERIAL RECORDING, SCHEDULING, AND DISTRIBUTING CLERKS, N.E.C.	13	
379	GENERAL OFFICE CLERKS	362	
385	DATA-ENTRY KEYERS	32	
389	ADMINISTRATIVE SUPPORT OCCUPATIONS, N.E.C.	1,513	
446	HEALTH AIDES, EXCEPT NURSING	203	142
447	NURSING AIDES, ORDERLIES, AND ATTENDANTS	46	46
449	MAIDS AND HOUSEMEN	55	
453	JANITORS AND CLEANERS	41,162	260
455	PEST CONTROL OCCUPATIONS	3,595	
503	SUPERVISORS, MECHANICS AND REPAIRERS	65	
505	AUTOMOBILE MECHANICS	17,614	
507	BUS, TRUCK, AND STATIONARY ENGINE MECHANICS	6,189	
514	AUTOMOBILE BODY AND RELATED REPAIRERS	3,595	
515	AIRCRAFT MECHANICS, EXC. ENGINE	308	
516	HEAVY EQUIPMENT MECHANICS	6,357	75
518	INDUSTRIAL MACHINERY REPAIRERS	2,993	
519	MACHINERY MAINTENANCE OCCUPATIONS	151	
523	ELECTRONIC REPAIRERS, COMMUNICATIONS AND INDUSTRIAL EQUIPMENT	382	
533	MISCELLANEOUS ELECTRICAL AND ELECTRONIC EQUIPMENT REPAIRERS	1,644	
534	HEATING, AIR CONDITIONING, AND REFRIGERATION MECHANICS	258	
538	OFFICE MACHINE REPAIRERS	2,169	
539	MECHANICAL CONTROLS AND VALVE REPAIRERS	37	
544	MILLWRIGHTS	2,127	
547	SPECIFIED MECHANICS AND REPAIRERS, N.E.C.	1,875	
549	NOT SPECIFIED MECHANICS AND REPAIRERS	17,844	8
558	SUPERVISORS, N.E.C.	1,317	
563	BRICKMASONS AND STONEMASONS	2,866	
567	CARPENTERS	5,261	
575	ELECTRICIANS	3,392	
579	PAINTERS, CONSTRUCTION AND MAINTENANCE	780	
585	PLUMBERS, PIPEFITTERS, AND STEAMFITTERS	34,135	96
587	PLUMBER, PIPEFITTER, AND STEAMFITTER APPRENTICES	460	
588	CONCRETE AND TERRAZZO FINISHERS	2,754	
593	INSULATION WORKERS	44	

595	ROOFERS	80	
596	SHEETMETAL DUCT INSTALLERS	3,086	
599	CONSTRUCTION TRADES, N.E.C.	878	
615	EXPLOSIVES WORKERS	306	
633	SUPERVISORS, PRODUCTION OCCUPATIONS	2,362	
634	TOOL AND DIE MAKERS	6,006	45
636	PRECISION ASSEMBLERS, METAL	16	
637	MACHINISTS	10,070	8
643	BOILERMAKERS	6,497	6
644	PRECISION GRINDERS, FITTERS, AND TOOL SHARPENERS	73	
645	PATTERNMAKERS AND MODEL MAKERS, METAL	514	
647	PRECIOUS STONES AND METALS WORKERS (JEWELERS)	765	
653	SHEET METAL WORKERS	11,716	71
655	MISCELLANEOUS PRECISION METAL WORKERS	364	
658	FURNITURE AND WOOD FINISHERS	1,234	608
675	HAND MOLDERS AND SHAPERS, EXCEPT JEWELERS	559	262
678	DENTAL LABORATORY AND MEDICAL APPLIANCE TECHNICIANS	1,581	
679	BOOKBINDERS	371	110
683	ELECTRICAL AND ELECTRONIC EQUIPMENT ASSEMBLERS	213	170
684	MISCELLANEOUS PRECISION WORKERS, N.E.C.	620	
689	INSPECTORS, TESTERS, AND GRADERS	28	
694	WATER AND SEWAGE TREATMENT PLANT OPERATORS	1,657	
695	POWER PLANT OPERATORS	35	28
696	STATIONARY ENGINEERS	70	
699	MISCELLANEOUS PLANT AND SYSTEM OPERATORS	855	
703	LATHE AND TURNING MACHINE SET-UP OPERATORS	459	
704	LATHE AND TURNING MACHINE OPERATORS	301	
705	MILLING AND PLANING MACHINE OPERATORS	162	
706	PUNCHING AND STAMPING PRESS MACHINE OPERATORS	2,531	336
707	ROLLING MACHINE OPERATORS	3,817	
708	DRILLING AND BORING MACHINE OPERATORS	14	
709	GRINDING, ABRADING, BUFFING, AND POLISHING MACHINE OPERATORS	4,486	146
713	FORGING MACHINE OPERATORS	350	
715	MISCELLANEOUS METAL, PLASTIC, STONE, AND GLASS WORKING MACHINE OPERATORS	122	
717	FABRICATING MACHINE OPERATORS, N.E.C.	379	57
719	MOLDING AND CASTING MACHINE OPERATORS	3,475	354
723	METAL PLATING MACHINE OPERATORS	3,652	207
724	HEAT TREATING EQUIPMENT OPERATORS	2,661	16
725	MISCELLANEOUS METAL AND PLASTIC PROCESSING MACHINE OPERATORS	1,693	
727	SAWING MACHINE OPERATORS	3	
734	PRINTING MACHINE OPERATORS	5,391	
745	SHOE MACHINE OPERATORS	2,193	1,571
748	LAUNDERING AND DRY CLEANING MACHINE OPERATORS	1,520	
753	CEMENTING AND GLUING MACHINE OPERATORS	198	198
754	PACKAGING AND FILLING MACHINE OPERATORS	337	145
755	EXTRUDING AND FORMING MACHINE OPERATORS	4,089	1,528
756	MIXING AND BLENDING MACHINE OPERATORS	2,235	250
757	SEPARATING, FILTERING, AND CLARIFYING MACHINE OPERATORS	4,681	8
758	COMPRESSING AND COMPACTING MACHINE OPERATORS	13	
759	PAINTING AND PAINT SPRAYING MACHINE OPERATORS	7,496	3,217
764	WASHING, CLEANING, AND PICKLING MACHINE OPERATORS	105	
766	FURNACE, KILN, AND OVEN OPERATORS, EXC. FOOD	2,960	

768	CRUSHING AND GRINDING MACHINE OPERATORS	828	35
769	SLICING AND CUTTING MACHINE OPERATORS	1,381	
777	MISCELLANEOUS MACHINE OPERATORS, N.E.C.	13,916	1,949
779	MACHINE OPERATORS, NOT SPECIFIED	8,753	918
783	WELDERS AND CUTTERS	104,631	1,471
784	SOLDERERS AND BRAZERS	8	
785	ASSEMBLERS	12,319	1,987
787	HAND MOLDING, CASTING, AND FORMING OCCUPATIONS	293	
796	PRODUCTION INSPECTORS, CHECKERS, AND EXAMINERS	2,154	342
797	PRODUCTION TESTERS	162	
798	PRODUCTION SAMPLERS AND WEIGHERS	827	
804	TRUCK DRIVERS, HEAVY	815	
844	OPERATING ENGINEERS	1,999	181
849	CRANE AND TOWER OPERATORS	1,086	
856	INDUSTRIAL TRUCK AND TRACTOR EQUIPMENT OPERATORS	1,131	
859	MISCELLANEOUS MATERIAL MOVING EQUIPMENT OPERATORS	1,894	
865	HELPERS, CONSTRUCTION TRADES	326	
869	CONSTRUCTION LABORERS	15,006	458
873	PRODUCTION HELPERS	774	
878	MACHINE FEEDERS AND OFFBEARERS	152	54
883	FREIGHT, STOCK, AND MATERIAL MOVERS, HAND, N.E.C.	85	
885	GARAGE AND SERVICE STATION RELATED OCCUPATIONS	684	
888	HAND PACKERS AND PACKAGERS	1,062	944
889	LABORERS, EXCEPT CONSTRUCTION	12,037	123
TOTAL		467,337	21,198

Agent Name	C, CARBON POWDER-MF UNKNOWN
CAS #	7440-44-0
RTECS #	FF5250000
Agent Code	X3261

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
073	CHEMISTS, EXCEPT BIOCHEMISTS	103	8
453	JANITORS AND CLEANERS	41	
563	BRICKMASONS AND STONEMASONS	123	
723	METAL PLATING MACHINE OPERATORS	65	
748	LAUNDERING AND DRY CLEANING MACHINE OPERATORS	185	
753	CEMENTING AND GLUING MACHINE OPERATORS	61	61
766	FURNACE, KILN, AND OVEN OPERATORS, EXC. FOOD	58	
779	MACHINE OPERATORS, NOT SPECIFIED	1,124	281
865	HELPERS, CONSTRUCTION TRADES	288	
888	HAND PACKERS AND PACKAGERS	1,124	422
889	LABORERS, EXCEPT CONSTRUCTION	37	
TOTAL		3,210	772

Agent Name	CARBON, FIBER
CAS #	7440-44-0
RTECS #	
Agent Code	X9503

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
224	CHEMICAL TECHNICIANS	36	
579	PAINTERS, CONSTRUCTION AND MAINTENANCE	318	297
653	SHEET METAL WORKERS	213	128

753	CEMENTING AND GLUING MACHINE OPERATORS	711	558
777	MISCELLANEOUS MACHINE OPERATORS, N.E.C.	43	
785	ASSEMBLERS	72	14
888	HAND PACKERS AND PACKAGERS	6	3
889	LABORERS, EXCEPT CONSTRUCTION	28	11
TOTAL		1,427	1,011

*(1) The estimates for each occupation apply across the surveyed industries in which the agent was observed. Not all industries were surveyed, and not all agents were observed in all surveyed industries. (2) When using the estimates,standard errors associated with estimates should be considered. (3) Potential exposures to a chemical agent are categorized as actual (i.e., the surveyor observed the use of the specific agent) or tradename (i.e., the surveyor observed the use of a tradename product known to contain the specific agent). The estimates presented in the table combine both categories.

As taken from NIOSH

2.2. Combustion products

No data available to us at this time

2.3. Ingredient(s) from which it originates

“A solid, porous, carbonaceous material prepared by carbonizing and activating organic substances. The raw materials, which include sawdust, peat, lignite, coal, cellulose residues, coconut shells, petroleum coke, etc., may be carbonized and activated at high temperature with or without the addition of inorganic salts in a stream of activating gases such as steam or carbon dioxide. Alternatively, carbonaceous matter may be treated with a chemical activating agent such as phosphoric acid or zinc chloride and the mixture carbonized at an elevated temperature, followed by removal of the chemical activating agent by water washing” (FAO/JECFA, 2010. Combined Compendium of Food Additive Specifications). As taken from http://www.fao.org/ag/agn/jecfa-additives/specs/monograph10/additive-006-m10.pdf

3. Status in legislation and other official guidance

NIOSH Recommendations:

NIOSH concluded that the documentation cited by OSHA was inadequate to support the proposed PEL (as an 8-hour TWA) of 10 mg/cu m for graphite (synthetic). [NIOSH. NIOSH Pocket Guide to Chemical Hazards & Other Databases CD-ROM. Department of Health & Human Services, Centers for Disease Prevention & Control. National Institute for Occupational Safety & Health. DHHS (NIOSH) Publication No. 2005-151 (2005)] **PEER REVIEWED**

FIFRA Requirements:

As the federal pesticide law FIFRA directs, EPA is conducting a comprehensive review of older pesticides to consider their health and environmental effects and make decisions about their future use. Under this pesticide reregistration program, EPA examines health and safety data for pesticide active ingredients initially registered before November 1, 1984, and determines whether they are eligible for reregistration. In addition, all pesticides must meet the new safety standard of the Food Quality Protection Act of 1996. Pesticides for which EPA had not issued Registration Standards prior to the effective date of FIFRA, as amended in 1988, were divided into three lists based upon

their potential for human exposure and other factors, with List B containing pesticides of greater concern and List D pesticides of less concern. Carbon is found on List D. Case No: 4019; Pesticide type: insecticide, rodenticide; Case Status: RED Approved 09/91; OPP has made a decision that some/all uses of the pesticide are eligible for reregistration, as reflected in a Reregistration Eligibility Decision (RED) document.; Active ingredient (AI): Carbon; AI Status: OPP has completed a Reregistration Eligibility Decision (RED) document for the case/AI. [United States Environmental Protection Agency/ Prevention, Pesticides and Toxic Substances; Status of Pesticides in Registration, Reregistration, and Special Review. (1998) EPA 738-R-98-002, p. 299] **PEER REVIEWED**

As taken from HSDB, 2009

"….This Account reviews the inhalation toxicity of manufactured nanomaterials and compares them with inhalation and intratracheal instillation studies of well-characterized fullerene and carbon nanotubes….The values of the acceptable exposure concentration in some countries were based on the data of subacute and subchronic inhalation and intratracheal instillation studies of well-characterized fullerene and carbon nanotubes. In Japan, the acceptable exposure concentration of fullerene is 0.39 mg/m³. In Europe, the proposal concentration is 44.4 μg/m³ for acute toxicity and 0.27 μg/m³ for chronic toxicity. The proposal acceptable exposure concentrations of carbon nanotubes are 0.03, 0.05, and 0.007 mg/m³ in Japan, Europe, and the United States, respectively." As taken from Morimoto Y et al. 2013. Acc. Chem. Res. 46(3), 770-81. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22574947

OSHA PEL (Gen Indu):8H TWA 15 mg/m3, total dust	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1910.1000,1994
OSHA PEL (Gen Indu):8H TWA 5 mg/m3, respirable fraction	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1910.1000,1994
OSHA PEL (Construc):8H TWA 15 mg/m3, total dust	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1926.55,1994
OSHA PEL (Construc):8H TWA 5 mg/m3, respirable fraction	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1926.55,1994
OSHA PEL (Shipyard):8H TWA 15 mg/m3, total dust	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1915.1000,1993
OSHA PEL (Shipyard):8H TWA 5 mg/m3, respirable fraction	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1915.1000,1993

Occupational Exposure Limits:

OEL-SWEDEN: TWA 3 mg/m3, JUN2005

OEL-UNITED KINGDOM: TWA 10 mg/m3 (inhal. dust), OCT2007

OEL-UNITED KINGDOM: TWA 4 mg/m3 (resp. dust), OCT2007

OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN check ACGIH TLV;

OEL IN SINGAPORE, VIETNAM check ACGIH TLV

As taken from RTECS, 2017.

UK 8-hr TWA for graphite (CAS RN 7440-44-0): 10 mg/m^3 (inhalable dust); 4 mg/m^3 (respirable dust) (HSE, 2013)

“Activated carbon should in addition comply with the same purity requirements as for Vegetable Carbon (E 153) set out by Commission Directive 95/45/EC with exception of ash content which can be up to 10 % (w/w)” (EFSA, 2012a, EFSA Journal 10(3):2643). As taken from http://www.efsa.europa.eu/en/efsajournal/doc/2643.pdf

E 153 Vegetable Carbon (EINECS 231-153-3) must comply with Comission Regulation (EU) No231/2012 laying down specification for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council.

The EFSA ANS Panel provides a scientific opinion re-evaluating the safety of vegetable carbon (E 153). Vegetable carbon has been evaluated previously by the SCF (1977, 1983) and by JECFA (1970, 1977, 1987). Neither Committee established an ADI for vegetable carbon, but the SCF concluded that vegetable carbon could be used in food The Panel considered the available toxicological data too limited to establish an ADI for vegetable carbon (EFSA 2012b).

Vegetable carbon (E 153) has been evaluated by JECFA in 1970, 1977 and 1987 (JECFA 1971, 1978,1987) and the SCF in 1977 and 1983 (SCF 1977, 1984). Both Committees did not establish an acceptable daily intake (ADI). However, “in view of its use as a traditional therapeutic agent”, the SCF recommended “the maintenance of the substance in the Directive for food use in general, despite the absence of extensive animal toxicological data” (SCF, 1977). In 1983, the Committee did not see any reason to change this evaluation (SCF, 1984). (EFSA 2012b)

There are REACH dossiers on “activated carbon - high density skeleton” and “activated carbon - low density skeleton” (CAS RNs not given for either) (ECHA, 2018a).

Carbon (CAS RN 7440-44-0) and “reaction mass of ACTIVATED CARBON and activated carbon” (no CAS RN provided) are pre-registered under REACH (“envisaged registration deadline 30 November 2010”) (ECHA, 2018b).

Neither “carbon” (CAS RN 7440-44-0), “activated carbon - high density skeleton” nor “activated carbon - low density skeleton” (CAS RNs not given for either) are classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2018c).

Carbon (CAS RN 7440-44-0) is listed in the US EPA Inert Finder Database (2018) as approved for food and non-food use pesticide products. For food use, it is regulated under 40 CFR Part 180.910 (Inert ingredients used pre- and post-harvest exemptions from the requirement of a tolerance) (US EPA, 2018).

Carbon (CAS RN 7440-44-0) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2012 CDR list (Chemical Data Reporting Rule) and 2012 and 2016 CDR Partial Exempt lists. The Chemical Data Reporting (CDR) Rule requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

The TSCA inventory and CDR lists are available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

Activated carbon (CAS RN 64365-11-3) is included on the FDA’s list of Everything Added to Food in the United States (EAFUS) and is included under 21 CFR 177.1210 (Indirect Food Additives: Polymers, Subpart B—Substances for Use as Basic Components of Single and Repeated Use Food Contact Surfaces, Closures with sealing gaskets for food containers) (FDA, 2013, 2018).

Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Activated carbon

Synonyms: CARBON, ACTIVATED VEGETABLE (FOOD GRADE), DECOLOURIZING CARBON

Chemical Names: CARBON

CAS number: 7440-44-0

Functional Class:
- Food Additives
  - ADSORBENT

BLEACHING_AGENT

Evaluation year: 1987

ADI: NOT LIMITED

Meeting: 31

Specs Code: R (1990)

Report: TRS 759-JECFA 31/25

Tox Monograph: FAS 70.39/NMRS 48A-JECFA 14/79 (1970)

Specification: COMPENDIUM ADDENDUM 11/FNP 52 Add. 11/89 (METALS LIMITS) (2003); FAO JECFA Monographs 1 vol.1/15

Previous Years: 1990, COMPENDIUM/21. R
1987, FNP 38-JECFA 31/43
1977, TRS 617-JECFA 21/28, NMRS 57-JECFA 21/4, FAS 70.39/NMRS 48A-JECFA 14/79 (1970). ADI NOT LIMITED. NL. R,T
1970, NMRS 48/TRS 462-JECFA 14/16, FAS 70.40/NMRS 48B-JECFA 14/39, FAS 70.39/NMRS 48A-JECFA

Vegetable carbon

Synonyms:	CARBON BLACK (VEGETABLE SOURCES), VEGETABLE BLACK
Chemical Names:	CARBON
CAS number:	7440-44-0; 1333-86-4 (CARBON BLACK)
INS:	○ 153
Functional Class:	Food Additives ○ COLOUR
Evaluation year:	1987
ADI:	NO ADI ALLOCATED
Meeting:	31
Specs Code:	R (1990)
Report:	TRS 759-JECFA 31/26
Tox Monograph:	NOT PREPARED
Specification:	COMPENDIUM ADDENDUM 10/FNP 52 Add.10/34 (METALS LIMITS) (2002). R; FAO JECFA Monographs 1 vol.3/587
Previous Years:	1990, COMPENDIUM/1579. R 1987, FNP 38-JECFA 31/47. R,T 1984, FNP 31/1-JECFA 28/43. R,T 1977, TRS 617-JECFA 21/17, NMRS VOL. II-IV/17 (1959). NOT PREPARED. DECISION POSTPONED. NO. S 1959, NMRS VOL. II-IV/17. N

As taken from JECFA, 2017.

Substance	Active carbon dust	
CAS No.	64365-11-3	
	Limit value - Eight hours	Limit value - Short term

	ppm	mg/m³	ppm	mg/m³
People's Republic of China		5 (1)		
	Remarks			
People's Republic of China	(1) Inhalable fraction			

Substance	Carbon fibres	
CAS No.		
	Limit value - Eight hours	Limit value - Short term

	ppm	mg/m³	ppm	mg/m³
Belgium	-	2 fibres per cm³	-	-
People's Republic of China		3 (1)		
	Remarks			
People's Republic of China	(1) Inhalable fraction			

As taken from GESTIS, 2017

Charcoal, activated (CAS RN 64365-11-3) is authorised for use as a food contact material in the EU under EU No 10/2011.

As taken from European Commission, 2009.

Vegetable carbon (E153) is authorised for use as a food additive in the EU under legislation nos 1129/2011, 738/2013 and, as a Group II, Food colour, under 380/2012, 438/2013 and 509/2013.

As taken from European Commission, 2018

Carbon is “not considered to pose an unreasonable risk to the health of workers and public health on the basis of the Tier I IMAP assessment” and is considered to be “an inorganic substance with low toxicity and/or low bioavailability” and “low concern to the environment” (NICNAS, 2018).

Substance	Carbon black
CAS #	1333-86-4 [1]/7440-44-0 [2]/- [3]/- [4]
EC #	215-609-9 [1]/231-153-3 [2]/931-328-0 [3]/931-334-3 [4]
Colour index Number / Name of Common Ingredients Glossary	77266
INN/ISO/AN	
Regulation	(EC) No 1120/2016
Regulated By	88/667/EEC
Other Directives/Regulations	
Annex/Ref #	IV/126
Colour	Black
Product Type, body parts	
Maximum concentration in ready for use preparation	
Other	Purity > 97 %, with the following impurity profile: Ash content ≤ 0,15 %, total sulphur ≤ 0,65 %, total PAH ≤ 500 ppb and benzo(a)pyrene ≤ 5 ppb, dibenz(a,h)anthracene ≤ 5 ppb, total As ≤ 3 ppm, total Pb ≤ 10 ppm, total Hg ≤ 1 ppm.
Wording of conditions of use and warnings	
SCCS opinions	
Chemical/IUPAC Name	Carbon black

Identified INGREDIENTS or substances e.g.	CARBON BLACK
Note	
Current Version	v.1

As taken from CosIng (Cosmetic substances and ingredients database). Accessed February 2018, available at http://ec.europa.eu/growth/tools-databases/cosing/

Carbon (CAS RN 7440-44-0) is included on the New Zealand Inventory of Chemicals with HSNO Approval Code HSR001271 (NZ EPA, 2006) and is classified according to the New Zealand authorities (NZ EPA CCID).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

"Carbon nanotubes (CNTs) consist of a family of carbon built nanoparticles, whose biological effects depend on their physical characteristics and other constitutive chemicals (impurities and functions attached)….Entrance into the body is physical, and usually few nanoparticles enter the body; however, once there, they are persistent due to their limited metabolisms, so their removal is slow…." As taken from Rodriguez-Yañez Y et al. 2013. Toxicol. Mech. Methods 23(3), 178-95. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23193995

4.2. Absorption, distribution and excretion

Exptl intravenous injection of pure carbon suspensions in rabbits produces no ocular inflammation, although carbon particles are deposited within the blood vessels. [Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 178] **PEER REVIEWED**

As taken from HSDB, 2009

"Further evidence for a correlation between geometric particle diameter and prolonged particle retention in airways was recently obtained from a study targeting 100 nm carbon particles to human airways by shallow aerosol bolus inhalation. In this study only 25% of the nanoparticles were removed by mucociliary clearance within 24 h, while 75% were retained for more than 48 h. Possible explanations for these findings are that the particles were no longer accessible to mucociliary clearance either because they penetrated through the mucus deep into the periciliary phase or that they were deposited in areas with reduced lung lining layer. In both cases, further interaction of particles with cells of the inner lung surface, i.e. macrophages, dendritic and epithelial cells is furthered and the probability for particle relocation beyond the epithelial barrier enhanced….There is evidence for translocation of gold…carbon nanoparticles in the size range of 5 - 100 nm across the air-blood barrier from animal experiments. Either, nanoparticles were found in the blood circulation and in secondary target organs, or thrombogenic effects were observed" (Geiser and Kreyling, 2010. Particle and Fibre Technology 7, 2). As taken from http://www.particleandfibretoxicology.com/content/pdf/1743-8977-7-2.pdf

"In this study, we prepared two-types of water-dispersible carbon nanotubes (CNTs) and investigated their biodistribution in mice as well as bio-/cyto-compatibility. After administration, their organs were excised at various post-injection times, then observed using both optical and transmission electron microscopy (TEM). The color of the liver and lung markedly darkened, suggesting that administered CNTs reached these organs. By TEM observation, the CNTs were found in the liver and lung. They were observed even in the kidney and spleen, though their

distributions in those organs were very low compared with that in liver and lung. Therefore, most of the administered CNTs would be accumulated in the liver or lung. However, the time profile of the body weight of CNT-administered mice was close to that of control mice. In addition, we estimated the cytocompatibility of the water-dispersible CNTs for hepatocytes. According to a TNF-alpha assay of the cells cultured with CNTs, the expression level was almost the same as that of the control. These results suggested that the water-dispersible CNTs have good bio-/cyto-compatibility under this condition" (Abe et al., 2012. Journal of Nanoscience and Nanotechnology 12, 700-706). As taken from *http://www.ncbi.nlm.nih.gov/pubmed/22524043*

"Lim and co-workers (Lim et al. 2011a and Lim et al. 2011b) administered 0 (control), 40, 200 or

1000 mg multi-wall CNTs (MWCNTs)/kg bw/day orally by gavage to pregnant Sprague-Dawley rats (N=12/group) from gestation days 6 through 19. The MWCNTs used were commercially available with a nominal diameter of 10-15 nm and length around 20 µm. The purity was stated to be 95% carbon and approximately 5% iron. The authors did not embark on any physico-chemical characterization and did not determine if aggregation of the CNTs occurred following the only 3 minutes ultrasound treatment in 0.1% carboxymethylcellulose (stabilizer) solution in water…..Conclusion: This study was not designed to be an absorption study, but, the toxic effects seen at the highest oral dose (1000 mg/kg bw/day), might give some indirect indication that material related to the MWCNTs was absorbed.

Awasthi and co-workers (Awasthi et al. 2013) administered male Swiss albino mice (N=6/group) single doses of 0 (vehicle control, distilled water), 60, or 100 mg/kg bw) of MWCNTs and studied hepatotoxicity on post dosing days 7, 14, 21 and 28 using liver SOD and CAT activity and microscopic examination as end-points. The tested MWCNTs, which were synthesised by chemical vapour deposition (CVD) technique, were purified and washed to remove metallic and carbonaceous impurities. Their size range was determined by SEM as 20–30 nm and length of 5–50 µm. The testing suspensions were made by physical mixing and ultrasonication of surface-oxidised material, but any further data on characterization or aggregation was missing…..Conclusion: The study does not support that any oral absorption of the test material occurred in mice.

Cicchetti and co-workers (Cicchetti et al. 2011) exposed human gingival fibroblasts in semiconfluent cultures to SWCNT concentrations between 50 and 150 µg SWCNTs/ml for 24 hours. The SWCNTs used were oxidized by treatment with a mixture of nitric and sulphuric acids. The surface area of was 407 m2/g, and the average external diameter was 1.58 nm ± 0.20 nm and the average length was 0.76 µm ± 0.70 µm. The SWCNTs were reported by the authors to have "a relatively high degree of crystallinity"….The effects seen in vitro indicated that SWCNT related material was absorbed into the cells, but did not prove the absorption of any intact nanomaterial.

Sachar and Saxena (Sachar and Saxena 2011) investigated the uptake of either SWCNTs or acid functionalized SWCNTs (AF-SWCNTs) in erythrocytes isolated from Swiss or C57BL76 female mice. The acid functionalized (AF)-SWCNTs were surface oxidized by a mixture of nitric and sulphuric acid under pressure at elevated temperature. The carboxylic acid moieties formed were derivatised by a fluorophor for imaging purposes, and were intensively purified to remove excess fluorescent dye. The particle size distribution and surface charge was not indicated. Particle size distribution and surface charge on AF-SWCNTs were reported before (Saxena et al. 2007 as cited in (Sachar and Saxena 2011)….Conclusion: This study suggested that some fluorescence related to exposure to fluorescence tagged AF-SWCNTs could enter erythrocytes, but no clear evidence about absorption of the intact NPs after oral exposure to SWCNT was provided.

In light of the occurrence of mainly negative data on absorption of CNT following oral exposure no evaluation of factors influencing their systemic absorption can be given."

As taken from Binderup et al. 2013.

"Multiwalled carbon nanotubes (MWCNT) are one of the most commonly produced nanomaterials,

and pulmonary exposure during production, use, and disposal is a concern for the developing nanotechnology field. The airway epithelium is the first line of defense against inhaled particles. In a mouse model, MWCNT were reported to reach the alveolar space of the lung after in vivo exposure, penetrate the epithelial lining, and result in inflammation and progressive fibrosis…." As taken from Snyder-Talkington BN et al. 2013a. Toxicol. Sci. 133(1), 79-89. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23377615

"BACKGROUND: SEVERAL PROPERTIES OF MULTI-WALLED CARBON NANOTUBES (MWCNT) HAVE THE POTENTIAL TO AFFECT THEIR BIOACTIVITY. THIS STUDY EXAMINED THE IN VITRO AND IN VIVO OUTCOMES OF THE INFLUENCE OF DIAMETER, LENGTH, PURIFICATION AND CARBOXYLATION (IN VITRO TESTING ONLY) OF MWCNT. METHODS: Three original 'as received' MWCNT that varied in size (diameter and length) were purified and functionalized by carboxylation. The resulting MWCNT were characterized and examined for cytotoxicity and inflammasome activation in vitro using THP-1 cells and primary alveolar macrophages from C57BL/6 mice. Oropharyngeal aspiration administration was used to deliver original MWCNT and in vivo bioactivity and lung retention was examined at 1 and 7 days. RESULTS:….Seven-day histology revealed that, consistent with the in vitro results, increasing width or length of MWCNT caused more severe pathology with the longest MWCNT causing the most severe inflammation. In addition, the same two larger MWCNT were retained more in the lung at 7 days…." As taken from Hamilton RF Jr et al. 2013. Part. Fibre Toxicol. 10(1), 57. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24225053

"The hallmark geometric feature of single-walled carbon nanotubes (SWCNT) and carbon nanofibers (CNF) - high length to width ratio - makes them similar to a hazardous agent - asbestos. Very limited data are available concerning long-term effects of pulmonary exposure to SWCNT or CNF. Here we compared inflammatory, fibrogenic and genotoxic effects of CNF, SWCNT or asbestos in mice one year after pharyngeal aspiration. In addition, we compared pulmonary responses to SWCNT by bolus dosing through pharyngeal aspiration and inhalation 5h/day for 4 days, to evaluate the effect of dose rate. The aspiration studies showed that, these particles can be visualized in the lung at one year post-exposure, while some translocate to lymphatics…." As taken from Shvedova AA et al. 2014. Am. J. Physiol. Lung Cell. Mol. Physiol. 306(2), L170-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24213921

"Understanding the excretion pathway is one of the most important prerequisites for the safe use of nanoparticles in biomedicine. However, the excretion of nanoparticles in human remains largely unknown, except for some particles very small in size. Here we report a novel natural pathway for nanoparticle excretion, the intestinal goblet cell (GC) secretion pathway (IGCSP). Direct live observation of the behavior of 30-200nm activated carbonnanoparticles (ACNP) demonstrated that ACNP microinjected into the yolk sac of zebrafish can be excreted directly through intestinal tract without involving the hepato-biliary (hap-bile) system. Histopathological examination in mice after ligation of the common bile duct (CBD) demonstrated that the intravenously-injected ACNP were excreted into the gut lumen through the secretion of intestinal GCs. ACNP in various secretion phases were revealed by histopathological examination and transmission electron microscopy (TEM). IGCSP, in combination with renal and hap-bile pathways, constitutes a complete nanoparticle excretion mechanism." As taken from Zhao B et al. 2014. Nanomedicine 10(4), 839-49. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24183999

"Carbon nanotubes have shown broad potential in biomedical applications, given their unique mechanical, optical, and chemical properties. Functionalized carbon nanotubes not only can deliver drug into specific organs but also can inherently produce heating by near-infrared laser radiation for cancer therapy. However, the toxicological and pharmacological profile of such carbon nanotube system will have to be determined prior to any clinical study undertaken. For providing a guide to

develop safe drug carriers, this review discusses the functionalization, toxicity and pharmacokinetics of carbon nanotubes. Lastly, the drug delivery and thermal ablation on carbon nanotubes are proposed." As taken from Luo E et al. 2013. Curr. Drug Metab. 14(8), 879-90. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24016108

"Because of their mechanical strength, chemical stability, and low molecular weight, carbon nanotubes (CNTs) are attractive biological implant materials. Biomaterials are typically implanted into subcutaneous tissue or bone; however, the long-term biopersistence of CNTs in these tissues is unknown. Here, tangled oxidized multi-walled CNTs (t-ox-MWCNTs) were implanted into rat subcutaneous tissues and structural changes in the t-ox-MWCNTs located inside and outside of macrophages were studied for 2 years post-implantation. The majority of the large agglomerates were present in the intercellular space, maintained a layered structure, and did not undergo degradation. By contrast, small agglomerates were found inside macrophages, where they were gradually degraded in lysosomes. None of the rats displayed symptoms of cancer or severe inflammatory reactions such as necrosis. These results indicate that t-ox-MWCNTs have high biopersistence and do not evoke adverse events in rat subcutaneous tissue in vivo, demonstrating their potential utility as implantable biomaterials." As taken from Sato Y et al. 2013. Sci. Rep. 3, 2516. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23981952

"In spite of the extreme rise to the knowledge of nanotechnology in pharmaceutical sciences, there are currently limited experimental works studying the interactions between nanoparticles (NPs) and the biological system. Adjustment of size and surface area plays the main role in the reaction between NPs and cells leading to their increased entrance into cells through skin, gastrointestinal and respiratory system. Moreover, change in physicochemical reactivity of NPs causes them to interact with circulatory and cellular proteins differentially leading to the altered parameters of their biokinetics, including adsorption, distribution, translocation, transformation, and elimination….Inhalation studies of some NPs have confirmed the translocation of inhaled materials to extra pulmonary organs such as central nervous system (CNS) via olfactory neurons and induction of inflammatory response. Injectable uncoated NPs have a tendency to remain on the injection site while the poly ethanol glycol (PEG)-coated NPs can be notably drained from the injection site to get as far as the lymph nodes where they accumulate. This confirms the existence of channels within the extracellular matrix for NPs to move along…." As taken from Mostafalou S et al. 2013. Daru 21(1), 14. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23432813

"….Entrance into the body is physical, and usually few nanoparticles enter the body; however, once there, they are persistent due to their limited metabolisms, so their removal is slow, and chronic cumulative health effects are studied…." As taken from Rodriguez-Yañez Y et al. 2013. Toxicol. Mech. Methods 23(3), 178-95. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23193995

"We summarized the findings of in vivo toxicity studies of single-walled carbon nanotubes (SWCNTs) in laboratory animals. …... Injected SWCNTs were distributed throughout most of the organs including the brain, mainly retained in the lungs, liver, and spleen, and eliminated through the kidney and bile duct. Orally administered SWCNTs are suggested to be absorbed from the gastrointestinal tract to the blood circulation in mice and rats. …... Overall, the available data provides initial information on SWCNT toxicity. To further clarify their toxicity and risk assessment, studies should be conducted using well-characterized SWCNTs, standard protocols, and the relevant route and doses of human exposure." As taken from Ema M et al. 2016. Regul. Toxicol. Pharmacol. 74, 42-63. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26619783

4.3. Interactions

“Etoposide is a semisynthetic, chemotherapeutic drug widely recommended to treat an extensive range of human cancers. Our studies indicate that, while etoposide is capable of killing human cancer cells, exposure to single-walled carbon nanotubes (SWCNTs) and etoposide results in enhanced cell death that appears to be synergistic and not merely additive. In this study, we used high pressure liquid chromatography and mass spectrometry to quantify the internal effective dose of etoposide when the human pancreatic cancer cell (PANC-1) was exposed to the combination of these agents. Our results unequivocally indicate that SWCNTs improve etoposide uptake and increase its capacity to kill cancer cells. We suggest that a combination of SWCNTs and etoposide may prove to be a more efficient chemotherapeutic protocol, especially because of the potential to lower toxicdrug doses to levels that may be useful in decreasing adverse side effects, as well as in lowering the probability of inducing chemoresistance in exposed cancer cells.” As taken from Mahmood M et al. 2013. Nanotechnology 24(4), 045102. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23291321

“In order to assess the in vivo efficacy of mycotoxin binders, specific toxicokinetic parameters should be measured according to European guidelines. For this purpose, an absorption model in pigs is described with emphasis on absorption kinetics. Pigs received a single oral bolus of the mycotoxin deoxynivalenol alone or in combination with active carbon (applied as mycotoxin binder). After administration of deoxynivalenol alone, significant plasma amounts of deoxynivalenol were detected and kinetic parameters were calculated using a one compartmental model. Activated carbon completely prevented the absorption of deoxynivalenol as no plasma amounts could be detected”. As taken from Devreese M et al. 2014. Toxins (Basel) 6(10), 2998-3004. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/25337799

“OBJECTIVES: Comparative in vivo studies were carried out to determine the adsorption characteristics of amitriptyline (AMT) on activated charcoal (AC) and sodium polystyrene sulfonate (SPS). AC has been long used as gastric decontamination agent for tricyclic antidepressants and SPS has showed to be highly effective on in-vitro drugs adsorption. MATERIALS AND METHODS: Sprague-Dawley male rats were divided into six groups. Group I: control, group II: AMT 200 mg/kg as single dose orally, group III and IV: AC 1g/kg as single dose orally 5 and 30 min after AMT administration respectively, and group 5 and 6: SPS 1 g/kg as single dose orally 5 and 30 min after AMT administration, respectively. 60 min after oral administration of AMT (Tmax of AMT determined in rats), Cmax plasma levels were determined by a validated GC-Mass method. RESULTS: The Cmax values for groups II to IV were determined as 1.1, 0.5, 0.6, 0.1 and 0.3 µg/ml, respectively. CONCLUSION: AC and SPS could significantly reduce Cmax of AMT when administrated either 5 or 30 min after AMT overdose ($P<0.05$). However, SPS showed to be more effective than AC in reducing Cmax when was administrated immediately (5 min) after AMT overdose. The results suggest a more efficient alternative to AC for AMT and probably other TCA overdoses.” As taken from Yousefi G et al. 2017. Iran. J. Basic Med. Sci. 20(1), 46-52. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28133524

5. Toxicity

5.1. Single dose toxicity

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
dog	LD	intraperitoneal	> 5gm/kg (5000mg/kg)		Gekkan Yakuji. Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.
dog	LD	oral	> 5gm/kg		Gekkan Yakuji. Pharmaceuticals Monthly.

dog	LD	oral	(5000mg/kg)		Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.
dog	LD	subcutaneous	> 5gm/kg (5000mg/kg)		Gekkan Yakuji. Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.
mouse	LD	intraperitoneal	> 5gm/kg (5000mg/kg)		Gekkan Yakuji. Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.
mouse	LD	oral	> 5gm/kg (5000mg/kg)		Gekkan Yakuji. Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.
mouse	LD	subcutaneous	> 5gm/kg (5000mg/kg)		Gekkan Yakuji. Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.
mouse	LD50	intravenous	440mg/kg (440mg/kg)		Toxicology and Applied Pharmacology. Vol. 24, Pg. 497, 1973.
rat	LD	intraperitoneal	> 5gm/kg (5000mg/kg)		Gekkan Yakuji. Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.
rat	LD	oral	> 5gm/kg (5000mg/kg)		Gekkan Yakuji. Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.
rat	LD	subcutaneous	> 5gm/kg (5000mg/kg)		Gekkan Yakuji. Pharmaceuticals Monthly. Vol. 34, Pg. 416, 1992.

As taken from ChemIDplus available at https://chem.nlm.nih.gov/chemidplus/

Species	**Route**	**Dose data**
Rat	Oral	LD_{50}: > 10000 mg/kg bw
Rat	Inhalation	LC_{50}: > 64.4 mg/L

As taken from IUCLID Dataset (2000), Carbon (7440-44-0).

“Probable oral lethal dose (human) > 15 g/kg; more than 1 quart (2.2 lb) for 70 kg person (150 lb).”

Mouse intravenous LD_{50}: 440 mg/kg bw

LD50 Rat oral > 10,000 mg/kg [European Chemicals Bureau; IUCLID Dataset, Carbon (7440-44-0) p.13 (2000 CD-ROM edition). Available from, as of July 18, 2008: http://ecb.jrc.it/esis/esis.php] **PEER REVIEWED**

LD50 Mouse iv 440 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 704] **PEER REVIEWED**

As taken from HSDB, 2009

“Three female Crl:CD(SD) rats/group were dosed with single wall carbon nanotube (SWCNT) or multi wall carbon nanotube (MWCNT) four times by gavage at a total of 50 mg/kg bw or 200 mg/kg bw (four equally divided doses at one-hour intervals). Acute oral doses of SWCNT and MWCNT caused neither death nor toxicological effects, and thus the oral LD50 values for SWCNT and MWCNT were considered to be greater than 50 mg/kg bw and 200 mg/kg bw, in rats respectively…..It was suggested that SWCNT and MWCNT dosed by gavage reached the gastro-intestinal tract as agglomerates and were mostly excreted via feces”. (Matsumoto et al., 2012. Journal of Toxicological Sciences 37, 463-474). As taken from http://www.ncbi.nlm.nih.gov/pubmed/22687986

“The present study was conducted to assess the pulmonary and systemic responses in rats after intratracheal instillation of highly pure, well-dispersed, and well-characterized SWCNTs. Exposure to SWCNTs up to 2 mg/kg did not produce mortality, changes in clinical signs, or body weights during the observation period. Dose-dependent changes were observed in the lung weight, BALF inflammatory cells, and biochemical parameters such as LDH value, protein content, IL-1β and IL-6 activity, and histopathology. In the 0.04 mg/kg SWCNT-exposed group, almost no changes were observed during the observation period. In the 0.2 mg/kg SWCNT-exposed group, pulmonary inflammatory responses were observed after instillation. In the 1 mg/kg and 2 mg/kg SWCNT-exposed group, acute lung inflammation and subsequent granuloma accompanied by increased lung weights were observed. Furthermore, the histopathological findings in the lungs of rats exposed to SWCNTs showed inflammatory responses related with the vital reaction to the foreign substance that was instilled intratracheally, and there were no fibrosis, atypical lesion, or tumor-related findings even at the highest dose (2 mg/kg) of SWCNT-exposed groups up to 6 months after instillation. For all groups, histopathological changes due to the instillation exposure of SWCNTs were observed only in the lungs and lung-associated lymph nodes and not in the other tissues examined (i.e. the liver, kidney, spleen, and cerebrum)” (Kobayashi N et al., 2011. Inhalation Toxicology 23, 814-828). As taken from http://www.ncbi.nlm.nih.gov/pubmed/22004357

“Sachar and Saxena (Sachar and Saxena 2011) administered single doses (100 μg/animal) of either SWCNTs or acid functionalized SWCNTs (AF-SWCNTs) to inbred Swiss and C57BL76 female mice (6–12 week old, weighing 20-25 g; number per group not reported) by either intratracheal instillation, intravenous (i.v.) or intra-peritoneal (i.p.) injections, or orally by gavage. The acid functionalized (AF)-SWCNTs were surface oxidized by a mixture of nitric and sulphuric acid under pressure at elevated temperature. The carboxylic acid moieties formed were derivatised by a fluorophor for imaging purposes, and were intensively purified to remove excess fluorescent dye. The particle size distribution and surface charge was not indicated. A transient decrease was observed in the number of erythrocytes and levels of blood haemoglobin (from 3 to 48 hours but not after 72 hours) after i.v. injection and to a lesser extent after i.p. injections of AF-SWCNTs as compared to SWCNTs. Administration of AF-SWCNTs through oral gavage and the i.p. route did not reduce erythrocyte count (haemoglobin was apparently not measured for these routes of as no information is given in the paper).”

As taken from Binderup et al. 2013.

“BACKGROUND: Engineered nanomaterials (ENMs) have potential benefits, but they also present safety concerns for human health. Interlaboratory studies in rodents using standardized protocols are needed to assess ENM toxicity. METHODS: Four laboratories evaluated lung responses in C57BL/6 mice to ENMs delivered by oropharyngeal aspiration (OPA), and three labs evaluated Sprague-Dawley (SD) or Fisher 344 (F344) rats following intratracheal instillation (IT). ENMs tested included three forms of titanium dioxide (TiO2) [anatase/rutile spheres (TiO2-P25), anatase spheres

(TiO2-A), and anatase nanobelts (TiO2-NBs)] and three forms of multiwalled carbonnanotubes (MWCNTs) [original (O), purified (P), and carboxylic acid "functionalized" (F)]. One day after treatment, bronchoalveolar lavage fluid was collected to determine differential cell counts, lactate dehydrogenase (LDH), and protein. Lungs were fixed for histopathology. Responses were also examined at 7 days (TiO2 forms) and 21 days (MWCNTs) after treatment. RESULTS:….All MWCNT types caused neutrophilia at 1 day in three of four mouse labs and in all rat labs. Three of four labs observed similar histopathology to O-MWCNTs and TiO2-NBs in mice. CONCLUSIONS: ENMs produced similar patterns of neutrophilia and pathology in rats and mice. Although interlaboratory variability was found in the degree of neutrophilia caused by the three types of TiO2 nanoparticles, similar findings of relative potency for the three types of MWCNTs were found across all laboratories, thus providing greater confidence in these interlaboratory comparisons." As taken from Bonner JC et al. 2013. Environ. Health Perspect. 121(6), 676-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23649427

"With the development and application of carbon nanotubes (CNTs), the potential hazards of CNTs to biological systems and the environment are getting more and more attention. This review evaluated the effects of physicochemical properties of CNTs on toxicity and summarized the advances on the mechanism of CNTs toxicity. We also proposed the possible hazards associated with CNTs and harmful effects resulting from exposure of aquatic animals, bacteria and higher plants to CNTs *in vitro* and *in vivo*. The current knowledge and gaps on CNTs were outlined as a potential problem for the environment and human health. The current research gaps on CNTs toxicity were identified and the further studying focus was proposed, too. This essay concluded with a set of recommendations for the advancement of understanding of the role of CNTs and future challenges in environmental and ecotoxicological research." As taken from Du J et al. 2013. Environ. Toxicol. Pharmacol. 36(2), 451-62. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23770455

"Background: several properties of multi-walled carbon nanotubes (mwcnt) have the potential to affect their bioactivity. this study examined the in vitro and in vivo outcomes of the influence of diameter, length, purification and carboxylation (in vitro testing only) of mwcnt. methods: three original 'as received' mwcnt that varied in size (diameter and length) were purified and functionalized by carboxylation. the resulting mwcnt were characterized and examined for cytotoxicity and inflammasome activation in vitro using thp-1 cells and primary alveolar macrophages from c57bl/6 mice. oropharyngeal aspiration administration was used to deliver original mwcnt and in vivo bioactivity and lung retention was examined at 1 and 7 days. results:…the in vivo studies demonstrated that all three original mwcnt caused similar neutrophil influx at one day, but increasing length or diameter resulted in the lavaged cells to release more inflammatory cytokines (il-6, tnf-alpha, and il-1beta) ex vivo. seven-day histology revealed that, consistent with the in vitro results, increasing width or length of mwcnt caused more severe pathology with the longest mwcnt causing the most severe inflammation. in addition, the same two larger mwcnt were retained more in the lung at 7 days. conclusions: taken together, the results indicated that in vitro and in vivo bioactivity of mwcnt increased with diameter and length. purification had no significant modifying effect from the original mwcnt. functionalization by carboxylation completely eliminated the bioactive potential of the mwcnt regardless of size in in vitro testing." As taken from hamilton rf jr et al. 2013. part. fibre toxicol. 10(1), 57. pubmed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24225053

"Carbon nanotubes have shown broad potential in biomedical applications, given their unique mechanical, optical, and chemical properties. Functionalized carbon nanotubes not only can deliver drug into specific organs but also can inherently produce heating by near-infrared laser radiation for cancer therapy. However, the toxicological and pharmacological profile of such carbon nanotube system will have to be determined prior to any clinical study undertaken. For providing a guide to develop safe drug carriers, this review discusses the functionalization, toxicity and

pharmacokinetics of carbon nanotubes. Lastly, the drug delivery and thermal ablation on carbon nanotubes are proposed." As taken from Luo E et al. 2013. Curr. Drug Metab. 14(8), 879-90. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24016108

"Carbon nanotubes (CNTs) find their extensive application as a promising material in medicine due to unique characteristics. However, such materials have been accompanied with potentially hazardous effects on human health. The toxicity of CNTs may vary depending on their structural characteristics, surface properties and chemical composition. To gain insight into the toxicity of CNTs in vivo and in vitro, we summarize contributing factors for the toxic effects of CNTs in this review. In addition, we elaborate on the toxic effects and mechanisms in target sites at systemic, organic, cellular, and biomacromolecule levels. Various issues are reported to be effected when exposed to CNTs including (1) blood circulation, (2) lymph circulation, (3) lung, (4) heart, (5) kidney, (6) spleen, (7) bone marrow, and (8) blood brain barrier. Though there have been published reports on thetoxic effects of CNTs to date, more studies will still be needed to gain full understanding of their potential toxicity and underlying mechanisms." As taken from Wang J et al. 2013a. Curr. Drug. Metab. 14(8), 891-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24016107

"We evaluated local inflammatory activity of oxidized multiwalled carbon nanotubes in rat experimental models of acute inflammation (paw edema and hyperalgesia) by analyzing their toxicity in non-mesoendothelial tissues. Subcutaneous injection of the nanotubes induced paw edema, that was maximal in the first 2 h after administration at 0.1 mg/kg (43.25 +/- 3.8 AUC) and 1 mg/kg (30.1 +/- 1.8 AUC) compared to saline (18.32 +/- 02.05 AUC). The histopathological analysis showed acute inflammation characterized by vasodilatation, edema formation, neutrophil infiltrate and tissue damage. The nanotubes also elicited hyperalgesic response, seen by the increase of animal paw withdrawal that was maximal in the first 3 hours. The data obtained at the 3rd h was: 75 +/- 9.3% (0.01 mg/kg), 58 +/- 8.3% (0.1 mg/kg) and 53 +/- 6.69% (1 mg/kg) in relation with saline (28 +/- 3.5%). In conclusion, the oxidized multiwalled carbon nanotubes elicit inflammatory and hyperalgesic effects associated to severe tissue damage in rats." As taken from Pinto NV et al. 2013. J. Nanosci. Nanotechnol. 13(8), 5276-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23882754

"We summarized the findings of in vivo toxicity studies of single-walled carbon nanotubes (SWCNTs) in laboratory animals. The large majority addressed the pulmonary toxicity of SWCNTs in rodents. Inhalation, pharyngeal aspiration, and intratracheal instillation studies revealed that SWCNTs caused acute and chronic inflammation, granuloma formation, collagen deposition, fibrosis, …. in the lungs. Pulmonary toxicity of well-dispersed SWCNTs was more potent than less dispersed ones. ……. Oxidative stress was caused by the administration of SWCNTs. …... Overall, the available data provides initial information on SWCNT toxicity. To further clarify their toxicity and risk assessment, studies should be conducted using well-characterized SWCNTs, standard protocols, and the relevant route and doses of human exposure." As taken from Ema M et al. 2016. Regul. Toxicol. Pharmacol. 74, 42-63. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26619783

"Human Health Assessment

Based on the available hazard information on the substance [multi-walled carbon nanotube], the substance has a low potential for acute toxicity by the oral, dermal and inhalation routes of exposure (oral and dermal LD50 > 2000 mg/kg bw; inhalation LC50 > 1.3 mg/m3). …...."

As taken from Environment Canada, 2015

5.2. Repeated dose toxicity

"While environmental particles are associated with mortality and morbidity related to pulmonary and

cardiovascular (CV) disease, the mechanisms involved in CV health effects are not known. Changes in systemic clotting factors have been associated with pulmonary inflammation. We hypothesized that inhaled ultrafine particles result in an inflammatory response which may stimulate systemic clotting factor release. Adult male Wistar rats were exposed to either fine or ultrafine carbon black (CB) for 7 h. The attained total suspended particle concentrations were 1.66 mg/m(3) for ultrafine CB and 1.40 mg/m(3) for fine CB. Particle concentration of ultrafine particles was more than 10 times greater than that of fine particles and the count median aerodynamic diameter averaged 114 nm for the ultrafine and 268 nm for the fine carbon particles. Data were collected immediately, 16 and 48 h following exposure. Only ultrafine CB caused an increase in total bronchoalveolar lavage (BAL) leukocytes, whereas both fine (2-fold) and ultrafine (4-fold) carbon particles caused an increase in BAL neutrophils at 16 h postexposure. Exposure to the ultrafine, but not fine, carbon was also associated with significant increases in the total numbers of blood leukocytes. Plasma fibrinogen, factor VII and von Willebrand factor (vWF) were unaffected by particle treatments as was plasma Trolox equivalent antioxidant status (TEAC). Macrophage inflammatory protein-2 mRNA was significantly increased in BAL cells 48 h following exposure to ultrafine CB. The data show that there is a small but consistent significant proinflammatory effect of this exposure to ultrafine particles that is greater than the effect of the same exposure to fine CB." As taken from Gilmour et al., (2004), Toxicol Appl Pharmacol. 2004 Feb 15;195(1):35-44, available at
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=14962503&query_hl=26&itool=pubmed_docsum

Mice, rats and guinea pigs were exposed to steam-activated carbon at 8.12 mg/m^3 for 7 h/day, 5 days/week for 1 year. In all species there was deposition of carbon particles, primarily in the alveoli; the main effects were oedema of the lungs and interstitial pneumonitis. In rats, lipid pneumonia also occurred (Gross and Nau, 1967. Archives Environmental Health 14, 450-460).
http://legacy.library.ucsf.edu/tid/awe80g00/pdf;jsessionid=7A619891C95CD1AA1B20A0503B9359F5.tobacco03

"Five or ten Crl:CD(SD) rats/sex were dosed with SWCNT [single wall carbon nanotubes] once daily by gavage at a dose of 0 (control), 0.125, 1.25 or 12.5 mg/kg bw/day for 28 days with a 14-day recovery period (0 and 12.5 mg/kg bw/day groups). Six or twelve Crl:CD(SD) rats/sex were dosed with MWCNT [multi wall carbon nanotubes] once daily by gavage at a dose of 0 (control), 0.5, 5.0 or 50 mg/kg bw/day for 28 days with a 14-day recovery period (0 and 50 mg/kg bw/day groups). Based on no toxicological effects, the no observed adverse effect levels (NOAELs) of repeated dose toxicity of SWCNT and MWCNT were considered to be 12.5 mg/kg bw/day and 50 mg/kg bw/day (the highest dose tested), respectively. It was suggested that SWCNT and MWCNT dosed by gavage reached the gastro-intestinal tract as agglomerates and were mostly excreted via feces" (Matsumoto et al., 2012. Journal of Toxicological Sciences 37, 463-474). As taken from http://www.ncbi.nlm.nih.gov/pubmed/22687986

"….In this Organization for Economic Cooperation and Development (OECD) 413 guideline inhalation study with VGCF-H carbon nanofibers (CNFs), rats were exposed to 0, 0.54, 2.5 or 25 mg/m(3) CNF for 13 weeks. The standard toxicology experimental design was supplemented with bronchoalveolar lavage (BAL) and respiratory cell proliferation (CP) endpoints. BAL fluid (BALF) recovery of inflammatory cells and mediators (i.e., BALF- lactate dehydrogenase [LDH], microprotein [MTP], and alkaline phosphatase [ALKP] levels) were increased only at 25 mg/m(3), 1 day after exposure. No differences versus control values in were measured at 0.54 or 2.5 mg/m(3) exposure concentrations for any BAL fluid endpoints. Approximately 90% (2.5 and 25 mg/m(3)) of the BAL-recovered macrophages contained CNF. CP indices at 25 mg/m(3) were increased in the airways, lung parenchyma, and subpleural regions, but no increases in CP versus controls were measured at 0.54 or 2.5 mg/m(3). Based upon histopathology criteria, the NOAEL was set at 0.54 mg/m(3), because at 2.5 mg/m(3), "minimal cellular inflammation" of the airways/lung parenchyma

was noted by the study pathologist; while the 25 mg/m(3) exposure concentration produced slight inflammation and occasional interstitial thickening. In contrast, none of the more sensitive pulmonary biomarkers such as BAL fluid inflammation/cytotoxicity biomarkers or CP turnover results at 2.5 mg/m(3) were different from air-exposed controls. Given the absence of convergence of the histopathological observations versus more quantitative measures at 2.5 mg/m(3), it is recommended that more comprehensive guidance measures be implemented for setting adverse effect levels in (nano)particulate, subchronic inhalation studies including a WOE approach for establishing no adverse effect levels; and a suggestion that some findings should be viewed as normal physiological adaptations (e.g., normal macrophage phagocytic responses-minimal inflammation) to long-term particulate inhalation exposures." As taken from Warheit DB et al. 2013. Toxicol. Pathol. 41(2), 387-94. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23242579

"With the development and application of carbon nanotubes (CNTs), the potential hazards of CNTs to biological systems and the environment are getting more and more attention. This review evaluated the effects of physicochemical properties of CNTs on toxicity and summarized the advances on the mechanism of CNTs toxicity. We also proposed the possible hazards associated with CNTs and harmful effects resulting from exposure of aquatic animals, bacteria and higher plants to CNTs *in vitro* and *in vivo*. The current knowledge and gaps on CNTs were outlined as a potential problem for the environment and human health. The current research gaps on CNTs toxicity were identified and the further studying focus was proposed, too. This essay concluded with a set of recommendations for the advancement of understanding of the role of CNTs and future challenges in environmental and ecotoxicological research." As taken from Du J et al. 2013. Environ. Toxicol. Pharmacol. 36(2), 451-62. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23770455

"Carbon nanotubes have shown broad potential in biomedical applications, given their unique mechanical, optical, and chemical properties. Functionalized carbon nanotubes not only can deliver drug into specific organs but also can inherently produce heating by near-infrared laser radiation for cancer therapy. However, the toxicological and pharmacological profile of such carbon nanotube system will have to be determined prior to any clinical study undertaken. For providing a guide to develop safe drug carriers, this review discusses the functionalization, toxicity and pharmacokinetics of carbon nanotubes. Lastly, the drug delivery and thermal ablation on carbon nanotubes are proposed." As taken from Luo E et al. 2013. Curr. Drug Metab. 14(8), 879-90. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24016108

"Carbon nanotubes (CNTs) find their extensive application as a promising material in medicine due to unique characteristics. However, such materials have been accompanied with potentially hazardous effects on human health. The toxicity of CNTs may vary depending on their structural characteristics, surface properties and chemical composition. To gain insight into the toxicity of CNTs in vivo and in vitro, we summarize contributing factors for the toxic effects of CNTs in this review. In addition, we elaborate on the toxic effects and mechanisms in target sites at systemic, organic, cellular, and biomacromolecule levels. Various issues are reported to be effected when exposed to CNTs including (1) blood circulation, (2) lymph circulation, (3) lung, (4) heart, (5) kidney, (6) spleen, (7) bone marrow, and (8) blood brain barrier. Though there have been published reports on thetoxic effects of CNTs to date, more studies will still be needed to gain full understanding of their potential toxicity and underlying mechanisms." As taken from Wang J et al. 2013a. Curr. Drug. Metab. 14(8), 891-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24016107

"To date, NIOSH is not aware of any reports of adverse health effects in workers using or producing CNT (carbon nanotubes) or CNF (carbon nanofibres) . However, there are studies of animals exposed to CNT and CNF that are informative in predicting potential human health effects

consistent with ways in which scientists traditionally have used such data in recommending risk management strategies. NIOSH systematically reviewed 54 laboratory animal studies, many of which indicated that CNT/CNF could cause adverse pulmonary effects including inflammation (44/54), granulomas (27/54), and pulmonary fibrosis (25/54). …

Critical effect levels for the noncancerous lung effects estimated from the animal dose-response data (e.g., BMD, benchmark dose and BMDL, the 95% lower confidence limit estimates of the BMD) have been extrapolated to humans by accounting for the factors influencing the lung dose in each animal species. The no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) estimates reported in the subchronic inhalation studies were also evaluated as the critical effect levels. Working-lifetime exposure concentrations were calculated based on estimates of either the deposited or retained alveolar lung dose of CNT assuming an 8-hour time-weighted average (TWA) exposure during a 40-hour workweek, 50 weeks per year, for 45 years. Based on BMD modeling of the subchronic animal inhalation studies with MWCNT [Ma-Hock et al. 2009; Pauluhn 2010a], a working lifetime exposure of 0.2–2 µg/m3 (8-hour TWA concentration) was estimated to be associated with a 10% excess risk of early-stage adverse lung effects (95% lower confidence limit estimates) (Tables 5–1 and A–5). Risk estimates derived from short-term animal studies (Tables A–3 and A–4) were consistent with these estimates.

In addition to the BMD-based risk estimates, NOAEL or LOAEL values were used as the critical effect level in animals. As with the BMD(L) estimates, the human-equivalent working lifetime concentrations were estimated, although using dosimetric adjustment and uncertainty factors (Section A.6.3). The estimated human-equivalent working lifetime concentrations based on this approach were approximately 4–18 µg/m3 (8-hr TWA), depending on the subchronic study and the interspecies dose retention and normalization factors used. Dividing these estimates by data-suitable uncertainty factors (e.g., UFs of 20–60), and assuming a threshold model, the estimated zero risk levels were <1 µg/m3 as working lifetime 8-hr TWA concentrations. A recent subchronic inhalation (13-wk exposure plus 3 months follow-up) study of CNF in rats [DeLorme et al. 2012] showed qualitatively similar lung response as in a shorter-term (28-day) study of CNF administered by pharyngeal aspiration in mice [Murray et al. 2012] (Sections 3.5 and A.7). Using the NOAEL-based approach, the human-equivalent working lifetime concentration estimates were 1–4 µg/m3 (8-hr TWA), depending on the data and assumptions used to estimate the human-equivalent dose."

As taken from NIOSH, 2013.

"We summarized the findings of in vivo toxicity studies of single-walled carbon nanotubes (SWCNTs) in laboratory animals. The large majority addressed the pulmonary toxicity of SWCNTs in rodents. Inhalation, pharyngeal aspiration, and intratracheal instillation studies revealed that SWCNTs caused acute and chronic inflammation, granuloma formation, collagen deposition, fibrosis, …. in the lungs. Pulmonary toxicity of well-dispersed SWCNTs was more potent than less dispersed ones. ….. Oxidative stress was caused by the administration of SWCNTs. ……. Overall, the available data provides initial information on SWCNT toxicity. To further clarify their toxicity and risk assessment, studies should be conducted using well-characterized SWCNTs, standard protocols, and the relevant route and doses of human exposure." As taken from Ema M et al. 2016. Regul. Toxicol. Pharmacol. 74, 42-63. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26619783

5.3. Reproduction toxicity

This study was undertaken to test novel genetic polymorphisms involved in 1-carbon metabolism for a potential association with increased risk of developing pregnancy complications associated with uteroplacental insufficiency. STUDY DESIGN: This was a prospective cohort study consisting of 50 women at low risk and 93 women at high risk for having a pregnancy complication develop. Maternal and fetal DNA samples were genotyped for methionine synthase (MTR) A2756G, methionine synthase reductase (MTRR) A66G and methylenetetrahydrofolate dehydrogenase (MTHFD1) G1958A. A chi squared or chi(2) analysis was used to compare genotypes and

pregnancy outcome, 1-way analysis of variance and linear regression were used to compare genotype with continuous variables. RESULTS: The fetal MTR 2756 G allele was associated with uteroplacental insufficiency (P = .022, likelihood ratio = 10.4) and maternal homocysteine (P = .017). The maternal MTR A2756G polymorphism was associated with uteroplacental insufficiency (P = .049, likelihood ratio = 6.0), but only in mothers not supplementing with high-dose B-vitamins. The maternal MTHFD1 AA genotype was associated with intrauterine growth restriction (P = .047, likelihood ratio = 5.8). CONCLUSION: This study suggests the maternal and fetal MTR 2756 G allele is an important risk factor in the development of uteroplacental insufficiency. In addition, the maternal MTHFD1 1958 AA genotype may be associated with intrauterine growth restriction. As taken from Furness DL et al. Am J Obstet Gynecol. 2008, Sep; 199(3):276.e1-8. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=18771981&dopt=AbstractPlus

Pregnant female C57BL/10JHir mice were irradiated whole-body at 9 days of gestation with a single acute dose of carbon-ion radiation. The average linear energy transfer (LET) of the carbon ions was 50 keV/microm within a spread-out Bragg peak (SOBP). The effects were studied by scoring changes in the postnatal development of the mice as well as in the pigmentation of the cutaneous coats and tail tips of their offspring 22 days after birth. The percentage of live births was reduced in mice exposed to carbon ions at doses greater than 0.5 Gy. The survival to day 22 was also reduced in mice exposed to carbon ions at doses greater than 0.75 Gy. Moreover, the body weight at day 22 was reduced in mice exposed to carbon ions at doses greater than 0.1 Gy. A comparison of the survival to day 22 after exposure to carbon ions with our previous results for 60Co gamma rays indicated that carbon ions were twice as effective as gamma rays. White spots were found in the mid-ventrum as well as in the tail tips of offspring exposed to carbon ions in utero. The frequency and the size of the white spots in the mid-ventrum and in the tail tips increased as the dose increased. Carbon ions appear to be slightly more effective than the gamma rays used in our previous study. In the ventral white spots, no melanocytes were observed in the epidermis, dermis and hair follicles. These results indicate that prenatal exposure to carbon ions has a greater effect on the postnatal development and survival of mice than does exposure to gamma rays, and that the relative biological effectiveness is greater than that for effects on melanocyte development. As taken from Hirobe T; Eguchi-Kasai K; Murakami M. Radiat Res. 2004, Nov; 162(5):580-4 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=15624313&dopt=AbstractPlus

In the present study, the effects of 290 Me V/u carbon-ion beams and X-rays on the development of rat brain were compared. Pregnant rats were exposed to carbon-ion beams of the 6-cm spread-out Bragg Peak (SOBP) at a single dose of 1.5 Gy on day 19.0 (midnight) of gestation. Three other groups of pregnant rats were exposed to X-rays on day 19.0 at single doses of 1.5, 2.0 and 2.5 Gy. Sham-exposed pregnant rats were used as controls. The offspring were deeply anesthetized at postnatal day 1, 7, 11, and 6 weeks of age, and perfused via the heart with 4% paraformaldehyde fixative. In rats with 6 weeks of age size of brain mantle exposed to 1.5 Gy carbon-ion beams was significantly smaller than that exposed to 1.5 Gy X-rays and larger than that exposed to 2.5 Gy. Local fiber distribution in the cerebral cortices of rats at 1, 7 and 11 postnatal days was examined using fluorescent dye, DiI. In rats exposed to carbon-ion beams, abnormal DiI-labeled fiber distribution was observed at three different postnatal stages. Similar but less irregular distribution of DiI-labeled fibers was observed in rats exposed to any dose of X-ray. Furthermore, in the control at postnatal day 11, DiI-labeled fibers in the cerebral cortex showed layer-dependent distribution. However, in rats exposed to carbon-ion beams or any dose of X-ray, the layer-dependent distribution of DiI-labeled fibers was not observed. Abnormal small clusters of DiI-labeled fibers were only observed in the cerebral cortex of rats exposed to 1.5 Gy carbon-ion beams. These findings suggest that the biological effects of 1.5 Gy carbon-ion beams on development of brain mantle are nearly equivalent to those of 2.0 Gy X-rays. However, subtle but more important

abnormalities such as local fiber distribution in the cerebral cortex seemed to be more complicated. Funahashi A; Inouye M; Nakamura E; Takahashi S; Kubota Y. Teratology 1999 May;59(5):34A.

As taken from DART powered by Toxnet, 2009 available at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?DARTETIC

"OBJECTIVE: To investigate the effects of fetal nanoparticle exposure on reproductive function in male mice offspring.

ANIMAL(S): Forty pregnant ICR mice and 120 male offspring.

INTERVENTION(S): Two hundred microg of 14-nm carbon nanoparticles was administered intratracheally on days 7 and 14 of gestation, and reproductive function of male offspring was determined at ages 5, 10, and 15 weeks after birth.

MAIN OUTCOME MEASURE(S): Maternal and fetal growth, histologic changes in the testes, and daily sperm production (DSP).

RESULT(S): Histologic examination showed partial vacuolation of seminiferous tubules. and cellular adhesion of seminiferous epithelia was reduced at all three ages. In addition, DSP was significantly decreased in fetal carbon nanoparticle-exposed mice. The DSP in the fetal carbon nanoparticle-exposed mice decreased by 47% at the age of 5 weeks, by 34% at the age of 10 weeks, and by 32% at the age of 15 weeks. On the other hand, nanoparticle administration had no marked effect on body weight, testicle weight, epididymis weight, or serum testosterone concentration.

CONCLUSION(S): These findings suggest that fetal nanoparticle exposure affects the reproductive function of male offspring. In the future, it would be necessary to clarify the onset mechanisms of nanoparticle-induced male reproductive disorders" (Yoshida et al., 2010. Fertility and Sterility 93, 1695-1699). As taken from http://www.ncbi.nlm.nih.gov/pubmed/19446808

"A possible teratogenicity of multi-wall carbon nanotube (MWCNT) was assessed using ICR mice. MWCNTs were suspended in 2% carboxymethyl cellulose and given intraperitoneally or intra-tracheally to pregnant ICR mice on day 9 of the gestation. All fetuses were removed from the uterus on day 18 of the gestation, and were examined for external and skeletal anomalies. In the intraperitoneal study, various types of malformation were observed in all MWCNT-treated groups (2, 3, 4 and 5 mg/kg body weight, intraperitoneal). In contrast, such malformations were observed in groups given 4 or 5 mg/kg body weight, but not in that treated with 3 mg/kg in the intratracheal study. In either study, the number of litters having fetuses with external malformation and that of litters having fetuses with skeletal malformations were both increased in proportion to the doses of MWCNT. The present results are the first to report that MWCNT possesses the teratogenicity at least under the present experimental conditions. Mechanism(s) to result such malformations is yet unclear and further experiment is necessary" (Fujitani et al., 2012. Journal of Toxicological Sciences 37, 81-89). As taken from http://www.ncbi.nlm.nih.gov/pubmed/22293413

Type of Test	Route of Exposure	Species Observed	Dose Data	Sex/Duration	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Subcutaneous	Rodent - rat	167 mg/kg	female 8 day(s) after conception	Reproductive - Fertility - post-implantation mortality (e.g. dead and/or resorbed implants per total number of implants)	TJADAB Teratology, The International Journal of Abnormal Development. (Alan R. Liss, Inc., 41 E. 11th St., New York, NY 10003) V.1- 1968- Volume(issue)/page/year: 4,327,1971

As taken from RTECS, 2017.

“Lim and co-workers (Lim et al. 2011a and Lim et al. 2011b) administered 0 (control), 40, 200 or 1000 mg multi-wall CNTs (MWCNTs)/kg bw/day orally by gavage to pregnant Sprague-Dawley rats (N=12/group) from gestation days 6 through 19. The MWCNTs used were commercially available with a nominal diameter of 10-15 nm and length around 20 µm. The purity was stated to be 95% carbon and approximately 5% iron. The authors did not embark on any physico-chemical characterization and did not determine if aggregation of the CNTs occurred following the only 3 minutes ultrasound treatment in 0.1% carboxymethylcellulose (stabilizer) solution in water. According to the authors the no-observed–adverse-effect-level (NOAEL) was 200 mg MWCNTs/kg bw/day for maternal toxicity and 200 mg MWCNs/kg bw/day for developmental toxicity."

As taken from Binderup et al. 2013.

“Carbon nanoparticles, with their high biocompatibility and low toxicity, have recently been considered for biomedical applications, including antiangiogenic therapy. Critical to normal development and tumor formation, angiogenesis is the process of forming capillary blood vessels from preexisting vessels. In the present study, we evaluated the effects of diamond and graphite nanoparticles on the development of chicken embryos, as well as vascularization of the chorioallantoic membrane and heart at the morphological and molecular level. Nanoparticles did not affect either body/heart weight or serum indices of the embryos' health. However, vascularization of the heart and the density of branched vessels were significantly reduced after treatment with diamond nanoparticles and, to a lesser extent, graphite nanoparticles. Application of nanoparticles significantly downregulated gene and protein expression of the proangiogenic basic fibroblast growth factor, indicating that both diamond and graphite nanoparticles inhibit angiogenesis.” As taken from Wierzbicki M et al. 2013. Int. J. Nanomedicine 8, 3427-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24039425

“In order to investigate the effect of SWCNTs in the embryo, we examined the outcome of SWCNTs in avian embryo at an early stage of development. We found that SWCNTs-treatment inhibits the angiogenesis of the chorioallantoic membrane (CAM) and in the chicken embryo. Moreover, we showed that SWCNTs can harm the normal development of the embryo since all SWCNTs-exposed embryos are smaller in comparison with their matched controls. We also found that the majority of SWCNTs-exposed embryos die before 12days of incubation. Macroscopic examination did not reveal any anomalies in these embryos. However, RT-PCR analysis of eleven genes, which are important regulators of cell proliferation, apoptosis, survival and angiogenesis, shows that these genes are deregulated in brain and liver tissues from SWCNTs-treated embryos in comparison with their matched controls. This study suggests that SWCNTs could have a very toxic effect on the normal development of the embryo.”As taken from Roman D et al. 2013. Nanomedicine 9(7), 945-50. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23563045

5.4. Mutagenicity

“Mutagenicity of activated carbon adsorbate from drinking water collected in Niigata City was assayed by the Ames assay. Adsorbate was extracted from activated carbon with benzene, and then with ethanol. Although the benzene extract was not mutagenic, the ethanol one showed the mutagenic activity for Salmonella typhimurium strains TA98 and TA100 with and without S9 mix. The ethanol extract was much more mutagenic on TA100 than TA98 both with and without S9 mix. The mutagenic activity per liter of water was found to be the strongest in winter and the weakest in summer.” As taken from Shibuya et al., (1993), Tohoku J Exp Med. 1993 Sep;171(1):89-95, available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=8122259&query_hl=13&itool=pubmed_docsum

“There is evidence that carbon nanotubes may induce aneuploidy by interaction with the mitotic spindle apparatus” (COM, 2012. COM/12/S1). As taken from http://www.iacom.org.uk/statements/documents/nanomaterialsfinal2012.pdf

“There is growing concern that gastrointestinal exposure to particles is associated with increased risk of toxicity to internal organs and carcinogenicity. The mechanism of action is related to particle-induced oxidative stress and oxidation of DNA. Observations from animal models indicate that gastrointestinal exposure to single-walled carbon nanotubes (SWCNT), fullerenes C60, carbon black, titanium dioxide and diesel exhaust particles generates oxidized DNA base lesions in organs such as the bone marrow, liver and lung. Oral exposure to nanosized carbon black has also been associated with increased level of lipid peroxidation derived exocyclic DNA adducts in the liver, suggesting multiple pathways of oxidative stress for particle-generated damage to DNA. At equal dose, diesel exhaust particles (SRM2975) generated larger levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine in rat liver than carbon black (Printex 90) did, whereas exposure to fullerenes C60 and SWCNT was the least potent. This ranking of samples was also observed for oxidatively damaged DNA in cultured cells. The extent of translocation from the gut is largely unresolved. However, there is evidence indicating that gastrointestinal exposure to particulate matter is associated with oxidative damage to DNA and this might be associated with increased risk of cancer” (Moller P et al. (2012). Current Molecular Medicine 12, 732-745. As taken from http://www.ncbi.nlm.nih.gov/pubmed/22292440

Nanomaterial	Nanoparticle Characteristics (Primary size)	Test System	Results
SWCNT	0.4-1.2 nm in diameter, 1-3μm in length.	Ames Salmonella assay using strains YG1024 and YG1029 without S9 mix.	-ve
MWCNT	Size unspecified.	Adenine phosphoribosyl-transferase (Aprt) mutation assay using Aprt-heterozygous mouse (C3H/Hej) embryonic stem cells.	+ve
SWCNT and MWCNTs	SWCNT (1.2-1.5 nm x 2.5μm) MWCNT (10-30 nm x 0.5-50μm).	Chromosome aberration assay in RAW264.7 cells (exposed for 24, 48, 72 h) to 1, 3 or 10 μg/ml.	+ve. Chromsome number in metaphases significantly different for both SWCNT and MWCNT. In addition chromosome breaks and irregularly condensed chromosomes were observed. Cytotoxicity was reported at concentrations of ≥50 μg/ml. ROS formation documented at 50 μg/ml..
SWCNT	0.4-1.2 nm in diameter, 1-3 μm in length composed of 99.7% carbon and 0.23% iron by weight.	Comet assay. Chinese hamster lung fibroblast V79 cells seeded into a medium containing 10% FCS.	After 3 h incubation, the highest concentration of SWCNT (96 μg/cm2) showed a 4.2-fold increase of Olive Tail Movement above the controls.
SWCNT	0.7-1.2 nm in diameter, 0.5-100 μm in length 96.7% carbon, 1.5% Co.	Alkaline murine assay in murine macrophage cell line RAW 264.7.	Comet +ve. Oxidized purines increased significantly, whereas pyrimidines showed a significant increase ($P<0.001$) only at the highest concentration (100 μg/ml)

SWCNT	1.1 nm in diameter, 50 µm in length composed of 96% carbon.	Alkaline comet assay in human peripheral blood lymphocytes cultured in a medium containing 15% FCS.	-ve.
SWCNT	Average diameter 1.4 nm, 2-5 µm in length 70-90% purity.	Comet assay.Normal and malignant human mesothelial cells cultured in a medium containing 5% FCS exposed to 25 or 50 µg/cm2 SWCNTs for 24 h.	Exposure of NM cells to 25 or 50 µg/cm2 SWCNTs resulted in a 5.2- and 6.6-fold increase in DNA tail length migration. Reactive oxygen species (ROS) scavengers only moderately reduced DNA damage.
SWCNT	1-4 nm in diameter, 1-3 µm in length composed of >99.7% carbon and 0.23% iron by weight.	Comet assay. Chinese hamster lung fibroblast V79 cells seeded into a medium containing 10% fetal calf serum (FCS).	After 24 h incubation, SWCNT (48 µg/cm2) showed a 3-fold increase of Olive Tail Movement above the controls.
SWCNT	2-5 µm in length composed of 72% carbon.	Comet assay. FE1 Muta^{TM}Mouse lung epithelial cells, cultured in a medium containing 2% FCS with and without FPG [a lesion-specific repair enzyme].	Comet -ve. Significantly increased the level of FPG sensitive sites/oxidized purines by 56%, respectively. No effect on mutant frequency in cII gene.
SWCNT and MWCNTs	SWCNT (1.2-1.5 nm x 2.5µm) MWCNT (10-30 nm x 0.5-50µm).	Comet assay using mouse macrophage RAW264.7 (in DMEM supplemented with 10% fetal bovine serum). Exposure for 24h. Intracellular ROS and uptake determined.	Comet +ve with SWCNT and MWCNT with concomitant ROS production
SWCNT and MWCNTs	SWCNT (<2nm x 5-15µm) (S4) MWCNT 20-60 nm x 5-15µm (M1) MWCNT 60-100 nmx 1-2 µm (M2) MWCNT <10 nm x 1-2 µm (M4).	Comet assay using A549 cells in DMEM supplemented with 10% FBS (3h exposure at 50 µg/ml).	Comet +ve for M1 and M2 but not M3 S4 (in-vivo studies showed M1 and M2 induced inflammation M3 and S4 did not). No effects on cell viability in this study. Authors concluded DNA damage was related to thickness of NT (characteristics of NTs in culture medium not reported).
MWCNT	MWCNT 1.5 nm x, 12 µm (purified and surface functionalised by carboxylation).	Comet assay in normal human dermal fibrblasts (48h exposure). DNA laddering and cytotoxicity determined.	Comet +ve at all doses used. Dose-dependent increase in cytotoxicity, and apoptosis reported. Increase DNA damage in laddering assay reported at highest dose level used.
MWCNT	MWCNT (average 81±5nm x 8.19±1.7 µm. (ultrasonication in RPMI 1640, 0.1% FBS. MWCNTs predominantly long loosely associated strands with small amounts of agglomeration.	Comet assay (24h) in normal meosthelial and malignat human mesothelial cells. ROS and cell viability measured.	Dose-dependent increase in DNA damage. Dose-related reduction in cell viability and increase in apoptosis reported. Small increase in ROS found. Activation of γ-H2AX (indicating double strand breasks) and Poly (ADP-ribose) polymerase (PARP) (indicating strand breaks) reported.
MWCNT	110-170 nm in diameter, 5-9 µm in length >98% carbon.	Alkaline comet assay in murine macrophage cell line RAW 264.7.	Comet positive. Increase in DNA migration due to the oxidative damage to purines was observed at a concentration of 1 and 10 µg/ml, whereas pyrimidines showed a significant increase only at the highest mass concentration tested

			(100 µg/ml).
SWCNT	0.4-1.2 nm in diameter, 1-3 µm in length composed of 99.7% carbon and 0.23% iron by weight.	Micronucleus assay in Chinese hamster lung fibroblast V79 cells incubated in a medium without fetal calf serum (FCS).	After 24 h incubation, the highest concentration of SWCNT (96 µg/cm^2) showed no significant increase in DNA damage.
SWCNT	0.7-1.2 nm in diameter, 0.5- 100 µm in length 96.7% carbon, 1.5% Co.	Micronucleus assay in murine macrophage cell line RAW 264.7 cultured in a medium containing 10% FCS with post-treatment with cytochalasin-B 20 h after the addition of MWCNT to culture.	Increase in number of micronuclei at doses above 0.1 µg/ml (P<0.05).
SWCNT	1-2 nm in diameter, 400-800 nm in length 98% purity and surface area of 585 m2/g.	Micronucleus assay in human bronchial epithelial BEAS-2B cells cultured in a medium containing either 2% or 10% FCS for 48 h and either post- or co-treatment, or absence of cytochalasin-B.	Increase in number of micronuclei at doses above 0.1 µg/ml (P<0.05).
SWCNT	1-4 nm in diameter, 1-3 µm in length composed of 99.7% carbon and 0.23% iron by weight.	Micronucleus assay in Chinese hamster lung fibroblast V79 cells incubated in a medium without both FCS and cytochalasin-B treatment.	After 24 h incubation, SWCNT (12 µg/cm2) showed significant (1.9-fold) micronucleus induction.
SWCNT	Dimensions not stated 70% purity functionalised with amides.	Micronucleus assay in human lymphocytes cultured in a medium containing 10% FCS treated with 24 h delayed co-treatment with cytochalasin-B. Enumeration of gamma H2AX foci as a measure of double strand breaks of the DNA in normal human dermal fibroblasts.	Increase in micronucleus induction in both cell types. In the fibroblasts there was a 2.7-fold increase in gamma H2AX foci above the control.
SWCNT and MWCNTs	SWCNT (1.2-1.5 nm x 2.5µm) MWCNT (10-30 nm x 0.5-50µm) suspended in serum free culture medium DMEM.	Cytokinesis-block micronucleus (CBMN) assay using mouse macrophage RAW264.7 cells. (48h exposure).	CBMN +ve (dose-related) for both SWCNT and MWCNT. Cellular ROS reported.
MWCNT	11.3 nm in diameter, 0.7 µm in length 98% carbon with traces of cobalt and iron catalysts.	Micronucleus assay in rat liver epithelial (RLE) cells suspended in a medium containing 5% FCS and MCF-7 breast cancer cells in medium containing 10% FCS were exposed separately. Post-treatment with cytochalasin-B.	There was a significant increase in micronuclei, up to 2-fold at the cytotoxic dose of 50 µg/ml, in RLE epithelial cells, and centromere-positive and negative micronuclei were produced in the MCF-7 cells.
MWCNT	20-40 nm in diameter, 1-5 µm in length 99% purity.	Human lymphocytes cultured in a medium containing 10% FCS treated with 24 h delayed co-treatment with cytochalasin-B. Enumeration of gamma H2AX foci as a measure of double strand breaks of the DNA in normal human dermal fibroblasts.	Induced lymphocyte micronuclei and anaphase bridges among nuclei in binucleated cells. Acted as a clastogen and aneugen simultaneously.
MWCNT	110-170 nm in	Murine macrophage cell line RAW	Increase in number of micronuclei at

	diameter, 5-9 μm in length >98% carbon.	264.7 cultured in a medium containing 10% FCS with post-treatment with cytochalasin-B 20 h after the addition of MWCNT to culture.	doses above 1 μg/ml (P<0.05).
SWCNT	Diameter 1-4 nm, length 0.5-1 μm.	Primary human respiratory epithelial cells (SEAC) examined for chromosome gain/loss. Mitotic disruption investigated in immortalised human bronchial epithelial cells (BEAS 2B) (medium contained 10% serum). Doses of 0.024, 0.24, 2.4, 24 μg/cm^2 with an exposure period of 24h.	Dose-dependent increase in aneuploidy reported at all dose levels (and using vanadium pentoxide as a positive control) SWCNT interacted and sirupted the mitotic spindle and was also associated with DNA and centrosomes.
SWCNT	0.9-1.7 nm x ≤1μm. Analyses for size distribution in dosing solution not achieved due to complex morphology and bundling of SWCNTs.	Intratracheal dose of 54 μg given to apolipoprotein E knockout mice C57BL(ApoE-/-) suspended by sonication in 0.9% NaCl MilliQ water with 10% Bronchiolar lavage fluid (BAL). Animals sacrificed 3 h post dose. BAlfluid obtained for Comet assay.	+ve (% DNA in tail), also increased tail length (p<0.001) Data not presented Evidence for inflammation (increase in neurtophils and protein in BAL).

As taken from: Committee on Mutagenicity (COM) of Chemicals in Food, Consumer Products and the Environment (2012). Statement on genotoxicity assessment of nanomaterials and experimental considerations. COM/12/S1. Available at

http://www.iacom.org.uk/statements/documents/nanomaterialsfinal2012.pdf

“Cicchetti and co-workers (Cicchetti et al. 2011) exposed human gingival fibroblasts in semiconfluent cultures to SWCNT concentrations between 50 and 150 μg SWCNTs/ml for 24 hours. The SWCNTs used were oxidized by treatment with a mixture of nitric and sulphuric acids. The surface area of was 407 m2/g, and the average external diameter was 1.58 nm ± 0.20 nm and the average length was 0.76 μm ± 0.70 μm. The SWCNTs were reported by the authors to have “a relatively high degree of crystallinity”. The authors reported a genotoxic effect ([DNA damage by the alkaline comet assay (from 75 μg/ml) and increase (at concentrations up to 100 μg/ml) or decrease (125 and 150 μg/ml) in the frequency of micronuclei]), decrease in cell proliferation and survival (125 and 150 μg/ml), increase in reactive oxygen species production (at all concentrations) and Hsp70 induction (at all concentrations).

Szendi and Varga (Szendi and Varga 2008) studied the possible genotoxicity of SWCNTs (<2nm x 4–15 μm, purity: 90%) and MWCNTs (10-30 nm x 1-2 μm; purity: 95% - 98%) dispersed in carbopol-based semiliquid gel. Urine samples obtained 24 hours after treatment by oral gavage of Fischer-344 male rats (N=3/group) with single doses of 0 (vehicle) or 50 mg/kg bw ofSWCNTs or MWCNTs, were 10x concentrated and were tested in bacterial mutation assay (Ames test) in Salmonella typhimurium TA98 and TA100 strains with and without metabolic activation. Oral exposure to the nanotubes did not increase urinary mutagenicity under the conditions of the assay. In addition, no genotoxic effects of SWCNTs or MWCNTs were found in the in vitro micronucleus and sister chromatid exchange assays using human lymphocytes.”

As taken from Binderup et al. 2013.

“The hallmark geometric feature of single-walled carbon nanotubes (SWCNT) and carbon nanofibers (CNF) - high length to width ratio - makes them similar to a hazardous agent - asbestos. …. Here we compared inflammatory, fibrogenic and genotoxic effects of CNF, SWCNT

or asbestos in mice one year after pharyngeal aspiration. …….SWCNT induced cytogenetic alterations seen as micronuclei formation and nuclear protrusions in vivo. Importantly, inhalation exposure to SWCNT showed significantly greater inflammatory, fibrotic and genotoxic effects than bolus pharyngeal aspiration. Finally, SWCNT and CNF, but not asbestos exposures, increased the incidence of K-ras oncogene mutations in the lung….Overall, our data suggest that long-term pulmonary toxicity of SWCNT, CNF and asbestos - is defined not only by their chemical composition but also by the specific surface area and type of exposure." As taken from Shvedova AA et al. 2014. Am. J. Physiol. Lung Cell. Mol. Physiol. 306(2), L170-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24213921

"In order to assess the safety of the carbon nanotubes to human health and the environment, we investigated the potential toxicity and ability of multi-walled carbon nanotubes (NT), to induce DNA damage by employing the Allium cepa genotoxicity/mutagenicity test and the Somatic Mutation and Recombination Test (SMART) in the fruitfly, Drosophila melanogaster. The results demonstrated that NT did not significantly induce genotoxic or mutagenic effects in the Allium cepa test. All concentrations evaluated in the SMART assay showed survival rates higher than 90 percent, indicating the absence of chronic toxicity for NT. Furthermore, the various treatments showed no significant increase in the NT mutation and recombination frequencies in mwh/flr(3) genotype compared to respective negative controls, demonstrating the absence of DNA damage caused by NT." As taken from de Andrade LR et al. 2014. Ecotoxicol. Environ. Saf. 99, 92-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24189313

"Peroxidase enzyme digests of oxidized single-wall carbon nanotubes (SWCNT) were shown to damage DNA in potentially genotoxic reactions for the first time using an electro-optical array with and without metabolic activation." As taken from Pan S et al. 2013. Toxicol. Res. 2(6), 375-378. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24159372

"….this study aims to evaluate the mutagenicity of multi-walled carbon nanotubes (MWCNTs) functionalized in somatic cells of Drosophila melanogaster, using the somatic mutation and recombination test (SMART). This assay detects the loss of heterozygosity of marker genes expressed phenotypically on the wings of the fly. Larvae of three days were used, resulting from ST cross, with basal levels of the cytochrome P450 and larvae of high metabolic bioactivity capacity (HB cross). They were treated with different concentrations of MWCNTs functionalized. The MH descendants, analyzed in both ST and HB crosses, had no significant effects on the frequency of mutant. Based on the results and on the experimental conditions mentioned in this study, it was concluded that MWCNTs were not mutagenic in D. melanogaster." As taken from Machado NM et al. 2013. Food Chem. Toxicol. 62, 355-60. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23994091

"Multiple-walled carbon nanotubes (MWCNTs) may cause carcinogenesis. We found that long-term exposure to MWCNTs can induce irreversible oncogenic transformation of human bronchial epithelial cells and tumorigenicity in vivo. A genome-wide array-comparative genomic hybridization (aCGH) analysis revealed global chromosomal aberration in MWCNTs-treated clones, predominantly at chromosome 2q31-32, where the potential oncogenes HOXD9 and HOXD13 are located. Functional assays confirmed that this variation can modulate oncogenic signaling and plays a part in MWCNTs-induced tumorigenesis, suggesting that MWCNTs are carcinogens that act by altering genomic stability and oncogenic copy numbers." As taken from Wu P et al. 2013. Nano. Lett. 13(10), 4632-41. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23984819

"The present study explored the ecotoxicology of single-walled carbon nanotubes (SWCNTs) and their likely interaction with dissolved metals, with a focus on the effect of in vivo exposure in marine

mussels….For the first time, the authors describe a potentiating toxicological effect, expressed as DNA strand breaks obtained using the comet assay, on divalent metals afforded by negatively charged SWCNT agglomerates in seawater at concentrations as low as 5 μg L^{-1}. This is supported by the observation that SWCNTs alone were only toxic at concentrations ≥100 μg L^{-1} and that the SWCNT-induced DNA damage was correlated with oxidative stress only in the absence of metals. If these laboratory experiments are confirmed in the natural environment, the present results will have implications for the understanding of the role of carbon nanotubes in environmental metal dynamics, toxicology, and consequently, regulatory requirements." As taken from Al-Shaeri M et al. 2013. Environ. Toxicol. Chem. 32(12), 2701-10. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23982896

"Toxicological characterization of manufactured nanomaterials (NMs) is essential for safety assessment, while keeping pace with innovation from their development and application in consumer products. The specific physicochemical properties of NMs, including size and morphology, might influence their toxicity and have impact on human health. The present work aimed to evaluate the genotoxicity of nanosized titanium dioxide (TiO2), synthetic amorphous silica (SAS) and multiwalled carbon nanotubes (MWCNTs), in human lymphocytes. The morphology and size of those NMs were characterized by transmission electron microscopy, while the hydrodynamic particle size-distributions were determined by dynamic light scattering. Using a standardized procedure to ensure the dispersion of the NMs and the cytokinesis-block micronucleus assay (without metabolic activation), we observed significant increases in the frequencies of micronucleated binucleated cells (MNBCs) for some TiO2 NMs and for two MWCNTs, although no clear dose-response relationships could be disclosed. In contrast, all forms of SAS analyzed in this study were unable to induce micronuclei. The present findings increase the weight of evidence towards a genotoxic effect of some forms of TiO2 and some MWCNTs. Regarding safety assessment, the differential genotoxicity observed for closely related NMs highlights the importance of investigating the toxic potential of each NM individually, instead of assuming a common mechanism and equal genotoxic effects for a set of similar NMs." As taken from Tavares AM et al. 2014. Toxicol. In Vitro 28(1), 60-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23811260

"OBJECTIVE: To compare the cytotoxicity and DNA strand breakage induced by multi-walled carbon nanotubes (MWCNTs) with different lengths and different surface modifications in human alveolar type II cells (A549 cells). METHODS: Two different lengths (5-15 μm, 350-700 nm) of MWCNTs and three different kinds of surface modified MWCNTs (COOH-MWCNTs, NH2-MWCNTs, and Tau-MWCNTs) were used in the experiments. The short MWCNTs were used as pristine MWCNTs to compare with the 3 surface modified MWCNTs. The cytotoxicity was determined by cell counting kit-8 (CCK-8) assay at the concentrations of 2, 8, and 32 mg/L at hours 12, 24, 36, and 48 respectively. Single cell gel electrophoresis (SCGE) assay was performed to evaluate DNA strand breakage in A549 cells after 24 h treatment of 8 mg/L of each tested material. RESULTS: Long multi-walled carbon nanotubes (Long-MWCNTs) and short multi-walled carbon nanotubes (Short-MWCNTs) showed a dose-dependent cytotoxicity within the exposure time 12-48 h. Especially, Long-MWCNTs showed greater cytotoxicity than Short-MWCNTs from 24 to 48 h at the same concentration. The relative cell viability of the 3 surface modified MWCNTs was higher than that of the pristine MWCNTs at h 12 at the concentration of 32 mg/L [COOH-MWCNTs (86.55±1.80)%, NH2-MWCNTs (84.67±1.32)%, Tau-MWCNTs (80.15±3.53)% and Pristine-MWCNTs (71.44±5.58)%], at h 24 at the concentration of 8 mg/L [COOH-MWCNTs (96.74±1.00)%, NH2-MWCNTs (96.74±3.35)%, Tau-MWCNTs (106.39±3.83)% and Pristine-MWCNTs (91.02±2.53)%], at h 24 at the concentration of 32 mg/L [COOH-MWCNTs (80.88±2.67)%, NH2-MWCNTs (82.90±3.25)%, Tau-MWCNTs (82.55±3.32)% and Pristine-MWCNTs (76.08±4.27)%] and at h 36 at the concentration of 8 mg/L [COOH-MWCNTs (96.87±1.05)%, NH2-MWCNTs (96.66±4.76)%, Tau-MWCNTs (100.23± 2.84)% and Pristine-MWCNTs (89.61±3.78)%], and the differences were statistically significant ($P<0.05$). Compared with

the Pristine-MWCNTs, the relative cell viability of the 3 surface modified MWCNTs didn't demonstrate a statistically significant difference (P>0.05) at other observation time and exposure concentrations. The DNA strand breakage of the 3 surface modified MWCNTs: the Olive tail moment of COOH-MWCNTs was 1.56±0.22, the Olive tail moment of NH2-MWCNTs 2.25±1.62 and the Olive tail moment of Tau-MWCNTs 2.23±0.94; the tail DNA% of COOH-MWCNTs was (3.96± 0.60)%, the tail DNA% of NH2-MWCNTs (6.16±4.68)% and the tail DNA% of Tau-MWCNTs (6.05±2.31)%, which were lower than that of the pristine MWCNTs (P<0.05), whose Olive tail moment was 3.00±0.64 and tail DNA% (8.23±2.27)%. Moreover, the COOH-MWCNTs induced the lowest DNA damage among the three modified MWCNTs. CONCLUSION: Long-MWCNTs compared with Short-MWCNTs demonstrated a greater cytotoxicity and lower DNA strand breakage damage. The surface modifications of MWCNTs can reduce the cytotoxicity and DNA strand breakage in A549 cells." As taken from Pu J et al. 2013. Beijing Da Xue Xue Bao. 45(3), 405-11. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23774918

"Novel materials are often commercialized without a complete assessment of the risks they pose to human health because such assessments are costly and time-consuming; additionally, sometimes the methodology needed for such an assessment does not exist. Carbon nanotubes have the potential for widespread application in engineering, materials science and medicine. However, due to the needle-like shape and high durability of multiwalled carbon nanotubes (MWCNTs), concerns have been raised that they may induce asbestos-like pathogenicity when inhaled. Indeed, experiments in rodents supported this hypothesis. Notably, the genetic alterations in MWCNT-induced rat malignant mesothelioma were similar to those induced by asbestos. Single-walled CNTs (SWCNTs) cause mitotic disturbances in cultured cells, but thus far, there has been no report that SWCNTs are carcinogenic. This review summarizes the recent noteworthy publications on the genotoxicity and carcinogenicity of CNTs and explains the possible molecular mechanisms responsible for this carcinogenicity. The nanoscale size and needle-like rigid structure of CNTs appear to be associated with their pathogenicity in mammalian cells, where carbon atoms are major components in the backbone of many biomolecules. Publishing adverse events associated with novel materials is critically important for alerting people exposed to such materials. CNTs still have a bright future with superb economic and medical merits. However, appropriate regulation of the production, distribution and secondary manufacturing processes is required, at least to protect the workers." As taken from Toyokuni S. 2013. Adv. Drug Deliv. Rev. 65(15), 2098-110. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23751780

"…..... Due to the current lack of hazardous effect information on SWNCTs, a standardized genotoxicity battery test was conducted to clarify the genetic toxicity potential of SWCNTs (diameter: 1-1.2 nm, length: ~20 μm) according to Organization for Economic Cooperation and Development test guidelines 471 (bacterial reverse mutation test), 473 (in vitro chromosome aberration test), and 474 (in vivo micronuclei test) with a good laboratory practice system. The test results showed that the SWCNTs did not induce significant bacterial reverse mutations at 31.3-500 μg/plate in Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 or in Escherichia coli strain WP2uvrA, with and without a metabolic activation system. Furthermore, the in vitro chromosome aberration test showed no significant increase in structural or numerical chromosome aberration frequencies at SWCNT dose levels of 12.5-50 μg/ml in the presence and absence of metabolic activation. However, dose-dependent cell growth inhibition was found at all the SWCNT dose levels and statistically significant cytotoxic effects observed at certain concentrations in the presence and absence of metabolic activation. Finally, the SWCNTs did not evoke significant in vivo micronuclei frequencies in the polychromatic erythrocytes of an imprinting control region mice at 25-100 mg/kg. Thus, according to the results of the present study, the SWCNTs were not found to have a genotoxic effect on the in vitro and in vivo test systems." As taken from Kim JS et al. 2015a. Toxicol. Ind. Health 31(8), 747-757. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/23552264

"Carbon nanotubes are unique one-dimensional macromolecules with promising application in biology and medicine. Since their toxicity is still under debate, here we describe an investigation

of genotoxic properties of purified single-walled carbon nanotubes (SWCNT), multiwall carbon nanotubes (MWCNT), and amide-functionalized purified SWCNT. We used two different cell systems: cultured human lymphocytes where we employed cytokinesis-block micronucleus test and human fibroblasts where we investigate the induction of DNA double-strand breaks (DSBs) employing H2AX phosphorylation assay." As taken from Nešković O et al. 2013. Methods Mol. Biol. 991, 315-23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23546681

"In order to study the effects of nanoparticles (NPs) with different physicochemical properties on cellular viability and structure, Saccharomyces cerevisiae were exposed to different concentrations of TiO2-NPs (1-3 nm), ZnO-NPs (<100 nm), CuO-NPs (<50 nm), their bulk forms, Ag-NPs (10 nm) and single-walled carbon nanotubes (SWCNTs). The GreenScreen assay was used to measure cyto- and genotoxicity, and transmission electron microscopy (TEM) used to assess ultrastructure….Two genotoxicity assays, GreenScreen and the comet assay, produced different results and the authors discuss the reasons for this discrepancy…." As taken from Bayat N et al. 2014. Nanotoxicology 8, 363-73. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23521755

"Single-wall carbon nanotubes (SWCNTs) and polyamidoamine dendrimers (PAMAM) have been proposed for a variety of biomedical applications. The combination of both molecules makes this new composite nanomaterial highly functionalizable and versatile to theranostic and drug-delivery systems. However, recent toxicological studies have shown that nanomaterials such as SWCNTs and PAMAM may have high toxicity in biological environments. Aiming to elucidate such behavior, in vitro studies with different cultured cells have been conducted in the past few years. This study focuses on the effects of SWCNT-PAMAM nanomaterials and their individual components on the C2C12 murine cell line, which is a mixed population of stem and progenitor cells. The interactions between the cells and the nanomaterials were studied with different techniques usually employed in toxicological analyses. The results showed that SWCNT-PAMAM and PAMAM inhibited the proliferation and caused DNA damage of C2C12 cells. Data from flow cytometry revealed a less toxicity in C2C12 cells exposed to SWCNT compared to the other nanomaterials. The results indicated that the toxicity of SWCNT, SWCNT-PAMAM and PAMAM in C2C12 cells can be strongly correlated with the charge of the nanomaterials." As taken from Cancino J et al. 2013. Toxicol. Lett. 219(1), 18-25. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23454831

"Although some types of carbon nanotubes (CNTs) have been described to induce mesothelioma in rodents and genotoxic effects in various cell systems, there are few previous studies on the genotoxicity of CNTs in mesothelial cells. Here, we examined in vitro DNA damage induction by short multi-wall CNTs (MWCNTs; 10-30 nm × 1-2 μm) and single-wall CNTs (SWCNTs; >50% SWCNTs, ~40% other CNTs; <2 nm × 1-5 μm) in human mesothelial (MeT-5A) cells and bronchial epithelial (BEAS 2B) cells, using the single cell gel electrophoresis (comet) assay and the immunoslot blot assay for the detection of malondialdehyde (M1dG) DNA adducts. In BEAS 2B cells, we also studied the induction of micronuclei (MN) by the CNTs using the cytokinesis-block method. The cells were exposed to the CNTs (5-200 μg/cm(2), corresponding to 19-760 μg/ml) for 24 and 48h in the comet assay and for 48 and 72 h in the MN and M1dG assays. Transmission electron microscopy (TEM) showed more MWCNT fibres and SWCNT clusters in BEAS 2B than MeT-5A cells, but no significant differences were seen in intracellular dose expressed as area of SWCNT clusters between TEM sections of the cell lines. In MeT-5A cells, both CNTs caused a dose-dependent induction of DNA damage (% DNA in comet tail) in the 48-h treatment and SWCNTs additionally in the 24-h treatment, with a statistically significant increase at 40 μg/cm(2) of SWCNTs and (after 48 h) 80 μg/cm(2) of both CNTs. SWCNTs also elevated the level of M1dG DNA adducts at 1, 5, 10 and 40 μg/cm(2) after the 48-h treatment, but both CNTs decreased M1dG adduct level at several doses after the 72-h treatment. In BEAS 2B cells, SWCNTs induced a

statistically significant increase in DNA damage at 80 and 120 μg/cm(2) after the 24-h treatment and in M1dG adduct level at 5 μg/cm(2) after 48 h and 10 and 40 μg/cm(2) after 72 h; MWCNTs did not affect the level of DNA damage but produced a decrease in M1dG adducts in the 72-h treatment. The CNTs did not affect the level of MN. In conclusion, MWCNTs and SWCNTs induced DNA damage in MeT-5A cells but showed a lower (SWCNTs) or no (MWCNTs) effect in BEAS 2B cells, suggesting that MeT-5A cells were more sensitive to the DNA-damaging effect of CNTs than BEAS 2B cells, despite the fact that more CNT fibres or clusters were seen in BEAS 2B than MeT-5A cells. M1dG DNA adducts were induced by SWCNTs but decreased after a 3-day exposure to MWCNTs and (in MeT-5A cells) SWCNTs, indicating that CNTs may lead to alterations in oxidative effects within the cells. Neither of the CNTs was able to produce chromosomal damage (MN).” As taken from Lindberg HK et al. 2013. Toxicology 313(1), 24-37. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23266321

“The genotoxicity of single-walled carbon nanotubes (SWCNTs) was determined using a battery of genotoxicity assays, comprising a bacterial reverse mutation test, an in vitro mammalian chromosomal aberration test and a mammalian erythrocytes micronucleus test. SWCNTs had no mutagenicityin S. typhimurium TA98, TA100, TA1535 or TA1537, or in E. coli WP2uvrA, in the absence or presence of metabolic activation. SWCNTs did not increase the number of structural or numerical chromosomal aberrations after short-term or continuous exposure. In the micronucleus test using CD-1 mice, SWCNTs did not affect the proportion of immature erythrocytes, the total proportion of erythrocytes or the number of micronuclei in immature erythrocytes. SWCNTs appear not to pose a genotoxic risk.” As taken from Ema M et al. 2013a. J. Appl. Toxicol. 33(9), 933-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22763644

“The genotoxicity of multi-walled carbon nanotubes (MWCNTs) was evaluated in vivo with comet assays using the lung cells of rats given MWCNTs. The MWCNTs were intratracheally instilled as a single dose at 0.2 or 1.0 mg kg(-1) or a repeated dose at 0.04 or 0.2 mg kg(-1) , once a week for 5 weeks, to male rats. The rats were sacrificed 3 or 24 h after the single instillation and were sacrificed 3 h after the last instillation in the repeated instillation groups. Histopathological examinations of the lungs revealed that MWCNTs caused inflammatory changes including the infiltration of macrophages and neutrophils after a single instillation and repeated instillation at both doses. In comet assays using rat lung cells, no changes in % Tail DNA were found in any group given MWCNTs. These findings indicate that MWCNTs do not have the potential to cause DNA damage in comet assays using the lung cells of rats given MWCNTs at doses causing inflammatory responses.” As taken from Ema M et al. 2013b. J. Appl. Toxicol. 33(10), 1053-60. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22936419

“The genotoxic effects of multi-walled carbon nanotubes (MWCNTs) were examined by using in vitro and in vivo assays. MWCNTs significantly induced micronuclei in A549 cells and enhanced the frequency of sister chromatid exchange (SCE) in CHO AA8 cells. When ICR mice were intratracheally instilled with a single dose (0.05 or 0.2 mg/animal) of MWCNTs, DNA damage of the lungs, analysed by comet assay, increased in a dose-dependent manner. Moreover, DNA oxidative damage, indicated by 8-oxo-7,8-dihydro-2'-deoxyguanosine and heptanone etheno-deoxyribonucleosides, occurred in the lungs of MWCNT-exposed mice. The gpt mutation frequencies significantly increased in the lungs of MWCNT-treated gpt delta transgenic mice. Transversions were predominant, and G:C to C:G was clearly increased by MWCNTs. Moreover, many regions immunohistochemically stained for inducible NO synthase and nitrotyrosine were observed in the lungs of MWCNT-exposed mice. Overall, MWCNTs were shown to be genotoxic both in in vitro and in vivo tests; the mechanisms probably involve oxidative stress and inflammatory responses.” As taken from Kato T et al. 2013. Nanotoxicology 7(4), 452-61. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22397533

“Single-walled carbon nanotubes (SWCNTs) have recently attracted great attention because of their fibrous structure and high aspect ratio. Here the genotoxic potential of 400-800 nm, 1-3 μm and 5-30 μm SWCNT with respect to their geometry and surface characteristics was studied. Following thorough physico-chemical characterisation, human bronchial epithelial (BEAS-2B) and lymphoblastoid (MCL-5) cells were treated with SWCNT for 24 or 48 h. This showed significant increases in micronucleus frequency in a time- and dose-dependent manner in both cell types in the absence of cytotoxicity. Over the same dose range, only 1-3 μm SWCNT gave rise to significant increases in hprt point mutations at doses ≥25 μg/ml. Cellular 2,7-dichlorodihydrofluoresceindiacetate (DCFH-DA) fluorescence assay and RT-PCR for oxidative pathway gene profiling revealed a possible oxidative mechanism for the genotoxicity observed in the 1-3 μm SWCNT. Consequently, this study has demonstrated that SWCNT genotoxicity is dependent on its secondary structure under experimental conditions and oxidative stress alone cannot account for the observed damage.” As taken from Manshian BB et al. 2013. Nanotoxicology 7(2), 144-56. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22263934

“Single walled carbon nanotubes were studied with respect to cytotoxic and genotoxic properties in cells of the gastrointestinal tract as exemplified for the human colon carcinoma cell line HT29….in subcytotoxic concentrations substantial DNA damaging effects were found in the alkaline comet assay, which were not associated with enhanced formation of formamidopyrimidine-DNA-glycosylase-sensitive sites. In addition, an increase of kinetochore-negative micronuclei (V79) and phosphorylation of the tumour suppressor protein p53 (HT29) underlined the genotoxic potential of these nanostructures.” As taken from Pelka J et al. 2013. Nanotoxicology 7(1), 2-20. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22007624

“In addition to the early-stage non-cancer lung effects in animals, some studies in cells or animals have shown genotoxic or carcinogenic effects. In vitro studies with human lung cells have shown that single-walled carbon nanotubes (SWCNT) can cause genotoxicity and abnormal chromosome number by interfering with mitosis (cell division) [Muller et al. 2008b; Sargent et al. 2009, 2011; Kisin et al. 2011]. Other in vitro studies did not show evidence of genotoxicity of some MWCNT [Wirnitzer et al. 2009; Kim et al. 2011].”

As taken from NIOSH, 2013.

Mutagenicity Studies:

Test System:	Chinese hamster V79 lung fibroblast cells
End Point:	In vitro chromosomal aberrations
Metabolic Activation:	None
Dose:	0; 2.5; 5; 10 ug/ml (Test material solvent: water)
Dose Regimen:	4 hr treatment, 18 hr recovery
Results:	Negative. Positive response at 10 ug/ml was considered not relevant by authors because it was within range of historical controls. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Chinese hamster V79 lung fibroblast cells

End Point:	In vitro chromosomal aberrations
Metabolic Activation:	None
Dose:	0; 2.5; 5; 10 ug/ml (Test material solvent: water)
Dose Regimen:	18 hr continuous treatment
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Chinese hamster V79 lung fibroblast cells
End Point:	In vitro chromosomal aberrations
Metabolic Activation:	Rat, Liver, S9, Aroclor 1254
Dose:	0; 2.5; 5; 10 ug/ml (Test material solvent: water)
Dose Regimen:	4 hr treatment, 18 hr recovery
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA98
Metabolic Activation:	Rat, Liver, S9, Aroclor 1254
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA100
Metabolic Activation:	Rat, Liver, S9, Aroclor 1254
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA102
Metabolic Activation:	Rat, Liver, S9, Aroclor 1254
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA1535
Metabolic Activation:	Rat, Liver, S9, Aroclor 1254
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA1537
Metabolic Activation:	Rat, Liver, S9, Aroclor 1254
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA1537
Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube

Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA1535
Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA102
Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA100
Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA98

Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 50; 158; 500; 1581; 5000 ug/tube
Results:	Negative. Material tested was baytubes, multi-walled carbon-nanotubes.
Reference:	[WIRNITZER,U, HERBOLD,B, VOETZ,M, RAGOT,J; STUDIES ON THE IN VITRO GENOTOXICITY OF BAYTUBES AGGLOMERATES OF ENGINEERED MULTI-WALLED CARBON-NANOTUBES (MWCNT). TOXICOL. LETT. 186(3): 160-165, 2009]

Test System:	Human bronchial epithelial BEAS 2B cells
End Point:	In vitro micronucleus
Metabolic Activation:	None
Dose:	0; 1; 5; 10; 20; 40; 60; 80; 100 ug/sq cm (chamber slide surface) corresponding to 0; 3.6; 18; 36; 72; 144; 216; 288; and 360 ug/ml
Dose Regimen:	24 hr continuous treatment; cytochalasin B added simultaneously with the particles
Results:	Negative. Test material was single-walled carbon nanotubes (>50%, ~40% other CNTs; 1.1 nm x 0.05-100 um).
Reference:	[LINDBERG,HK, FALCK,GC, SUHONEN,S, VIPPOLA,M, VANHALA,E, CATALAN,J, SAVOLAINEN,K, NORPPA,H; GENOTOXICITY OF NANOMATERIALS: DNA DAMAGE AND MICRONUCLEI INDUCED BY CARBON NANOTUBES AND GRAPHITE NANOFIBRES IN HUMAN BRONCHIAL EPITHELIAL CELLS IN VITRO. TOXICOL. LETT. 186(3): 166-173, 2009]

Test System:	Human bronchial epithelial BEAS 2B cells
End Point:	In vitro micronucleus
Metabolic Activation:	None
Dose:	0; 1; 5; 10; 20; 40; 60; 80; 100 ug/sq cm (chamber slide surface) corresponding to 0; 3.6; 18; 36; 72; 144; 216; 288; and 360 ug/ml
Dose Regimen:	72 hr continuous treatment; cytochalasin B added simultaneously with the particles
Results:	Negative. Test material was single-walled carbon nanotubes (>50%, ~40% other CNTs; 1.1 nm x 0.05-100 um).
Reference:	[LINDBERG,HK, FALCK,GC, SUHONEN,S, VIPPOLA,M, VANHALA,E, CATALAN,J, SAVOLAINEN,K, NORPPA,H; GENOTOXICITY OF NANOMATERIALS: DNA DAMAGE AND MICRONUCLEI INDUCED BY CARBON NANOTUBES AND GRAPHITE NANOFIBRES IN HUMAN BRONCHIAL EPITHELIAL CELLS IN VITRO. TOXICOL. LETT. 186(3): 166-173, 2009]

Test System:	Human bronchial epithelial BEAS 2B cells
End Point:	In vitro micronucleus
Metabolic Activation:	None
Dose:	0; 1; 5; 10; 20; 40; 60; 80; 100 ug/sq cm (chamber slide surface) corresponding to 0; 3.6; 18; 36; 72; 144; 216; 288; and 360 ug/ml

Dose Regimen:	48 hr continuous treatment; cytochalasin B added simultaneously with the particles
Results:	Positive. Test material was single-walled carbon nanotubes (>50%, ~40% other CNTs; 1.1 nm x 0.05-100 um)
Reference:	[LINDBERG,HK, FALCK,GC, SUHONEN,S, VIPPOLA,M, VANHALA,E, CATALAN,J, SAVOLAINEN,K, NORPPA,H; GENOTOXICITY OF NANOMATERIALS: DNA DAMAGE AND MICRONUCLEI INDUCED BY CARBON NANOTUBES AND GRAPHITE NANOFIBRES IN HUMAN BRONCHIAL EPITHELIAL CELLS IN VITRO. TOXICOL. LETT. 186(3): 166-173, 2009]

As taken from CCRIS, 2010.

The Panel concluded that, altogether, tests with carbon black particles do not indicate a genotoxic hazard. However, the positive results obtained in tests in vitro with carbon black solvent extracts point to the presence of genotoxic compounds, mainly PAHs and nitro and sulphur containing PAHs (IARC, 2010), absorbed onto the surface of the particles. The Panel noted that the PAHs extracted by solvent are tightly bound to carbon particles, and may have limited bioavailability in physiological condition. (EFSA 2012b)

“Graphene and its derivatives are promising candidates for important biomedical applications because of their versatility. The prospective use of graphene-based materials in a biological context requires a detailed comprehension of the toxicity of these materials. Moreover, due to the expanding applications of nanotechnology, human and environmental exposures to graphene-based nanomaterials are likely to increase in the future. Because of the potential risk factors associated with the manufacture and use of graphene-related materials, the number of nanotoxicological studies of these compounds has been increasing rapidly in the past decade. These studies have researched the effects of the nanostructural/biological interactions on different organizational levels of the living system, from biomolecules to animals. This review discusses recent results based on in vitro and in vivo cytotoxicity and genotoxicity studies of graphene-related materials and critically examines the methodologies employed to evaluate their toxicities. ……..” As taken from Seabra AB et al. 2014. Chem. Res. Toxicol. 27(2), 159-168. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24422439

“Carbon-based nanomaterials have attracted great interest in biomedical applications such as advanced imaging, tissue regeneration, and drug or gene delivery. The toxicity of the carbon nanotubes and graphene remains a debated issue although many toxicological studies have been reported in the scientific community. In this review, we summarize the biological effects of carbon nanotubes and graphene in terms of in vitro and in vivo toxicity, genotoxicity and toxicokinetics. .....”. As taken from Zhang Y et al. 2014. Drug Metab. Rev. 46(2), 232-46. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24506522

“Long carbon nanotubes (CNTs) resemble asbestos fibers due to their high length to diameter ratio and they thus have genotoxic effects. Another parameter that might explain their genotoxic effects is contamination with heavy metal ions. On the other hand, short (1-2 μm) CNTs do not resemble asbestos fibers, and, once purified from contaminations, they might be suitable for medical applications. To identify the role of fiber thickness and surface properties on genotoxicity, well-characterized short pristine and carboxylated single-walled (SCNTs) and multi-walled (MCNTs) CNTs of different diameters were studied for cytotoxicity, the cell's response to oxidative stress (immunoreactivity against hemoxygenase 1 and glutathione levels), and in a hypoxanthine guanine phosphoribosyltransferase (HPRT) assay using V79 chinese hamster fibroblasts and human lung adenocarcinoma A549 cells. DNA repair was demonstrated by measuring immunoreactivity against activated histone H2AX protein. The number of micronuclei as well as the number of multinucleated cells was determined. CNTs acted more cytotoxic in V79 than in A549 cells. Plain and carboxylated thin (<8 nm) SCNTs and MCNTs showed greater cytotoxic potential and carboxylated CNTs showed indication for generating oxidative stress. Multi-walled CNTs did not cause HPRT mutation, micronucleus formation, DNA damage, interference with cell division, and oxidative stress. Carboxylated, but not plain, SCNTs showed indication for in vitro DNA damage according to increase of H2AX-immunoreactive cells and HPRT mutation. Although short CNTs presented a low

in vitro genotoxicity, functionalization of short SCNTs can render these particles genotoxic." As taken from Mrakovcic M et al. 2015. Toxicol. Sci. 144(1), 114-27. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25505129

"We summarized the findings of in vivo toxicity studies of single-walled carbon nanotubes (SWCNTs) in laboratory animals. The large majority addressed the pulmonary toxicity of SWCNTs in rodents. Inhalation, pharyngeal aspiration, and intratracheal instillation studies revealed that SWCNTs caused ….. genotoxic effects in the lungs. ….. Overall, the available data provides initial information on SWCNT toxicity. To further clarify their toxicity and risk assessment, studies should be conducted using well-characterized SWCNTs, standard protocols, and the relevant route and doses of human exposure." As taken from Ema M et al. 2016. Regul. Toxicol. Pharmacol. 74, 42-63. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26619783

"Human Health Assessment

….. It is not an in vitro mutagen (negative in a mammalian cell gene mutation test and in a mammalian chromosome aberration test). Therefore the substance is unlikely to cause genetic damage. ….."

As taken from Environment Canada, 2015

5.5. Cytotoxicity

This study investigated the cytotoxic potential of novel activated carbon adsorbents (MAST Carbon International Ltd.) developed for medical applications such as extracorporeal therapies. Carbon adsorbents were assessed for their in vitro cytotoxicity against a V79 cell line using a material extraction method in combination with a colony formation assay. Results were compared to those from a commercially available cellulose-coated carbon adsorbent, developed for direct haemoperfusion. Initial findings demonstrated an inhibition of colony formation and an apparent cytotoxic effect. However, it was found that this inhibition occurred as a result of protein and ion adsorption by carbon materials possessing large surface area and highly developed porous structure. Consequently, these essential nutrients were unavailable to the cells during colony formation. Modifications to the cytotoxicity assessment methods were required in order to take into account nutrient loss. Subsequently it was determined that the carbon materials do not show a cytotoxic response towards the V79 cell line under the modified conditions employed. The suggested approach may be useful in the assessment of other biomaterials such as carbon nanotubes and other nanoparticles which possess large surface area. The preliminary data support the ongoing investigation of these adsorbents as candidates for use in extracorporeal therapies**.** BARNES Lara-Marie et al. Carbon ISSN 0008-6223 CODEN CRBNAH. 2009, vol. 47, n°8, pp. 1887-1895 [9 page(s) (article)] (33 ref.)

The success or failure of medical implants often depends on the cell-surface behavior after implantation of the device. This study investigated the use of woven carbon fabric, which had been sonoelectrochemically coated with calcium phosphate, to enhance bone cell attachment and proliferation in vitro. Human osteoblast-like cells, MG63, were used to study the interactions between cells and the material and assess the cytotoxicity of the substrates. The cytotoxicity of the materials was assessed using an MTS ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt)) assay to determine the viability of the osteoblast-like MG63 cells in direct contact with the carbon fabric or calcium phosphate coated carbon fabrics, and to assess the cytotoxicity of extracts from these materials. The morphology of the surface adherent cells was assessed by scanning electron microscopy (SEM). Results showed that neither carbon fabrics nor calcium phosphate coated materials were cytotoxic. Furthermore, cell attachment and proliferation were enhanced by coating carbon fabrics with calcium phosphate. SEM showed that the cells had a normal morphology and were well spread similar to those seen in the tissue culture plate control. These flexible calcium phosphate coated fabrics could, therefore, have uses in the

reconstruction of bone in orthopaedic and dental surgery. New Carbon Materials, Volume 23, Issue 2, June 2008, Pages 139-143. Hong-mei HAN et al.

“Engineered nanomaterials offer numerous and tantalizing opportunities in many sectors of society, including medicine. Needless to say, attention should also be paid to the potential for unexpected hazardous effects of these novel materials. To date, much of the nanotoxicology literature has focused on the assessment of cell viability or cell death using primitive assays for the detection of plasma membrane integrity or mitochondrial function or assessment of cellular morphology. However, when assessing the cytotoxic effects of engineered nanomaterials, researchers need not only to consider whether cells are dead or alive but also to assess which of the numerous, highly specific pathways of cell death might be involved. Moreover, it is important to diagnose cell death based not only on morphological markers but on the assessment and quantification of biochemical alterations specific to each form of cell death. In this Account, we provide a description of the three major forms of programmed cell death in mammalian cells: apoptosis, autophagic cell death, and regulated necrosis, sometimes referred to as necroptosis. Apoptosis can be activated via the extrinsic (death receptor-dependent) or via the intrinsic (mitochondria-dependent) route. Apoptotic cell death may or may not require the activation of cytosolic proteases known as caspases. Autophagy (self-eating) has an important homeostatic role in the cell, mediating the removal of dysfunctional or damaged organelles thereby allowing the recycling of cellular building blocks. However, unrestrained autophagy can kill cells. Studies in recent years have revealed that necrosis that depends on activation of the kinases RIP1 and RIP3 is a major form of programmed cell death with roles in development and immunity. We also discuss recent examples of the impact of engineered nanoparticles on the three different pathways of programmed cell death. For example, acute exposure of cells to carbon nanotubes (CNTs) can induce apoptosis whereas chronic exposure to CNTs may yield an apoptosis-resistant and tumorigenic phenotype in lung epithelial cells. Several reports show that nanoparticles, including polystyrene particles, are routed to the lysosomal compartment and trigger cell death through the destabilization of lysosomal membranes with engagement of the intrinsic apoptosis pathway. In addition, a number of studies have demonstrated that nanomaterials such as CNTs, quantum dots, and gold nanoparticles can affect cellular autophagy. An improved understanding of the complexities of the nanomaterial-induced perturbation of different cell death pathways may allow for a better prediction of the consequences of human exposure” (Andón and Fadeel, 2013. Accounts of Chemical Research 46(3), 733-42. As taken from http://www.ncbi.nlm.nih.gov/pubmed/22720979

“The present study aimed to evaluate the potential toxicity and the general mechanism involved in multi-walled carbon nanotubes (MWCNT)-induced cytotoxicity in C6 rat glioma cell line. Two kinds of MWCNT, which were coded as MWCNT1 (measured 10-20nm in diameter and 2μm in average length) and MWCNT2 (measured 40-100nm in diameter and 10μm in average length), were used in this study. To elucidate the possible mechanisms of cytotoxicity induced by MWCNT, MTT assay and flow cytometry analysis for apoptosis and cell cycle, MDA and SOD assays for oxidative stress were quantitatively assessed. The exposure of C6 rat glioma cells to different sizes of two kinds of carbon nanotubes at concentrations between 25 and 400μg/ml decreased the cell viability in a concentration- and time-dependent manner. The exposure of C6 rat glioma cells to MWCNT (200-400μg/ml) resulted in a concentration dependent cell apoptosis and G1 cell cycle arrest, and increased the level of oxidative stress. Results demonstrate that smaller size of MWCNT seems to be more toxic than that of larger one. MWCNT-induced cytotoxicity in C6 cells is probably due to the increased oxidative stress. “ (Han YG et al., 2012. Neurotoxicology 33(5), 1128-34. As taken from http://www.ncbi.nlm.nih.gov/pubmed/22728153

“Sachar and Saxena (Sachar and Saxena 2011) investigated the uptake of either SWCNTs or acid functionalized SWCNTs (AF-SWCNTs) in erythrocytes isolated from Swiss or C57BL76 female mice. The acid functionalized (AF)-SWCNTs were surface oxidized by a mixture of nitric and sulphuric acid under pressure at elevated temperature. The carboxylic acid moieties formed were

derivatised by a fluorophor for imaging purposes, and were intensively purified to remove excess fluorescent dye. The particle size distribution and surface charge was not indicated. Particle size distribution and surface charge on AF-SWCNTs were reported before (Saxena et al. 2007 as cited in (Sachar and Saxena 2011). A dose and time dependent decline (70 to 90%) in erythrocyte recovery was recorded in cultures treated with AF-SWCNTs (concentrations of 10, 25 or 50 µg/ml), while treatment with SWCNTs (50 µg/ml) had no effect on erythrocyte recovery as compared to the untreated control groups. Furthermore, the authors reported an increase in the binding of 8-anilino naphthalene sulfonic acid to erythrocytes treated with AF-SWCNTs, which according to the authors indicated a significant damage of the erythrocyte membrane after exposure to the AF-SWCNT NPs.

Cicchetti and co-workers (Cicchetti et al. 2011) exposed human gingival fibroblasts in semiconfluent cultures to SWCNT concentrations between 50 and 150 µg SWCNTs/ml for 24 hours. The SWCNTs used were oxidized by treatment with a mixture of nitric and sulphuric acids. The surface area of was 407 m2/g, and the average external diameter was 1.58 nm ± 0.20 nm and the average length was 0.76 µm ± 0.70 µm. The SWCNTs were reported by the authors to have “a relatively high degree of crystallinity”. The authors reported a….decrease in cell proliferation and survival (125 and 150 µg/ml), increase in reactive oxygen species production (at all concentrations) and Hsp70 induction (at all concentrations).”

As taken from Binderup et al. 2013.

/ALTERNATIVE and IN VITRO TESTS/ ... The impact of /carbon/ single-walled nanotubes (SWNT) on rat aortic smooth muscle cells (SMC) was examined for SWNT (0.0-0.1 mg/mL) over a 3.5-day time-course. Cell culture medium was filtered to remove the aggregate material and both nanomaterial (un-filtered) and filtered SWNT media were used to examine cell growth. In general, the removal of SWNT aggregates from cell culture test medium by filtration increased the SMC number in comparison to unfiltered medium at pre-filtered SWNT dosages below 0.1 mg/mL. However, at 0.1 mg/mL, both filtered and unfiltered media exhibited a similar decrease in cell number relative to the control medium. The filtered medium was characterized and contained both suspended nanoparticles as well as a small quantity of SWNT, which may have contributed to the observed cell growth inhibition. As a comparison to the SWNT, activated carbon (0.1 mg/mL), a nanoporous, microparticulate carbon material, was found to be less inhibitory to SMC growth than the SWNT at the same dosage, implying an inverse proportionality between carbon nanomaterial size regimes and cell growth inhibition. [Raja PM et al; Toxicol Lett 169 (1): 51-63 (2007)] **PEER REVIEWED**

As taken from HSDB, 2009

“Carbon nanotubes (CNTs) have been used in orthopaedic applications because of their exceptional mechanical properties. However, the influence of CNTs on the behaviour of bone-forming cells and on the ability of these cells to respond to growth factors, such as bone morphogenetic proteins (BMPs), remains poorly known. Therefore, in the present study, single-walled CNTs (SWCNTs) were synthesised using an induction thermal plasma process and purified using a multistep procedure. The impact of these purified SWCNTs on the Smad activation, cell proliferation and differentiation, with or without BMP-2 and BMP-9 (1.92 nM), was also studied using western blot, mitochondrial enzymatic activity, TUNEL, RT-PCR and alkaline phosphatase activity analyses. Pre-treatment of MC3T3-E1 preosteoblasts with SWCNTs accelerated the Smad1/5/8 activation, induced by both BMP-2 and BMP-9, within 15 min. It also slightly affected their proliferation at 48 h without apoptosis. Interestingly, at 72 h, BMP-9 favoured the differentiation of MC3T3-E1 preosteoblasts pretreated with SWCNTs to a larger extent than BMP-2 did. Therefore, the combination of BMP-9 with SWCNTs appears to be a promising avenue for bone applications.” As taken from Alinejad Y et al. 2013. J. Biomed. Nanotechnol. 9(11), 1904-13. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24059089?dopt=AbstractPlus

“BACKGROUND: Differences in interlaboratory research protocols contribute to the conflicting data

in the literature regarding engineered nanomaterial (ENM) bioactivity. OBJECTIVES: Grantees of a National Institute of Health Sciences (NIEHS)-funded consortium program performed two phases of in vitro testing with selected ENMs in an effort to identify and minimize sources of variability. METHODS: Consortium program participants (CPPs) conducted ENM bioactivity evaluations on zinc oxide (ZnO), three forms of titanium dioxide (TiO2), and three forms of multiwalled carbon nanotubes (MWCNTs). In addition, CPPs performed bioassays using three mammalian cell lines (BEAS-2B, RLE-6TN, and THP-1) selected in order to cover two different species (rat and human), two different lung epithelial cells (alveolar type II and bronchial epithelial cells), and two different cell types (epithelial cells and macrophages). CPPs also measured cytotoxicity in all cell types while measuring inflammasome activation [interleukin-1β (IL-1β) release] using only THP-1 cells. RESULTS:….MWCNTs did not produce cytotoxicity, but stimulated lower levels of IL-1β production in THP-1 cells, with the original MWCNT producing the most IL-1β. CONCLUSIONS: The results provide justification for the inclusion of mechanism-linked bioactivity assays along with traditional cytotoxicity assays for in vitro screening. In addition, the results suggest that conducting studies with multiple relevant cell types to avoid false-negative outcomes is critical for accurate evaluation of ENM bioactivity." As taken from Xia T et al. 2013. Environ. Health Perspect. 121(6), 683-90. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23649538

"The applicability of rat precision-cut lung slices (PCLuS) in detecting nanomaterial (NM) toxicity to the respiratory tract was investigated evaluating sixteen OECD reference NMs (TiO_2, ZnO, CeO_2, SiO_2, Ag, multi-walled carbon nanotubes (MWCNTs)). Upon 24-hour test substance exposure, the PCLuS system was able to detect early events of NM toxicity: total protein, reduction in mitochondrial activity, caspase-3/-7 activation, glutathione depletion/increase, cytokine induction, and histopathological evaluation. Ion shedding NMS (ZnO and Ag) induced severe tissue destruction detected by the loss of total protein. Two anatase TiO_2 NMs, CeO_2 NMs, and two MWCNT caused significant (determined by trend analysis) cytotoxicity in the WST-1 assay. At non-cytotoxic concentrations, different TiO_2 NMs and one MWCNT increased GSH levels, presumably a defense response to reactive oxygen species, and these substances further induced a variety of cytokines. One of the SiO_2 NMs increased caspase-3/-7 activities at non-cytotoxic levels, and one rutile TiO_2 only induced cytokines. Investigating these effects is, however, not sufficient to predict apical effects found in vivo. Reproducibility of test substance measurements was not fully satisfactory, especially in the GSH and cytokine assays. Effects were frequently observed in negative controls pointing to tissue slice vulnerability even though prepared and handled with utmost care. Comparisons of the effects observed in the PCLuS to in vivo effects reveal some concordances for the metal oxide NMs, but less so for the MWCNT. The highest effective dosages, however, exceeded those reported for rat short-term inhalation studies." As taken from Sauer UG et al. 2014. Toxicol. Appl. Pharmacol. 276(1), 1-20. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24382512

"BACKGROUND: SEVERAL PROPERTIES OF MULTI-WALLED CARBON NANOTUBES (MWCNT) HAVE THE POTENTIAL TO AFFECT THEIR BIOACTIVITY. This study examined the in vitro and in vivo outcomes of the influence of diameter, length, purification and carboxylation (in vitro testing only) of MWCNT. METHODS: Three original 'as received' MWCNT that varied in size (diameter and length) were purified and functionalized by carboxylation. The resulting MWCNT were characterized and examined for cytotoxicity and inflammasome activation in vitro using THP-1 cells and primary alveolar macrophages from C57BL/6 mice. Oropharyngeal aspiration administration was used to deliver original MWCNT and in vivo bioactivity and lung retention was examined at 1 and 7 days. RESULTS: Studies with THP-1 macrophages demonstrated that increased length or diameter corresponded with increased bioactivity as measured by inflammasome activation. Purification had little effect on the original MWCNT, and functionalization completely eliminated bioactivity. Similar results were obtained using alveolar macrophages isolated from C57BL/6 mice…." As taken from Hamilton RF Jr et al. 2013. Part. Fibre Toxicol. 10(1), 57.

PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24225053

"Double-walled carbon nanotubes (DWCNT) are a rather new and unexplored variety of carbon nanotubes. Previously conducted studies established that exposure to a variety of carbon nanotubes produced lung inflammation and fibrosis in mice after pharyngeal aspiration. However, the bioactivity of double-walled carbon nanotubes (DWCNT) has not been determined. In this study, the hypothesis that DWCNT would induce pulmonary toxicity was explored by analyzing the pulmonary bioactivity of DWCNT. To test this hypothesis, C57Bl/6 mice were exposed to DWCNT by pharyngeal aspiration. Mice underwent whole-lung lavage (WLL) to assess pulmonary inflammation and injury, and lung tissue was examined histologically for development of pulmonary disease as a function of dose and time…DWCNT exposure also produced a dose-dependent rise in lactate dehydrogenase (LDH) activity, as well as albumin levels, in WLL fluid, indicating that DWCNT exposure promoted cytotoxicity as well as decreases in the integrity of the blood-gas barrier in the lung, respectively…." As taken from Sager TM et al. 2013. J. Toxicol. Environ. Health A. 76(15), 922-36. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24156695

"The intranasal drug delivery route provides exciting expectations regarding the application of engineered nanomaterials as nano-medicines or drug-delivery vectors into the brain. Among nanomaterials, multiwalled CNTs (MWCNTs) are some of the best candidates for brain cancer therapy since they are well known to go across cellular barriers and display an intrinsic ability to block cancer cell proliferation triggering apoptosis. This study reveals that microglial cells, the brain macrophages and putative vehicles for MWCNTs into the brain, undergo a dose-dependent cell division arrest and apoptosis when treated with MWCNTs. Moreover, it is shown that MWCNTs severely interfere with both cell migration and phagocytosis in live microglia. These results lead to a re-evaluation of the safety of inhaled airborne CNTs and provide strategic clues of how to biocompatibilize MWCNTs to reduce brain macrophage damage and to develop new nanodrugs." As taken from Villegas JC et al. 2014. Adv. Healthc. Mater. 3(3), 424-32. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23950018

"Background: nanotechnology, particularly the use of multi-walled carbon nanotubes (mwcnt), is a rapidly growing discipline with implications for advancement in a variety of fields. A major route of exposure to MWCNT during both occupational and environmental contact is inhalation. While many studies showed adverse effects to the vascular endothelium upon MWCNT exposure, in vitro results often do not correlate with in vivo effects. This study aimed to determine if an alveolar-capillary co-culture model could determine changes in the vascular endothelium after epithelial exposure to MWCNT. METHODS: A co-culture system in which both human small airway epithelial cells and human microvascular endothelial cells were separated by a Transwell membrane so as to resemble an alveolar-capillary interaction was used. Following exposure of the epithelial layer to MWCNT, the effects to the endothelial barrier were determined. RESULTS: Exposure of the epithelial layer to MWCNT induced multiple changes in the endothelial cell barrier, including an increase in reactive oxygen species, actin rearrangement, loss of VE-cadherin at the cell surface, and an increase in endothelial angiogenic ability. Overall increases in secreted VEGFA, sICAM-1, and sVCAM-1 protein levels, as well as increases in intracellular phospho-NF-κB, phospho-Stat3, and phospho-p38 MAPK, were also noted in HMVEC after epithelial exposure. CONCLUSION: The co-culture system identified that alveolar-capillary exposure to MWCNT induced multiple changes to the underlying endothelium, potentially through cell signaling mediators derived from MWCNT-exposed epithelial cells. Therefore, the co-culture system appears to be a relevant in vitro method to study the pulmonary toxicity of MWCNT." As taken from Snyder-Talkington BN et al. 2013b. Part. Fibre Toxicol. 10, 35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23903001

"OBJECTIVE: To compare the cytotoxicity and dna strand breakage induced by multi-walled carbon nanotubes (mwcnts) with different lengths and different surface modifications in

human alveolar type ii cells (A549 CELLS). METHODS: TWO DIFFERENT LENGTHS (5-15 μm, 350-700 nm) of MWCNTs and three different kinds of surface modified MWCNTs (COOH-MWCNTs, NH2-MWCNTs, and Tau-MWCNTs) were used in the experiments. The short MWCNTs were used as pristine MWCNTs to compare with the 3 surface modified MWCNTs. The cytotoxicity was determined by cell counting kit-8 (CCK-8) assay at the concentrations of 2, 8, and 32 mg/L at hours 12, 24, 36, and 48 respectively. Single cell gel electrophoresis (SCGE) assay was performed to evaluate DNA strand breakage in A549 cells after 24 h treatment of 8 mg/L of each tested material. RESULTS: Long multi-walled carbon nanotubes (Long-MWCNTs) and short multi-walled carbon nanotubes (Short-MWCNTs) showed a dose-dependent cytotoxicity within the exposure time 12-48 h. Especially, Long-MWCNTs showed greater cytotoxicity than Short-MWCNTs from 24 to 48 h at the same concentration. The relative cell viability of the 3 surface modified MWCNTs was higher than that of the pristine MWCNTs at h 12 at the concentration of 32 mg/L [COOH-MWCNTs (86.55±1.80)%, NH2-MWCNTs (84.67±1.32)%, Tau-MWCNTs (80.15±3.53)% and Pristine-MWCNTs (71.44±5.58)%], at h 24 at the concentration of 8 mg/L [COOH-MWCNTs (96.74±1.00)%, NH2-MWCNTs (96.74±3.35)%, Tau-MWCNTs (106.39±3.83)% and Pristine-MWCNTs (91.02±2.53)%], at h 24 at the concentration of 32 mg/L [COOH-MWCNTs (80.88±2.67)%, NH2-MWCNTs (82.90±3.25)%, Tau-MWCNTs (82.55±3.32)% and Pristine-MWCNTs (76.08±4.27)%] and at h 36 at the concentration of 8 mg/L [COOH-MWCNTs (96.87±1.05)%, NH2-MWCNTs (96.66±4.76)%, Tau-MWCNTs (100.23± 2.84)% and Pristine-MWCNTs (89.61±3.78)%], and the differences were statistically significant ($P<0.05$). Compared with the Pristine-MWCNTs, the relative cell viability of the 3 surface modified MWCNTs didn't demonstrate a statistically significant difference ($P>0.05$) at other observation time and exposure concentrations. The DNA strand breakage of the 3 surface modified MWCNTs: the Olive tail moment of COOH-MWCNTs was 1.56±0.22, the Olive tail moment of NH2-MWCNTs 2.25±1.62 and the Olive tail moment of Tau-MWCNTs 2.23±0.94; the tail DNA% of COOH-MWCNTs was (3.96± 0.60)%, the tail DNA% of NH2-MWCNTs (6.16±4.68)% and the tail DNA% of Tau-MWCNTs (6.05±2.31)%, which were lower than that of the pristine MWCNTs ($P<0.05$), whose Olive tail moment was 3.00±0.64 and tail DNA% (8.23±2.27)%. Moreover, the COOH-MWCNTs induced the lowest DNA damage among the three modified MWCNTs. CONCLUSION: Long-MWCNTs compared with Short-MWCNTs demonstrated a greater cytotoxicity and lower DNA strand breakage damage. The surface modifications of MWCNTs can reduce the cytotoxicity and DNA strand breakage in A549 cells." As taken from Pu J et al. 2013. Beijing Da Xue Xue Bao. 45(3), 405-11. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23774918

"The growing use of engineered nanoparticles (NPs) in commercial and medical applications raises the urgent need for tools that can predict NP toxicity. Global transcriptome and proteome analyses were conducted on three human cell types, exposed to two high aspect ratio NP types, to identify patterns of expression that might indicate high versus low NP toxicity. Three cell types representing the most common routes of human exposure to NPs, including macrophage-like (THP-1), small airway epithelial and intestinal (Caco-2/HT29-MTX) cells, were exposed to TiO2 nanobelts (TiO2-NB; high toxicity) and multi-walled carbon nanotubes (MWCNT; low toxicity) at low (10 μg/mL) and high (100 μg/mL) concentrations for 1 and 24 h. Unique patterns of gene and protein expressions were identified for each cell type, with no differentially expressed ($p < 0.05$, 1.5-fold change) genes or proteins overlapping across all three cell types. While unique to each cell type, the early response was primarily independent of NP type, showing similar expression patterns in response to both TiO2-NB and MWCNT. The early response might, therefore, indicate a general response to insult. In contrast, the 24 h response was unique to each NP type. The most significantly ($p < 0.05$) enriched biological processes in THP-1 cells indicated TiO2-NB regulation of pathways associated with inflammation, apoptosis, cell cycle arrest, DNA replication stress and genomic instability, while MWCNT-regulated pathways indicated increased cell proliferation, DNA repair and anti-apoptosis. These two distinct sets of biological pathways might, therefore, underlie cellular responses to high and low NP toxicity, respectively." As taken from Tilton SC et al. 2014. Nanotoxicology 8, 533-48. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23659652

“Nanoparticles (NPs) can cause respiratory and cardiovascular problems, furthermore small carboxyl polystyrene NPs induce hemolysis, activate platelets and induce inflammation in human blood. Carbon nanoparticles (CNPs) are known to interfere with cellular metabolism, specific cellular functions and moreover may cause cellular toxicity. We aimed to study the influence of CNPs on oxidative stress, mitochondrial membrane damage and intracellular gene expression in human mesenchymal stem cells (hMSCs). CNPs cause a dose and time dependent growth inhibition in hMSCs at a dose range from 50 to 400µg/mL. Exposure of CNPs toxic doses viz., 50µg/mL (D1) and 100µg/mL (D2) decreased intracellular mitochondrial membrane potential compared to control. CNPs treated cells were found to lose their morphology due to cell membrane damage have been confirmed by propidium iodide staining and fluorescence microscopic analysis. Oxidative stress responsive genes like GSTM3 and GSR1 expression have increased a fold when compared to control, interim there is no change were observed in SOD and GPx. We found an increased expression of CYP1A and POR genes by at least 2- fold, which is involved in mitochondrial trans-membrane potential. In conclusion, routine and high exposure of CNPs to hMSCs increased oxidative stress and mitochondrial membrane damage.” As taken from Alshatwi AA et al. 2013. Environ. Toxicol. Pharmacol. 36(1), 215-22. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23624273

“With the advancements in nanotechnology, studies on the synthesis, modification, application, and toxicology evaluation of nanomaterials are gaining increased attention. In particular, the applications of nanomaterials in biological systems are attracting considerable interest because of their unique, tunable, and versatile physicochemical properties. Artificially engineered nanomaterials can be well controlled for appropriate usage, and the tuned physicochemical properties directly influence the interactions between nanomaterials and cells. This review summarizes recently synthesized major nanomaterials that have potential biomedical applications. Focus is given on the interactions, including cellular uptake, intracellular trafficking, andtoxic response, while changing the physicochemical properties of versatile materials. The importance of physicochemical properties such as the size, shape, and surface modifications of the nanomaterials in their biological effects is also highlighted in detail….” As taken from Cheng LC et al. 2013. Nanoscale 5(9), 3547-69. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23532468

“In order to study the effects of nanoparticles (NPs) with different physicochemical properties on cellular viability and structure, Saccharomyces cerevisiae were exposed to different concentrations of TiO2-NPs (1-3 nm), ZnO-NPs (<100 nm), CuO-NPs (<50 nm), their bulk forms, Ag-NPs (10 nm) and single-walled carbon nanotubes (SWCNTs). The GreenScreen assay was used to measure cyto- and genotoxicity, and transmission electron microscopy (TEM) used to assess ultrastructure. CuO-NPs were highly cytotoxic, reducing the cell density by 80% at 9 cm(2)/ml, and inducing lipid droplet formation. Cells exposed to Ag-NPs (19 cm(2)/ml) and TiO2-NPs (147 cm(2)/ml) contained dark deposits in intracellular vacuoles, the cell wall and vesicles, and reduced cell density (40 and 30%, respectively). ZnO-NPs (8 cm(2)/ml) caused an increase in the size of intracellular vacuoles, despite not being cytotoxic. SWCNTs did not cause cytotoxicity or significant alterations in ultrastructure, despite high oxidative potential….” As taken from Bayat N et al. 2014. Nanotoxicology 8, 363-73. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23521755

“Single-wall carbon nanotubes (SWCNTs) and polyamidoamine dendrimers (PAMAM) have been proposed for a variety of biomedical applications. The combination of both molecules makes this new composite nanomaterial highly functionalizable and versatile to theranostic and drug-delivery systems. However, recent toxicological studies have shown that nanomaterials such as SWCNTs and PAMAM may have high toxicity in biological environments. Aiming to elucidate such behavior, in vitro studies with different cultured cells have been conducted in the past few years. This study focuses on the effects of SWCNT-PAMAM nanomaterials and their individual components on the

C2C12 murine cell line, which is a mixed population of stem and progenitor cells. The interactions between the cells and the nanomaterials were studied with different techniques usually employed in toxicological analyses. The results showed that SWCNT-PAMAM and PAMAM inhibited the proliferation and caused DNA damage of C2C12 cells. Data from flow cytometry revealed a less toxicity in C2C12 cells exposed to SWCNT compared to the other nanomaterials. The results indicated that the toxicity of SWCNT, SWCNT-PAMAM and PAMAM in C2C12 cells can be strongly correlated with the charge of the nanomaterials." As taken from Cancino J et al. 2013. Toxicol. Lett. 219(1), 18-25. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23454831

….Considering the potential application of carbon as scaffold materials and the lack of understanding of compatibility of amorphouscarbon with neuronal cells, the carbon-based materials in the forms of carbon films and continuous electrospun carbon nanofibers having average diameter of ~200 nm are being investigated with or without ultraviolet (UV) and oxy-plasma (OP) treatments for cytocompatibility property using mouse Neuroblastoma (N2a) and rat Schwann cells (RT4-D6P2T). The use of Raman spectroscopy in combination with Fourier transform infrared (FTIR) and X-ray diffraction establishes the amorphous nature and surface-bonding characteristics of the studied carbon materials. Although both UV and OP treatments make carbon surfaces more hydrophilic, the cell viability of N2a cells is statistically more significant on OP treated fibers/films compared to UV fiber/film substrates after 4 days in culture. The electrospun carbon fibrous substrate provides the physical guidance to the cultured Schwann cells. Overall, the experimental results of this study demonstrate that the electrospun amorphous carbon nanofibrous scaffolds can be used as a suitable biomaterial substrate for supporting cell adhesion and proliferation of neuronal cells in the context of their applications as artificial nerve implants" As taken from Jain S et al. 2013. J. Biomed. Mater. Res. B. Appl. Biomater. 101(4), 520-31. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23359403

"Multiwalled carbon nanotubes (MWCNTs) possess unique properties rendering them a potentially useful biomaterial for neurobiological applications such as providing nanoscale contact-guidance cues for directing axon growth within peripheral nerve repair scaffolds. The in vitro biocompatibility of MWCNTs with postnatal mouse spinal sensory neurons was assessed for this application. Cell culture medium conditioned with MWCNTs was not significantly toxic to dissociated cultures of postnatal mouse dorsal root ganglia (DRG) neurons. However, exposure of DRG neurons to MWCNTs dispersed in culture medium resulted in a time- and dose-dependent reduction in neuronal viability. At 250 μg mL^{-1}, dispersed MWCNTs caused significant neuronal death and unusual neurite morphologies illustrated by immunofluorescent labelling of the cytoskeletal protein beta (III) tubulin, however, at a dose of 5 μg mL^{-1} MWCNTs were nontoxic over a 14-day period. DRG neurons grown on fabricated MWCNT substrates produced neurite outgrowths with abnormal morphologies that were significantly inferior in length to neurons grown on the control substrate laminin. This evidence demonstrates that to be utilized as a biomaterial in tissue scaffolds for nerve repair, MWCNTs will require robust surface modification to enhance biocompatibility and growth promoting properties." As taken from Gladwin KM et al. 2013. Adv. Healthc. Mater. 2(5), 728-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23184463

"Carbon nanotubes (CNTs) have potential as not only electrical materials but also biomedical devices. However, some findings have been reported indicating that the use of CNTs is accompanied by a risk of the development of certain diseases such as pulmonary fibrosis and pleura mesothelioma; and one of the reasons for this risk may be macrophage cell death. In the present study, to elucidate the mechanism of macrophage cell death by CNTs, we focused on biomembrane damage caused by multi-walled CNTs (MWCNTs). When the distribution of MWCNTs in RAW264 cells was observed under a light microscope, MWCNTs were located on the surface of the plasma membrane; and a portion of them seemed to stick into it. The acute cytotoxicity toward RAW264 cells was examined by performing the LDH cytotoxic test, and LDH release was detected after exposure to 100 μg/ml CNT. To examine the physical damage to biomembranes by CNT

exposure, we conducted a calcein release assay using calcein-encapsulated liposomes. The results indicated that an increase in the permeability of the lipid bilayer was induced by MWCNTs. The present study thus demonstrated for the first time that a high concentration of MWCNTs was cytotoxic to macrophages and suggested that the direct physical perturbation of biomembranes by MWCNTs plays a role in this activity." As taken from Shimizu K et al. 2013. J. Toxicol. Sci. 38(1), 7-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23358135

"Etoposide is a semisynthetic, chemotherapeutic drug widely recommended to treat an extensive range of human cancers. Our studies indicate that, while etoposide is capable of killing human cancer cells, exposure to single-walled carbon nanotubes (SWCNTs) and etoposide results in enhanced cell death that appears to be synergistic and not merely additive. In this study, we used high pressure liquid chromatography and mass spectrometry to quantify the internal effective dose of etoposide when the human pancreatic cancer cell (PANC-1) was exposed to the combination of these agents. Our results unequivocally indicate that SWCNTs improve etoposide uptake and increase its capacity to kill cancer cells. We suggest that a combination of SWCNTs and etoposide may prove to be a more efficient chemotherapeutic protocol, especially because of the potential to lower toxicdrug doses to levels that may be useful in decreasing adverse side effects, as well as in lowering the probability of inducing chemoresistance in exposed cancer cells." As taken from Mahmood M et al. 2013. Nanotechnology 24(4), 045102. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23291321

"Despite the great use of nanomaterials for engineering and medical applications, nanomaterials may have adverse consequences by accidental exposure, because of their nanoscale size, composition and shape. Like many nanomaterials, carbon nanotubes (CNTs) have been used for many proven applications, but the size of the CNTs makes them more readily become airborne and can therefore create the risk of being inhaled by a worker. In this study, we evaluated single-walled CNT (SWCNT)-induced effects on cellular responses such as cell proliferation, inflammatory response and oxidative stress in dynamic cell growth condition. A dynamic cell growth environment was established to mimic the dynamic changes in the amount of circumferential and longitudinal expansion and contraction occurred during normal breathing movement in the lung. Two different length (short: outer diameter (OD) 1-2 nm, length 0.5-2 μm; long: OD 1-2 nm, length 5-30 μm) of SWCNTs were used at different exposure concentrations (5, 10 and 20 μg/ml) during the different exposure duration (24, 48 and 72 h). Dynamic environment facilitated altered interaction between SWCNTs and A549 monolayer. Cellular responses in dynamic condition were significantly different from those in static condition. Moreover, cellular responses were dependent on the length of SWCNTs both in static and dynamic cell growth conditions." As taken from Patel HJ & Kwon S. 2013. J. Expo. Sci. Environ. Epidemiol. 23(1), 101-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22854519

"Effects on the liver C3A cell line treated with a panel of engineered nanomaterials (NMs) consisting of two zinc oxide particles (ZnO; coated 100 nm and uncoated 130 nm), two multi-walled carbon nanotubes (MWCNTs), one silver (Ag < 20 nm), one 7 nm anatase, two rutile TiO2 nanoparticles (10 and 94 nm) and two derivatives with positive and negative covalent functionalisation of the 10 nm rutile were evaluated. The silver particles elicited the greatest level of cytotoxicity (24 h LC50 - 2 μg/cm(2)). The silver was followed by the uncoated ZnO (24 h LC50 - 7.5 μg/cm(2)) and coated ZnO (24 h LC50 - 15 μg/cm(2)) particles with respect to cytotoxicity. The ZnO NMs were found to be about 50-60% soluble which could account for their toxicity. By contrast, the Ag was <1% soluble. The LC50 was not attained in the presence of any of the other engineered NMs (up to 80 μg/cm(2)). All NMs significantly increased IL-8 production. Meanwhile, no significant change in TNF-α, IL-6 or CRP was detected. Urea and albumin production were measured as indicators of hepatic function. These markers were only altered by the coated and uncoated ZnO, which significantly decreased albumin production." As taken from Kermanizadeh A et al. 2013. Nanotoxicology 7(3), 301-13. PubMed, 2014 available at

http://www.ncbi.nlm.nih.gov/pubmed/22263564

“Single walled carbon nanotubes were studied with respect to cytotoxic and genotoxic properties in cells of the gastrointestinal tract as exemplified for the human colon carcinoma cell line HT29. No effect on cell growth in the sulphorhodamine B assay was observed after 24 h of incubation, whereas growth inhibitory properties were found after 48 and 72 h. After 24 h incubation a decrease of mitochondrial activity (WST-1) was measured (≥0.1 µg/ml), whereas membrane integrity (lactate dehydrogenase) was not affected. In cytotoxic concentrations, the formation of reactive oxygen species and a slight increase of total glutathione and nuclear Nrf2 were observed….” As taken from Pelka J et al. 2013. Nanotoxicology 7(1), 2-20. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22007624

“…Various experiments have shown that lysosomal damage is one of the main reasons for CNTs to trigger apoptosis. It has been suggested that the exposure of CNT to the cell's environment results in destabilisation of lysosomal membranes leading to apoptotic as well as necrotic cell death. Mitochondrial damage, which eventually leads to lysosomal damage, is also another way, which has been suggested by other research groups, as a method for the induction of apoptosis by the CNT exposure to the cell. The injured lysosome releases digestive enzymes, which damage entire cells…” As taken from Madani SY et al. 2013. Nano. Rev. Dec 3, 4. PubMed, 2014, available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3851535/?report=classic

“Some studies also indicate that CNT containing certain metals (nickel, 26%) [Lam et al. 2004] or higher metal content (17.7% vs. 0.2% iron) are more cytotoxic in vitro and in vivo [Shvedova et al. 2003, 2008].”

As taken from NIOSH, 2013.

“Graphene and its derivatives are promising candidates for important biomedical applications because of their versatility. The prospective use of graphene-based materials in a biological context requires a detailed comprehension of the toxicity of these materials. Moreover, due to the expanding applications of nanotechnology, human and environmental exposures to graphene-based nanomaterials are likely to increase in the future. Because of the potential risk factors associated with the manufacture and use of graphene-related materials, the number of nanotoxicological studies of these compounds has been increasing rapidly in the past decade. These studies have researched the effects of the nanostructural/biological interactions on different organizational levels of the living system, from biomolecules to animals. This review discusses recent results based on in vitro and in vivo cytotoxicity and genotoxicity studies of graphene-related materials and critically examines the methodologies employed to evaluate their toxicities. ……..” As taken from Seabra AB et al. 2014. Chem. Res. Toxicol. 27(2), 159-168. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24422439

“The biomedical application of graphene quantum dots (GQDs) is a new emerging area. However, their safety data are still in scarcity to date. Particularly, the effect of GQDs on the immune system remains unknown. This study aimed to elucidate the interaction of GQDs with macrophages and the underlying mechanisms. Our results showed that GQDs slightly affected the cell viability and membrane integrity of macrophages, whereas GQDs significantly increased reactive oxygen species (ROS) generation and apoptotic and autophagic cell death with an increase in the expression level of Bax, Bad, caspase 3, caspase 9, beclin 1, and LC3-I/II and a decrease in that of Bcl-2. Furthermore, low concentrations of GQDs significantly increased the expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-8, whereas high concentrations of GQDs elicited opposite effects on the cytokines production. SB202190, a selective inhibitor of p38 mitogen-activated protein kinase (MAPK), abolished the cytokine-inducing effect of GQDs in macrophages. Moreover, GQDs significantly increased the phosphorylation of p38 MAPK and p65,

and promoted the nuclear translocation of nuclear factor-κB (NF-κB). Taken together, these results show that GQDs induce ROS generation, apoptosis, autophagy, and inflammatory response via p38MAPK and NF-κB mediated signaling pathways in THP-1 activated macrophages." As taken from Qin Y et al. 2015. Toxicology 327, 62-76. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25446327

"An in vitro model resembling the respiratory epithelium was used to investigate the biological response to laboratory-made pristine and functionalised multi-walled carbon nanotubes (pMWCNT and MWCNT-COOH). Cell uptake was analysed by MWCNT-COOH, FITC labelled and the effect of internalisation was evaluated on the endocytic apparatus, mitochondrial compartment and DNA integrity. In the dose range 12.5-100μgml(-1), cytotoxicity and ROS generation were assayed, evaluating the role of iron (the catalyst used in MWCNTs synthesis). We observed a correlation between MWCNTs uptake and lysosomal dysfunction and an inverse relationship between these two parameters and cell viability ($P<0.01$). In particular, pristine-MWCNT caused a time- and dose-dependent ROS increase and higher levels of lipid hydroperoxides compared to the controls. Mitochondrial impairment was observed. Conversely to the functionalised MWCNT, higher micronuclei (MNi) frequency was detected in mono- and binucleate pMWCNT-treated cells, underlining an aneugenic effect due to mechanical damage. Based on the physical and chemical features of MWCNTs, several toxicological pathways could be activated in respiratory epithelium upon their inhalation. The biological impacts of nano-needles were imputable to their efficient and very fast uptake and to the resulting mechanical damages in cell compartments. Lysosomal dysfunction was able to trigger further toxic effects." As taken from Visalli G et al. 2015. Toxicol. In Vitro 29(2), 352-62. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25499066

"Long carbon nanotubes (CNTs) resemble asbestos fibers due to their high length to diameter ratio and they thus have genotoxic effects. Another parameter that might explain their genotoxic effects is contamination with heavy metal ions. On the other hand, short (1-2 μm) CNTs do not resemble asbestos fibers, and, once purified from contaminations, they might be suitable for medical applications. To identify the role of fiber thickness and surface properties on genotoxicity, well-characterized short pristine and carboxylated single-walled (SCNTs) and multi-walled (MCNTs) CNTs of different diameters were studied for cytotoxicity, the cell's response to oxidative stress (immunoreactivity against hemoxygenase 1 and glutathione levels), and in a hypoxanthine guanine phosphoribosyltransferase (HPRT) assay using V79 chinese hamster fibroblasts and human lung adenocarcinoma A549 cells. DNA repair was demonstrated by measuring immunoreactivity against activated histone H2AX protein. The number of micronuclei as well as the number of multinucleated cells was determined. CNTs acted more cytotoxic in V79 than in A549 cells. Plain and carboxylated thin (<8 nm) SCNTs and MCNTs showed greater cytotoxic potential and carboxylated CNTs showed indication for generating oxidative stress. Multi-walled CNTs did not cause HPRT mutation, micronucleus formation, DNA damage, interference with cell division, and oxidative stress. Carboxylated, but not plain, SCNTs showed indication for in vitro DNA damage according to increase of H2AX-immunoreactive cells and HPRT mutation. Although short CNTs presented a low in vitro genotoxicity, functionalization of short SCNTs can render these particles genotoxic." As taken from Mrakovcic M et al. 2015. Toxicol. Sci. 144(1), 114-27. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25505129

"Recent research on porous silica materials as drug carriers for amorphous and controlled drug delivery has shown promising results. However, due to contradictory literature reports on toxicity and high costs of production, it is important to explore alternative safe and inexpensive porous carriers. In this study, the potential of activated carbon (AC) as an amorphous drug carrier was investigated using paracetamol (PA) and ibuprofen (IBU) as model drugs. The solution impregnation method was used for drug loading, with loading efficiency determined by UV spectroscopy and drug release kinetics studied using USP II dissolution apparatus. The physical state of the drug in the complex was characterised using differential scanning calorimetry and X-ray diffractions techniques, whilst sites of drug adsorption were studied using Fourier transform infrared spectroscopy and N_2 adsorption techniques. In addition, the cytotoxicity of AC on human colon

carcinoma (Caco-2) cells was assessed using the MTT assay. Results presented here reveal that, for PA/AC and IBU/AC complexes, the saturation solubility of the drug in the loading solvent appears to have an effect on the drug loading efficiency and the physical state of the drug loaded, whilst drug release kinetics were affected by the wettability of the activated carbon particles. Furthermore, activated carbon microparticles exhibited very low cytotoxicity on Caco-2 cells at the concentrations tested (10-800µg/mL). This study, therefore, supports the potential of activated carbon as a carrier for amorphous drug delivery." As taken from Miriyala N et al. 2017. Eur. J. Pharm. Biopharm. 115, 197-205. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28284728

5.6. Carcinogenicity

Dermal carcinogenicity was evaluated in groups of male C3H/HeJ mice (40/group) receiving 25ul applications of either CF carbon fibers (100% carbon), MAT carbon fibers (98% carbon), PAN carbon fibers (94% carbon) or PAN oxidized carbon fibers (64% carbon) suspended in benzene to the skin of the back three times weekly until death, beginning at age 51 to 76 days. The mean survival time of the CF carbon fibers treatment group and the PAN oxide treatment group were significantly greater ($p < 0.05$) than the solvent control group. In the CF carbon fiber treatment group, one mouse was observed with a papilloma on the skin of the back, and one mouse diagnosed with a squamous cell carcinoma on the skin of the back, and a third was diagnosed with an hemangiosarcoma in the subcutis of the right side. In the PAN oxidized treatment group, one mouse was diagnosed with a leiomyosarcoma of the skin and subcutis of the shoulder. The mean survival time of the MAT carbon fiber treatment group was 518 days. In the MAT carbon fiber treatment group, one mouse was observed with a squamous cell carcinoma of the skin in the inguinal region, one with fibrosarcomas on the skin of the axilla and one with a hemangiosarcoma in the subcutis of the abdomen. No skin or subcutaneous tumors were observed in the PAN carbon fiber treatment group and mean survival time was 522 days. The solvent control (benzene) group had a mean survival time of 435 days and no tumors were observed. [Bushy Run Research Center: Evaluation of the Dermal Carcinogenicity of Four Carbon Fiber Materials in Male C3H/HeJ Mice, Final Report, (1982), EPA Document No. 88-8200392, Fiche No. OTS0503334] **UNREVIEWED**

EPIDEMIOLOGY STUDIES/ /The objective was/ to investigate the risk of cancer and non-neoplastic respiratory diseases among workers who manufacture carbon electrodes, as this industry entails exposure to mixtures of polycyclic aromatic hydrocarbons. ... A historical cohort study was carried out of 1006 male workers employed for at least 1 year between 1945 and 1971 in a carbon (graphite) electrode production plant in central Italy, who were followed up for mortality between 1955 and 1996. The ratio of observed to expected deaths (standardised mortality ratios, SMRs) was computed from both national and (for the period 1964-96) regional age and period specific mortalities. A multivariate Poisson regression analysis was performed to investigate the relative risk (RR) of death according to duration of employment and time since first employment in the factory. ... A total of 424 workers had died, 538 were still alive, and 44 were lost to follow up. Mortalities from all causes, all cancers, and respiratory tract cancer were in line with the regional figure. An excess was found over the expected deaths from skin cancer including melanoma (SMR 3.16, 95% confidence interval (95% CI) 0.65 to 9.23) and from non-neoplastic respiratory diseases (SMR 1.58, 95% CI 1.16 to 2.11). Poisson regression analysis including age as a covariate showed an increased risk of dying from gastric cancer with increasing duration of employment, and an increase in the RR of dying from lung cancer and from non-neoplastic respiratory diseases with increasing time since first employment, although the linear trend was not significant. ... This study supports previous findings that working in the carbon electrode manufacturing industry may not increase the risk of dying from respiratory cancer.

As taken from HSDB, 2009

Tumor Inhibition Studies:

Species:	RAT
Number of Animals Tested:	(21,14)/(21,13)
Strain/Sex:	LIO/FEMALE
Dose (Inhibitor):	100 MG/KG BW AQUALEN (ACTIVATED CARBON FIBER ADSORBENT) IN DIET 5X/WK FOR 16 MONTHS (STUDY DURATION: 16 MONTHS)
Route (Inhibitor):	ORAL
Carcinogen:	N-METHYL-N'-NITRO-N-NITROSOGUANIDINE [70-25-7]
Route (Carcinogen):	ORAL
Dose (Carcinogen):	100 MG/L IN DRINKING WATER 5X/WK FOR 12 MONTHS (STUDY DURATION: 16 MONTHS)
Promoter:	NONE
Target Tissue: Type of Lesion:	STOMACH: CARCINOMA IN SITU
Endpoint (Incidence):	1/14 (7.1%), 4/13 (30.8%), -334%, NOT SIGNIFICANT
Reference:	[ANISIMOV,VN ZABEZHINSKI,MA POPOVICH,IG LIEBERMAN,AI AND SHMIDT,JL; PREVENTION OF SPONTANEOUS AND CHEMICALLY INDUCED CARCINOGENESIS USING ACTIVATED CARBON FIBER ADSORBENT. II.INHIBITORY EFFECT OF THE ACTIVATED CARBON FIBER ADSORBENT 'AQUALEN' ON N-METHYL-N'-NITRO-N-NITROSOGUANIDINE-INDUCED GASTRIC CARCINOGENESIS IN RATS; CANCER LETT. (SHANNON, IREL.) 138(1-2):23-26, 1999]

Species:	RAT
Number of Animals Tested:	(21,14)/(21,13)
Strain/Sex:	LIO/FEMALE
Dose (Inhibitor):	100 MG/KG BW AQUALEN (ACTIVATED CARBON FIBER ADSORBENT) IN DIET 5X/WK FOR 16 MONTHS (STUDY DURATION: 16 MONTHS)
Route (Inhibitor):	ORAL
Carcinogen:	N-METHYL-N'-NITRO-N-NITROSOGUANIDINE [70-25-7]
Route (Carcinogen):	ORAL
Dose (Carcinogen):	100 MG/L IN DRINKING WATER 5X/WK FOR 12 MONTHS (STUDY DURATION: 16 MONTHS)
Promoter:	NONE
Target Tissue: Type of Lesion:	STOMACH: INVASIVE ADENOCARCINOMA
Endpoint (Incidence):	5/14 (35.7%), 1/13 (7.7%), 78%, P<0.05
Reference:	[ANISIMOV,VN ZABEZHINSKI,MA POPOVICH,IG LIEBERMAN,AI AND

	SHMIDT,JL; PREVENTION OF SPONTANEOUS AND CHEMICALLY INDUCED CARCINOGENESIS USING ACTIVATED CARBON FIBER ADSORBENT. II.INHIBITORY EFFECT OF THE ACTIVATED CARBON FIBER ADSORBENT 'AQUALEN' ON N-METHYL-N'-NITRO-N-NITROSOGUANIDINE-INDUCED GASTRIC CARCINOGENESIS IN RATS; CANCER LETT. (SHANNON, IREL.) 138(1-2):23-26, 1999]

Species:	RAT
Number of Animals Tested:	(21,14)/(21,13)
Strain/Sex:	LIO/FEMALE
Dose (Inhibitor):	100 MG/KG BW AQUALEN (ACTIVATED CARBON FIBER ADSORBENT) IN DIET 5X/WK FOR 16 MONTHS (STUDY DURATION: 16 MONTHS)
Route (Inhibitor):	ORAL
Carcinogen:	N-METHYL-N'-NITRO-N-NITROSOGUANIDINE [70-25-7]
Route (Carcinogen):	ORAL
Dose (Carcinogen):	100 MG/L IN DRINKING WATER 5X/WK FOR 12 MONTHS (STUDY DURATION: 16 MONTHS)
Promoter:	NONE
Target Tissue: Type of Lesion:	DUODENUM: ADENOCARCINOMA
Endpoint (Incidence):	2/14 (14.3%), 0/13 (0.0%), 100%, NOT SIGNIFICANT
Reference:	[ANISIMOV,VN ZABEZHINSKI,MA POPOVICH,IG LIEBERMAN,AI AND SHMIDT,JL; PREVENTION OF SPONTANEOUS AND CHEMICALLY INDUCED CARCINOGENESIS USING ACTIVATED CARBON FIBER ADSORBENT. II.INHIBITORY EFFECT OF THE ACTIVATED CARBON FIBER ADSORBENT 'AQUALEN' ON N-METHYL-N'-NITRO-N-NITROSOGUANIDINE-INDUCED GASTRIC CARCINOGENESIS IN RATS; CANCER LETT. (SHANNON, IREL.) 138(1-2):23-26, 1999]

Species:	RAT
Number of Animals Tested:	(21,14)/(21,13)
Strain/Sex:	LIO/FEMALE
Dose (Inhibitor):	100 MG/KG BW AQUALEN (ACTIVATED CARBON FIBER ADSORBENT) IN DIET 5X/WK FOR 16 MONTHS (STUDY DURATION: 16 MONTHS)
Route (Inhibitor):	ORAL
Carcinogen:	N-METHYL-N'-NITRO-N-NITROSOGUANIDINE [70-25-7]
Route (Carcinogen):	ORAL
Dose (Carcinogen):	100 MG/L IN DRINKING WATER 5X/WK FOR 12 MONTHS (STUDY DURATION: 16 MONTHS)
Promoter:	NONE

Target Tissue: Type of Lesion:	LIVER: CYSTOCHOLANGIOMA
Endpoint (Incidence):	2/14 (14.3%), 0/13 (0.0%), 100%, NOT SIGNIFICANT
Reference:	[ANISIMOV,VN ZABEZHINSKI,MA POPOVICH,IG LIEBERMAN,AI AND SHMIDT,JL; PREVENTION OF SPONTANEOUS AND CHEMICALLY INDUCED CARCINOGENESIS USING ACTIVATED CARBON FIBER ADSORBENT. II.INHIBITORY EFFECT OF THE ACTIVATED CARBON FIBER ADSORBENT 'AQUALEN' ON N-METHYL-N'-NITRO-N-NITROSOGUANIDINE-INDUCED GASTRIC CARCINOGENESIS IN RATS; CANCER LETT. (SHANNON, IREL.) 138(1-2):23-26, 1999]

As taken from CCRIS, 2010.

“One of the key obstacles against the success in cancer chemotherapy is the toxic and side effects of the chemotherapeutic agents. The avoidance of these toxic and side effects will greatly improve the therapeutic effects of anticancer drugs while decrease the pains of the patients. Here we show that activated carbon nanoparticles (ACNP), one of the mesoporous nanoparticles, can decrease the genotoxicity and teratogenicity of mitomycin C (MMC). To study the effects of ACNP on genotoxicity and teratogenicity of MMC, methods of PCE micronucleus test, Chinese hamster lung cell chromosome aberration experiment and rat teratogenicity were employed to observe the differences in genotoxicity and teratogenicity between ACNP-adsorbed MMC (ACNP-MMC) and free MMC. Results demonstrated that free MMC 0.16-5.0 microg/kg significantly increased the positive rate of PCE micronucleus test, the chromosome aberration rate and rat teratogenecity, but ACNP-MMC did not increase these heredity and reproduction toxicological indexes in a dose range of 0.625-10.0 microg/kg. From these results, it can be concluded that ACNP-MMC have significant effects to decrease the genotoxicity and teratogenicity effects of MMC. These results will have a considerable impact on the strategy of anticancer chemotherapy”. Zhong et al. 2010). Journal of Nanoscience and Nanotechnology 10, 8603-8609). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21121372

“Etoposide is a semisynthetic, chemotherapeutic drug widely recommended to treat an extensive range of human cancers. Our studies indicate that, while etoposide is capable of killing human cancer cells, exposure to single-walled carbon nanotubes (SWCNTs) and etoposide results in enhanced cell death that appears to be synergistic and not merely additive. In this study, we used high pressure liquid chromatography and mass spectrometry to quantify the internal effective dose of etoposide when the human pancreatic cancer cell (PANC-1) was exposed to the combination of these agents. Our results unequivocally indicate that SWCNTs improve etoposide uptake and increase its capacity to kill cancer cells. We suggest that a combination of SWCNTs and etoposide may prove to be a more efficient chemotherapeutic protocol, especially because of the potential to lower toxicdrug doses to levels that may be useful in decreasing adverse side effects, as well as in lowering the probability of inducing chemoresistance in exposed cancer cells.” As taken from Mahmood M et al. 2013. Nanotechnology 24(4), 045102. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23291321

“The aim of this study was to investigate the potential toxic mechanisms associated with multiwall carbon nanotubes (MWCNT) in normal mouse lung. A total of 100 μg of two types of MWCNT, namely, pristine MWCNT (PMWCNT) and acid-treated-MWCNT (TMWCNT), was administered to male C57BL/6 mice via intratracheal (IT) instillation for a period of 6 mo. Our results indicated that PMWCNT induced pulmonary autophagy accumulation and resulted in more potent tumorigenic effects compared to TMWCNT. Accordingly, MWCNT may exert differential toxicity attributed to various physicochemical properties. Data emphasize the need for careful regulation of production and use of CNT.” As taken from Yu KN et al. 2013. J. Toxicol. Environ. Health A. 76(23), 1282-92. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24283420

“Background: diesel engine exhaust (dee) has recently been classified as a known human carcinogen. objective: to derive a meta-exposure-response curve (erc) for dee and lung cancer mortality and estimate lifetime excess risks (elrs) of lungcancer mortality based on assumed occupational and environmental exposure scenarios. methods: we conducted a meta-regression of lung cancer mortality and cumulative exposure to elemental carbon (ec), a proxy measure of dee, based on relative risk (rr) estimates reported by three large occupational cohort studies (including two studies of workers in the trucking industry and one study of miners). Based on the derived risk function, we calculated ELRs for several lifetime occupational and environmental exposure scenarios, and also calculated the fractions of annual lung cancer deaths attributable to DEE. RESULTS: We estimated a lnRR of 0.00098 (95% CI: 0.00055, 0.0014) for lung cancer mortality with each 1-μg/m^3-year increase in cumulative EC based on a linear meta-regression model. Corresponding lnRRs for the individual studies ranged from 0.00061 to 0.0012. Estimated numbers of excess lung cancer deaths through age 80 for lifetime occupational exposures of 1, 10, and 25 μg/m^3 EC were 17, 200, and 689 per 10,000, respectively. For lifetime environmental exposure to 0.8 μg/m^3 EC, we estimated 21 excess lung cancer deaths per 10,000. Based on broad assumptions regarding past occupational and environmental exposures we estimate that approximately 6% of annual lung cancer deaths may be due to DEE exposure. CONCLUSIONS: Combined data from three US occupational cohort studies suggest that DEE at levels common in the workplace and in outdoor air appear to pose substantial excess lifetime risks of lung cancer, above usually acceptable limits in the US and Europe, which are generally set at 1/1,000 and 1/100,000 based on lifetime exposure for the occupational and general population, respectively.” As taken from Vermeulen R et al. 2014. Environ. Health Perspect. 122(2), 172-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24273233

“The hallmark geometric feature of single-walled carbon nanotubes (SWCNT) and carbon nanofibers (CNF) - high length to width ratio - makes them similar to a hazardous agent - asbestos. Very limited data are available concerning long-term effects of pulmonary exposure to SWCNT or CNF. Here we compared inflammatory, fibrogenic and genotoxic effects of CNF, SWCNT or asbestos in mice one year after pharyngeal aspiration. In addition, we compared pulmonary responses to SWCNT by bolus dosing through pharyngeal aspiration and inhalation 5h/day for 4 days, to evaluate the effect of dose rate….No increased lung tumor incidence occurred after 1 year post exposure to SWCNT, CNF and asbestos. Overall, our data suggest that long-term pulmonary toxicity of SWCNT, CNF and asbestos - is defined not only by their chemical composition but also by the specific surface area and type of exposure.” As taken from Shvedova AA et al. 2014. Am. J. Physiol. Lung Cell. Mol. Physiol. 306(2), L170-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24213921

“Metastatic establishment and growth of Lewis lung carcinoma is promoted by single-walled carbon nanotubes (SWCNT) in C57BL6/J mice. The effect is mediated by increased local and systemic accumulation of myeloid-derived suppressor cells (MDSC), as their depletion abrogated pro-tumor activity in vivo. These data are important for the design of novel theranostics platforms with modules capable of depleting or functionally suppressing MDSC to ensure effective immunosurveillance in the tumor microenvironment.” As taken from Shvedova AA et al. 2013. Small 9(9-10), 1691-5. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22996965

“Multiple-walled carbon nanotubes (MWCNTs) may cause carcinogenesis. We found that long-term exposure to MWCNTs can induce irreversible oncogenic transformation of human bronchial epithelial cells and tumorigenicity in vivo. A genome-wide array-comparative genomic hybridization (aCGH) analysis revealed global chromosomal aberration in MWCNTs-treated clones, predominantly at chromosome 2q31-32, where the potential oncogenes HOXD9 and HOXD13 are located. Functional assays confirmed that this variation can modulate oncogenic signaling and plays

a part in MWCNTs-induced tumorigenesis, suggesting that MWCNTs are carcinogens that act by altering genomic stability and oncogenic copy numbers." As taken from Wu P et al. 2013. Nano Lett. 13(10), 4632-41. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23984819

"Malignant mesothelioma is one of the most aggressive forms of cancer known. Recent studies have shown that carbon nanotubes (CNTs) are biopersistent and induce mesothelioma in animals, but the underlying mechanisms are not known. Here, we investigate the effect of long-term exposure to high aspect ratio CNTs on the aggressive behaviors of human pleural mesothelial cells, the primary cellular target of human lung mesothelioma. We show that chronic exposure (4 months) to single- and multiwalled CNTs induced proliferation, migration, and invasion of the cells similar to that observed in asbestos-exposed cells. An up-regulation of several key genes known to be important in cell invasion, notably matrix metalloproteinase-2 (MMP-2), was observed in the exposed mesothelial cells as determined by real-time PCR. Western blot and enzyme activity assays confirmed the increased expression and activity of MMP-2. Whole genome microarray analysis further indicated the importance of MMP-2 in the invasion gene signaling network of the exposed cells. Knockdown of MMP-2 in CNT and asbestos-exposed cells by shRNA-mediated gene silencing effectively inhibited the aggressive phenotypes. This study demonstrates CNT-induced cell invasion and indicates the role of MMP-2 in the process." As taken from Lohcharoenkal W et al. 2013. ACS Nano. 7(9), 7711-23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23924264

"Accumulating evidence indicates that carbon nanotubes (CNTs) are biopersistent and can cause lung damage. With similar fibrous morphology and mode of exposure to asbestos, a known human carcinogen, growing concern has arisen for elevated risk of CNT-induced lung carcinogenesis; however, relatively little is known about the long-term carcinogenic effect of CNT. Neoplastic transformation is a key early event leading to carcinogenesis. We studied the ability of single- and multi-walled CNTs to induce neoplastic transformation of human lung epithelial cells compared to asbestos. Long-term (6-month) exposure of the cells to occupationally relevant concentrations of CNT in culture caused a neoplastic-like transformation phenotype as demonstrated by increased cell proliferation, anchorage-independent growth, invasion and angiogenesis. Whole-genome expression signature and protein expression analyses showed that single- and multi-walled CNTs shared similar signaling signatures which were distinct from asbestos. These results provide novel toxicogenomic information and suggest distinct particle-associated mechanisms of neoplasia promotion induced by CNTs and asbestos" As take from Wang L et al. 2014. Nanotoxicology 8, 485-507. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23634900

"Novel materials are often commercialized without a complete assessment of the risks they pose to human health because such assessments are costly and time-consuming; additionally, sometimes the methodology needed for such an assessment does not exist. Carbon nanotubes have the potential for widespread application in engineering, materials science and medicine. However, due to the needle-like shape and high durability of multiwalled carbon nanotubes (MWCNTs), concerns have been raised that they may induce asbestos-like pathogenicity when inhaled. Indeed, experiments in rodents supported this hypothesis. Notably, the genetic alterations in MWCNT-induced rat malignant mesothelioma were similar to those induced by asbestos. Single-walled CNTs (SWCNTs) cause mitotic disturbances in cultured cells, but thus far, there has been no report that SWCNTs are carcinogenic. This review summarizes the recent noteworthy publications on the genotoxicity and carcinogenicity of CNTs and explains the possible molecular mechanisms responsible for this carcinogenicity. The nanoscale size and needle-like rigid structure of CNTs appear to be associated with their pathogenicity in mammalian cells, where carbon atoms are major components in the backbone of many biomolecules. Publishing adverse events associated with novel materials is critically important for alerting people exposed to such materials. CNTs still have

a bright future with superb economic and medical merits. However, appropriate regulation of the production, distribution and secondary manufacturing processes is required, at least to protect the workers." As taken from Toyokuni S. 2013. Adv. Drug Deliv. Rev. 65(15), 2098-110. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23751780

"In the NIOSH study, a group of laboratory mice were injected with a chemical that is a known cancer initiator, methylcholanthrene. Another group of mice were injected with a saline solution as a control group. The mice then were exposed by inhalation either to air or to a concentration of MWCNT. These protocols enabled the researchers to investigate whether MWNCT alone would initiate cancer in mice, or whether MWCNT would promote cancer where the initiator, methylcholanthrene, had already been applied.

Mice receiving both the initiator chemical plus exposure to MWCNT were significantly more likely to develop tumors (90% incidence) and have more tumors (an average of 3.3 tumors/mouse lung) than mice receiving the initiator chemical alone (50% of mice developing tumors with an average of 1.4 tumors/lung). Additionally, mice exposed to MWCNT and to MWCNT plus the initiator chemical had larger tumors than the respective control groups. The number of tumors per animal exposed to MWCNT alone was not significantly elevated compared with the number per animal in the controls. These results indicate that MWCNT can increase the risk of cancer in mice exposed to a known carcinogen. The study does not suggest that MWCNTs alone cause cancer in mice.

Several earlier studies in the scientific literature indicated that MWCNT could have the potential to initiate or promote cancer. The new NIOSH study is the first to show that MWCNT is a cancer promoter in a laboratory experiment, and reports the growth of lung tumors in laboratory mice following inhalation exposure to MWCNT rather than injection, instillation, or aspiration. Inhalation exposure most closely resembles the exposure route of greatest concern in the workplace. In the study, laboratory mice were exposed to one type of MWCNT through inhalation at a concentration of 5 milligrams per cubic meter of air for five hours per day for a period of 15 days.

Risk of occupational cancer depends on the potency of a given substance to cause or promote cancer and the concentration and duration of worker exposure to that substance. This research is an important step in our understanding of the hazard associated with MWCNT, but before we can determine whether MWCNT pose an occupational cancer risk, we need more information about actual exposure levels and the types and nature of MWCNT being used in the workplace, and how that compares to the material used in this study. We also need to identify what work processes, tasks, and physical forms of the MWCNT are associated with exposure. Workplace studies are underway at NIOSH to learn more about actual worker exposure and to develop guidance on how to contain and control MWCNT processes to eliminate exposures, based on advancing knowledge about exposures. Further, similar research is needed for understanding the potential health effects and potential occupational risk of other types of carbon nanotubes and nanofibers, as well as other nanomaterials."

As taken from Castranova V et al. 2013. NIOSH Science Blog. Available at http://blogs.cdc.gov/niosh-science-blog/2013/03/11/mwcnt/

"We summarized the findings of in vivo toxicity studies of single-walled carbon nanotubes (SWCNTs) in laboratory animals. ….. Although no definitive study on the carcinogenicity of SWCNTs is available at present, evidence of carcinogenicity has not been reported in toxicity studies cited in this review. Overall, the available data provides initial information on SWCNT toxicity. To further clarify their toxicity and risk assessment, studies should be conducted using well-characterized SWCNTs, standard protocols, and the relevant route and doses of human exposure." As taken from Ema M et al. 2016. Regul. Toxicol. Pharmacol. 74, 42-63. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26619783

"Human Health Assessment

…… Hazards related to substances used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS). However, based on the available information on structurally related nanomaterials, the substance may cause ….. carcinogenicity

following oral and inhalation exposure. …….”

As taken from Environment Canada, 2015

5.7. Irritation/immunotoxicity

/IMMUNOTOXICITY/ Human epidermal keratinocytes (HEKs) were dosed with 6-Aminohexanoic acid-derivatized single-wall carbon nanotubes (AHA-SWNTs) ranging in concentration from 0.00000005 to 0.05 mg/mL. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability decreased significantly ($p < .05$) from 0.00005 to 0.05 mg/mL after 24 hr. The proinflammatory mediators of inflammation cytokines interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-alpha, IL-10, and IL-1beta were also assessed. Cytokine analysis did not show a significant increase in IL-6 and IL-8 in the medium containing 0.000005 mg/mL of AHA-SWNTs from 1 to 48 hr. IL-6 increased in cells treated with 0.05 mg/mL of AHA-SWNTs from 1 to 48 hr, whereas IL-8 showed a significant increase at 24 and 48 hr. No significant difference ($p < .05$) was noted with TNF-alpha, IL-10, and IL-1beta expression at any time point. Transmission electron microscopy of HEKs treated with 0.05 mg/mL AHA-SWNTs for 24 hr depicted AHA-SWNTs localized within intracytoplasmic vacuoles in HEKs. Treatment with the surfactant 1% Pluronic F127 caused dispersion of the AHA-SWNT aggregates in the culture medium and less toxicity. These data showed that the lower concentration of 0.000005 mg/mL of AHA-SWNTs maintains cell viability and induces a mild cytotoxicity, but 0.05 mg/mL of AHA-SWNTs demonstrated an irritation response by the increase in IL-8. [Zhang LW et al; Int J Toxicol 26 (2): 103-13 (2007)] **PEER REVIEWED**

“One of priority approaches in occupational medicine and health risk evaluation is study of immune system features in individuals exposed to occupational chemical hazards. The studies revealed reliable changes in immune parameters (positive annexin tag, disorders of cytokine profile)-- that proves retarded apoptosis precesses in workers engaged into activated carbon and coagulants production. Marked disorders of cellular regulation in machinery operators of activated carbon and coagulants production are seen with observed normal content of phenol in the air of workplace” (Zaĭtseva et al., 2011. Meditsina truda i promyshlennaia ekologiia 2, 21-23). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21506374

Skin, Eye and Respiratory Irritations:

It can cause a dust irritation, particularly to the eyes and mucous membranes. [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 704] **PEER REVIEWED**

As taken from HSDB, 2009

“Carbon has been found to be neither irritating nor sensitizing.”

As taken from IUCLID Dataset (2000), Carbon (7440-44-0)

“It can ... cause conjunctivitis epithelial hyperplasia of cornea, as well as eczematous inflammation of eyelids.”

“In the form of graphite ... it can cause a dust irritation, particularly to the eyes ... Some forms of carbon dust can cause irritation of eyes and mucous membranes.”

“Exptl intravenous injection of pure carbon suspensions in rabbits produces no ocular inflammation, although carbon particles are deposited within the blood vessels.”

“Small quantities of carbon suspensions in the form of graphite or India ink injected into the anterior chamber of rabbits is mostly taken up by leukocytes and by the corneal endothelium, producing essentially no signs of inflammation. Large quantities may obstruct aqueous outflow mechanically.”

After 4 h of inhalation, mainly heat shock proteins were induced, whereas after 24 h, different immunomodulatory proteins (osteopontin, galectin-3 and lipocalin-2) were upregulated in alveolar macrophages and septal cells. In conclusion, these data indicate that inhalation of ultrafine carbon

particles triggers a biphasic pro-inflammatory process in the lung, involving the activation of macrophages and the upregulation of immunomodulatory proteins." As taken from Andre et al., (2006), Eur Respir J. 2006 Aug;28(2):275-85.

"Inhalation of carbon dust ... can immediately give rise to an increased mucociliary transport ... & airway resistance mediated by the vagus."

As taken from HSDB, 2009

"In the present study, we investigated the immunomodulatory activity of multi-walled carbon nanotubes (MWCNTs) in peripheral blood mononuclear cells (PBMCs) from healthy donors and mite-allergic subjects. Freshly prepared PBMCs, stimulated or not with Toll-like receptor (TLR)1-9 agonists, a T cell mitogen (phytohemagglutinin A) or mite allergen extract were cultured in the presence or absence of MWCNTs. Secretion of TNF-α, IL-2, IL-5, IL-6, IL-12/23p40 or IFN-γ was quantified in the culture supernatants by ELISA. Basal secretion of all the cytokines was not altered by MWCNTs in PBMCs from both healthy donors and allergic subjects. In PBMCs from healthy donors, TNF-α, IL-6 and IL-12/23p40 secretion in response to the TLR4 agonist, lipopolysaccharide was however increased in a dose-dependent manner by MWCNTs. Significant increases in the release of these cytokines were also observed in PBMCs stimulated with a TLR2 or TLR3 agonist. MWCNTs also increased the release of IL-2 and IFN-γ by PBMCs stimulated with a T cell mitogen. In contrast, MWCNTs inhibited allergen-induced IL-5 secretion by PBMCs from mite-allergic subjects. As well, MWCNTs altered the capacity of PBMC-derived monocytes to differentiate into functional dendritic cells. All together, our data suggest that according to its immune cell target, MWCNTs may either promote or suppress immune responses in humans. Further investigations are necessary to fully understand the complexity behind interactions of engineered nanoparticles with the immune system." As taken from Laverny G et al. 2013. Toxicol. Lett. 217(2), 91-101. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23266719

"Aerosolized or aspirated manufactured carbon nanotubes have been shown to be cytotoxic, cause pulmonary lesions, and demonstrate immunomodulatory properties. CD-1 mice were used to assess pulmonary toxicity of helical carbon nanotubes (HCNTs) and alterations of the immune response to subsequent infection by Pseudomonas aeruginosa in mice. HCNTs provoked a mild inflammatory response following either a single exposure or 2X/week for three weeks (multiple exposures) but were not significantly toxic. Administering HCNTs 2X/week for three weeks resulted in pulmonary lesions including granulomas and goblet cell hyperplasia. Mice exposed to HCNTs and subsequently infected by P. aeruginosa demonstrated an enhanced inflammatory response to P. aeruginosa and phagocytosis by alveolar macrophages was inhibited. However, clearance of P. aeruginosa was not affected. HCNT exposed mice depleted of neutrophils were more effective in clearing P. aeruginosa compared to neutrophil-depleted control mice, accompanied by an influx of macrophages. Depletion of systemic macrophages resulted in slightly inhibited bacterial clearance by HCNT treated mice. Our data indicate that pulmonary exposure to HCNTs results in lesions similar to those caused by other nanotubes and pre-exposure to HCNTs inhibit alveolar macrophage phagocytosis of P. aeruginosa. However, clearance was not affected as exposure to HCNTs primed the immune system for an enhanced inflammatory response to pulmonary infection consisting of an influx of neutrophils and macrophages." As taken from Walling BE et al. 2013. PLoS One 8(11), e80283. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24324555

"Carbon-based nanomaterials (CBN), such as graphene nanosheets (GNS) and multiwalled carbon nanotubes (MWCNT), have been proposed for potential nanomedicine applications such as biomedical devices and carriers for drug delivery. However, our current understanding regarding the systemic toxicity of these CBN through intravenous (iv) injection is limited. In this study, we compare the immune response resulting from GNS and MWCNT exposure. We hypothesize that iv administration of GNS and MWCNT would result in divergent systemic

inflammatory responses due to physicochemical differences between these two CBN. In the lungs of C57BL/6 mice, GNS actuate a Th2 immune response 1 day following iv administration, which consists of neutrophilic influx and a significant increase in interleukin (IL)-5, IL-13, IL-33, and its soluble receptor (sST2) in the bronchoalveolar lavage fluid. MWCNT elicited a significant increase in the messenger ribonucleic acid expression of cytokines in the spleen including IL-4 and IL-33, which are associated with an increase in splenic cell differentiation (CD)4(+) and CD8(+) T-cells in C57BL/6 mice following iv injection. The observed Th2 responses in both the lung and spleen are absent in ST2(-/-) mice administrated GNS or MWCNT, suggesting a critical role for IL-33. In conclusion, the use of GNS or MWCNT as nanocarriers for drug delivery may result in Th2 immune responses that are mediated through the IL-33/ST2 axis and therefore may promote adverse allergic reactions." As aken from Wang X et al. 2013. Int. J. Nanomedicine 8, 1733-48. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23662055

"It is increasingly important to understand the single-walled carbon nanotubes' (SWCNTs) immune response as their increasingly biomedical researches and applications. Macrophages and T cells play important roles in scavenging foreign materials and pathogens and regulating immune response. In this work, primarily cultured murine peritoneal macrophages and purified splenic T cells were utilised to determine the toxic effects of SWCNTs and acid-functionalised SWCNTs (AF-SWCNTs) on the immune system, especially on macrophage functions. Macrophages were exposed to 0-50 μg/ml of CNTs for 24 h and no significant cytotoxicity was found by live/dead and annexin-V-FITC/PI analyses. The TEM images revealed that AF-SWCNTs were engulfed mostly through phagocytosis and located in lysosomes of macrophages. Measurement of mitochondrial membrane potential and proteasome subunit gene expression demonstrated that 10 and 50 μg/ml AF-SWCNTs could damage mitochondrial function and proteasome formation in a concentration-dependent manner. Functional analyses revealed that the percentage of phagocytic cells were affected significantly by 20 μg/ml CNTs, and 5 μg/ml AF-SWCNTs inhibited the phagocytic efficiency of latex beads in macrophages. The accessory cell function was affected by both AF-SWCNTs and SWCNTs at concentrations of 10 and 50 μg/ml, respectively. Furthermore, AF-SWCNT biased naïve T-cell differentiation to Th1 type by inducing the production of IFN-γ and TNF, implying the potential risk of Th1-associated diseases (e.g. autoimmune diseases and inflammation) on AF-SWCNT exposure." As taken from Dong PX et al. 2013. Nanotoxicology 7(5), 1028-42. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22632544

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…. It is a severe eye irritant (MAS score = 68), a mild skin irritant (PII = 1.08) and at most a weak sensitizer (because the positive control was tested at a concentration 10X higher than the test substance). …... Hazards related to substances used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS). However, based on the available information on structurally related nanomaterials, the substance may cause …. immunotoxicity, …. following oral and inhalation exposure….."

As taken from Environment Canada, 2015

"The biomedical application of graphene quantum dots (GQDs) is a new emerging area. However, their safety data are still in scarcity to date. Particularly, the effect of GQDs on the immune system remains unknown. This study aimed to elucidate the interaction of GQDs with macrophages and the underlying mechanisms. Our results showed that GQDs slightly affected the cell viability and membrane integrity of macrophages, whereas GQDs significantly increased reactive oxygen species (ROS) generation and apoptotic and autophagic cell death with an increase in the expression level of Bax, Bad, caspase 3, caspase 9, beclin 1, and LC3-I/II and a decrease in that of Bcl-2. Furthermore, low concentrations of GQDs significantly increased the expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-8, whereas high concentrations of GQDs elicited opposite effects on the cytokines production. SB202190, a selective inhibitor of p38 mitogen-activated protein kinase (MAPK), abolished the cytokine-inducing effect of GQDs in macrophages. Moreover, GQDs significantly increased the phosphorylation of p38 MAPK and p65,

and promoted the nuclear translocation of nuclear factor-κB (NF-κB). Taken together, these results show that GQDs induce ROS generation, apoptosis, autophagy, and inflammatory response via p38MAPK and NF-κB mediated signaling pathways in THP-1 activated macrophages." As taken from Qin Y et al. 2015. Toxicology 327, 62-76. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25446327

"The potential of carbon nanotubes (CNTs) in medical applications has been attracting constant research interest as well as raising concerns related to toxicity. The immune system serves as the first line of defense against invasion. In this work, interactions of oxidized multiwalled carbon nanotubes (MWCNT) with macrophages were investigated to unravel the activation profile of macrophages, using cytokine array, ELISA assay, transwell assay, confocal microscopy, and reactive oxygen species examination. Results show that MWCNT initiate phagocytosis of macrophages and upregulate CD14, CD11b, TLR-4/MD2, and CD206, which does not alter the MHCII expression of the macrophages. The macrophages engulfing MWCNT (MWCNT-RAW) secrete a large amount of MIP-1α and MIP-2 to recruit naïve macrophages and produce angiogenesis-related cytokines MMP-9 and VEGF, while inducing much lower levels of proinflammatory cytokines than those activated by LPS. In conclusion, MWCNT activate macrophages into a M1/M2 mixed status, which allows the cells to recruit naïve macrophages and support angiogenesis." As taken from Meng J et al. 2015. ACS Appl. Mater. Interfaces 7(5), 3180-8. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25591447

"The interleukin-1 (IL-1) family has been implicated in cellular responses to nanoparticles including carbon nanotubes (CNTs). IL-1α and β are key proinflammatory cytokines important in inflammatory and oxidative stress responses. The aim of this study was to characterize the role of IL-1 in cellular responses of CNTs in cells from IL-1α/β wild type (IL1-WT) mice and cells with reduced inflammatory potential from IL-1α/β deficient (IL1-KO) mice. Two multi-walled CNTs, CNT-1 containing long and thick fibers and CNT-2 containing short and thin fibers, were compared to UICC crocidolite asbestos fibers. Upon CNT exposure toxicity and apoptosis were affected differently in IL1-WT and IL1-KO cells. Upregulation of TNFα and IL-1α mRNA expression in IL1-WT cells was dependent on the type of CNT. On the contrary precursor IL-1α protein was downregulated after 24h. The mitogen-activated protein kinase (MAPK) c-Jun N-terminal kinase (JNK) was activated in IL1-KO cells and regulated by CNTs, whereas no significant changes of extracellular regulated kinase (ERK) were observed when comparing IL1-WT and IL1-KO cells. In summary, the results presented here indicate that IL-1 contributes to the cellular and molecular effects of CNT exposure and that the type of CNT has an important effect on the cellular response." As taken from Arnoldussen YJ et al. 2015. Cytokine 73(1), 128-37. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25748835

"OBJECTIVE: Activated carbon (AC) has been used in wound therapy as an active substance inside dressings. Applying AC directly on a wound is a new concept. The aim of this study was to analyse the outcomes of chronic wounds which were managed with directly applied activated carbon knitted cloth (ACC, Zorflex) in Swiss patients. METHOD: A retrospective analysis of the records of all patients with chronic wounds treated with ACC between 1 October 2013 and 31 December 2015 in an outpatient wound clinic. Chronic was defined as a wound being present for >3 weeks. Malignant wounds were excluded. The main outcome was the time to complete closure or readiness for spilt-thickness skin grafting (STSG). Descriptive data, including nutritional status and angiology results were obtained. RESULTS: There were 36 women and 34 men, median age 68 years old. The median body mass index (BMI) 28.1kg/m^2 and 76% (n=53) of patients had comorbidities. Angiology exam results showed signs of reduced arterial perfusion in 13% (n=9) of patients and malnutrition in 11% (n=8). Of the wounds included 34% (n=24) were on the trunk and 66% (n=46) on the extremities. The median wound size was 6.9cm^2 (range: 0.1-300cm^2). The wounds on the trunk were larger than wounds on extremities (10 versus 2cm^2). Overall, median time to wound closure was 51 days. In 94% (n=66) of patients, wounds closed without further intervention and 6% (n=4) underwent STSG. Patients with comorbidities showed longer wound healing times compared with those without. No adverse events such as allergies or skin irritation occurred. Cost analysis, including personnel and material and stratified according known wound closure times, showed ACC (US$ 1252) to be like hydrocolloids (US$ 1128), but substantially lower

than white gauze (US$ 3026) and negative pressure wound therapy (NPWT) (US$ 2578). CONCLUSION: ACC applied directly on chronic wounds of different aetiology is safe with short closure times. The cost efficiency is high. It combines the positive features of other wound dressings, such as hydrocolloids and NPWT, without their disadvantages. The dressing change of ACC is easy and non-specialised nurses or even patients themselves can be taught to perform it." As taken from Scheer HS et al. 2017. J. Wound Care 26(8), 476-481. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28795884

5.8. All other relevant types of toxicity

ALTERNATIVE and IN VITRO TESTS/ ... The toxicity of single-walled carbon nanotubes (SWCNT) was assessed in human keratinocyte cells. The results show increased oxidative stress and inhibition of cell proliferation in response to treatment of keratinocytes with SWCNT particles. In addition, the signaling mechanism in keratinocytes upon exposure to SWCNT particles was investigated. Results from the study suggest that SWCNT particles activate NF-kappaB in a dose-dependent manner in human keratinocytes. Further, the mechanism of activation of NF-kappaB was due to the activation of stress-related kinases by SWCNT particles in keratinocytes. [Manna SK et al; Nano Lett 5 (9): 1676-84 (2005)] **PEER REVIEWED**

/ALTERNATIVE and IN VITRO TESTS/ Carbon nanotube films were grown using a microwave plasma enhanced chemical vapor deposition system. Human epidermal keratinocytes (HEK) were exposed to 0.1, 0.2, and 0.4 mg/mL of multi-walled carbon nanotubes (MWCNT) for 1, 2, 4, 8, 12, 24 and 48 hr. HEK were examined by transmission electron microscopy for the presence of MWCNT. ... Chemically unmodified MWCNT were present within cytoplasmic vacuoles of the HEK at all time points. The MWCNT also induced the release of the proinflammatory cytokine interleukin 8 from HEKs in a time dependent manner. These data clearly show that MWCNT, not derivatized nor optimized for biological applications, are capable of both localizing within and initiating an irritation response in a target epithelial cell that composes a primary route of occupational exposure for manufactured nanotubes. [Monteiro-Riviere NA et al; Toxicol Lett 155 (3): 377-84 (2005)] **PEER REVIEWED**

/ALTERNATIVE and IN VITRO TESTS/ ... Recent studies in skin and lung reveal that carbon nanoparticles can cause toxicity. To generate a preliminary protein profile of nanotube exposure, ... human epidermal keratinocytes (HEKs) exposed to multi-walled carbon nanotubes (MWCNTs) in cell culture /were analyzed/ using large-format, two-dimensional (2D) gel electrophoresis and mass spectrometry (MS). Compared with controls, 24 hours of MWCNT exposure altered the expression of 36 proteins ($P < .01$), whereas 106 were altered at 48 hours. At both time points, roughly 67% of the affected proteins were significantly down-regulated. Peptide mass fingerprinting identified most of the differentially expressed proteins, and the various protein identities reflected a complex cellular response to MWCNT exposure. In addition to proteins associated with metabolism, cell signaling, and stress, we observed a consistent effect on the expression of cytoskeletal elements and vesicular trafficking components. These data clearly show that MWCNTs are capable of altering protein expression in a target epithelial cell that constitutes a primary route of occupational exposure for manufactured nanotubes. [Witzmann FA et al; Nanomedicine 2 (3): 158-68 (2006)] **PEER REVIEWED**

/ALTERNATIVE and IN VITRO TESTS/ ... Adverse effects of single-wall carbon nanotubes (SWCNT) /were investigated/ using a cell culture of immortalized human epidermal keratinocytes (HaCaT). After 18 hr of exposure of HaCaT to SWCNT, oxidative stress and cellular toxicity were indicated by formation of free radicals, accumulation of peroxidative products, antioxidant depletion, and loss of cell viability. Exposure to SWCNT also resulted in ultrastructural and morphological changes in cultured skin cells. These data indicate that dermal exposure to unrefined SWCNT may lead to dermal toxicity due to accelerated oxidative stress in the skin of exposed workers. [Shvedova AA et al; J Toxicol Environ Health A 66 (20): 1909-26 (2003)] **PEER REVIEWED**

/OTHER TOXICITY INFORMATION/ **...** Human exposure is expected to be negligible for carbon

when it is used as one component in gas-producing cartridges placed in animal burrows. Ignited cartridges are to be quickly placed into burrows which are then covered to entrap the generated fumes. Improperly covered burrows could result in inhalation exposure to the fumes if the applicator remains in close proximity to the burrow. [USEPA/Office of Pesticide Programs; Reregistration Eligibility Decision Document - Carbon and Carbon Dioxide p.6 (September 1991). Available from, as of July 19, 2008: http://www.epa.gov/pesticides/reregistration/status.htm] **PEER REVIEWED**

As taken from HSDB, 2009

"BACKGROUND: REPETITIVE ELEMENTS TAKE UP >40% OF THE HUMAN GENOME AND CAN CHANGE DISTRIBUTION THROUGH TRANSPOSITION, THUS GENERATING SUBFAMILIES. Repetitive element DNA methylation has associated with several diseases and environmental exposures, including exposure to airborne pollutants. No systematic analysis has yet been conducted to examine the effects of exposures across different repetitive element subfamilies. The purpose of the study is to evaluate sensitivity of DNA methylation in differentially–evolved LINE, Alu, and HERV subfamilies to different types of airborne pollutants. METHODS: We sampled a total of 120 male participants from three studies (20 high-, 20 low-exposure in each study) of steel workers exposed to metal-rich particulate matter (measured as PM10) (Study 1); gas-station attendants exposed to air benzene (Study 2); and truck drivers exposed to traffic-derived elemental carbon (Study 3). We measured methylation by bisulfite-PCR-pyrosequencing in 10 differentially–evolved repetitive element subfamilies. RESULTS: High-exposure groups exhibited subfamily-specific methylation differences compared to low-exposure groups: L1PA2 showed lower DNA methylation in steel workers (P=0.04) and gas station attendants (P=0.03); L1Ta showed lower DNA methylation in steel workers (P=0.02); AluYb8 showed higher DNA methylation in truck drivers (P=0.05). Within each study, dose-response analyses showed subfamily-specific correlations of methylation with exposure levels. Interaction models showed that the effects of the exposures on DNA methylation were dependent on the subfamily evolutionary age, with stronger effects on older LINEs from PM10 (p–interaction=0.003) and benzene (p–interaction=0.04), and on younger Alus from PM10 (p-interaction=0.02). CONCLUSIONS: The evolutionary age of repetitive element subfamilies determines differential susceptibility of DNA methylation to airborne pollutants. As taken from Byun HM et al. 2013. Part. Fibre. Toxicol. 10, 28. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23855992

"Nanomaterials (NMs) are engineered for commercial purposes such as semiconductors, building materials, cosmetics, and drug carriers, while natural nanoparticles (NPs) already exist in the environment….This review will summarize and discuss recent reports derived from cell lines or animal models concerning the effects of NMs on, and their application in, the endocrine system of mammalian and other species. It will present an update on current studies of the effects of some typical NMs-such as metal-based NMs, carbon-based NMs, and dendrimers-on endocrine functions, in which some effects are adverse or unwanted and others are favorable or intended. Disruption of endocrine function is associated with adverse health outcomes including reproductive failure, metabolic syndrome, and some types of cancer. Further investigations are therefore required to obtain a thorough understanding of any potential risk of pathological endocrine disruption from products containing NMs. This review aims to provide impetus for further studies on the interactions of NMs with endocrine functions." As taken from Lu X et al. 2013. Small 9(9-10), 1654-71. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23401134

"Carbon nanotubes (CNTs) consist of a family of carbon built nanoparticles, whose biological effects depend on their physical characteristics and other constitutive chemicals (impurities and functions attached)….Oxidative stress is the main mechanism of toxicity but size, agglomeration, chirality as well as impurities and functionalization are some of the structural and chemical characteristic contributing to the CNTs toxicity outcomes. Among the many toxicity pathways, interference with cytoskeleton and fibrous mechanisms, cell signaling, membrane perturbations and the production of

cytokines, chemokines and inflammation are some of the effects resulting from exposure to CNTs. The aim of this review is to offer an up-to-date scope of the effects of CNTs on biological systems with attention to mechanisms of toxicity." As taken from Rodriguez-Yañez Y et al. 2013. Toxicol. Mech. Methods 23(3), 178-95. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23193995

"Graphene and its derivatives are promising candidates for important biomedical applications because of their versatility. The prospective use of graphene-based materials in a biological context requires a detailed comprehension of the toxicity of these materials. Moreover, due to the expanding applications of nanotechnology, human and environmental exposures to graphene-based nanomaterials are likely to increase in the future. Because of the potential risk factors associated with the manufacture and use of graphene-related materials, the number of nanotoxicological studies of these compounds has been increasing rapidly in the past decade. These studies have researched the effects of the nanostructural/biological interactions on different organizational levels of the living system, from biomolecules to animals. This review discusses recent results based on in vitro and in vivo cytotoxicity and genotoxicity studies of graphene-related materials and critically examines the methodologies employed to evaluate their toxicities. The environmental impact from the manipulation and application of graphene materials is also reported and discussed. Finally, this review presents mechanistic aspects of graphene toxicity in biological systems. More detailed studies aiming to investigate the toxicity of graphene-based materials and to properly associate the biological phenomenon with their chemical, structural, and morphological variations that result from several synthetic and processing possibilities are needed. Knowledge about graphene-based materials could ensure the safe application of this versatile material. Consequently, the focus of this review is to provide a source of inspiration for new nanotoxicological approaches for graphene-based materials." As taken from Seabra AB et al. 2014. Chem. Res. Toxicol. 27(2), 159-168. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24422439

"Carbon-based nanomaterials have attracted great interest in biomedical applications such as advanced imaging, tissue regeneration, and drug or gene delivery. The toxicity of the carbon nanotubes and graphene remains a debated issue although many toxicological studies have been reported in the scientific community. In this review, we summarize the biological effects of carbon nanotubes and graphene in terms of in vitro and in vivo toxicity, genotoxicity and toxicokinetics. The dose, shape, surface chemistry, exposure route and purity play important roles in the metabolism of carbon-based nanomaterials resulting in differential toxicity. Careful examination of the physico-chemical properties of carbon-based nanomaterials is considered a basic approach to correlate the toxicological response with the unique properties of the carbon nanomaterials. The reactive oxygen species-mediated toxic mechanism of carbon nanotubes has been extensively discussed and strategies, such as surface modification, have been proposed to reduce the toxicity of these materials. Carbon-based nanomaterials used in photothermal therapy, drug delivery and tissue regeneration are also discussed in this review. The toxicokinetics, toxicity and efficacy of carbon-based nanotubes and graphene still need to be investigated further to pave a way for biomedical applications and a better understanding of their potential applications to humans". As taken from Zhang Y et al. 2014. Drug Metab. Rev. 46(2), 232-46. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24506522

"Human Health Assessment

…... Hazards related to substances used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS). ……. t. Based on the low potential for direct and indirect exposure of the general population under the industrial uses identified in this submission, the substance is not likely to pose a significant health risk to the general population, and is therefore unlikely to be harmful to human health. However, based on the current understanding of carbon nanotubes and of nanomaterials in general, the risk arising from

the use of the substance in consumer products is not known at this time. The use of the substance in consumer products or in products intended for use by or for children may significantly alter the exposure of the general population resulting in the substance becoming harmful to human health. Similarly, the import or manufacture of the substance in quantities greater than 10 000 kg/yr may significantly increase the exposure levels of the general population resulting in the substance becoming harmful to human health. Consequently, more information is necessary to better characterize potential health risks."

As taken from Environment Canada, 2015.

Activated carbons are effective adsorbents for many volatile organic compounds and are used in cigarette filters to remove selected smoke toxicants. Polymer-derived carbon is more effective in removing many vapour phase toxicants found in cigarette smoke than coconut-shell-derived carbon. We compared mouth-level exposure to "tar", nicotine and five vapour phase constituents (1,3-butadiene, benzene, toluene, isoprene, acrylonitrile) in two groups of Romanian smokers of 4-mg or 8-mg International Organization for Standardization (ISO) "tar" bands. Test cigarettes with 4 and 8 mg ISO "tar" were manufactured for the study with two target levels of polymer-derived carbon (30 mg and 56 mg), along with control cigarettes containing a target level of 56 mg of coconut-shell-derived carbon in both "tar" bands. No significant differences were found between mouth-level exposure to "tar" or nicotine yields obtained from control and test products ($p > 0.05$) in either ISO "tar" band. Mouth-level exposure to each of the five vapour phase constituents was significantly lower from the test products with polymer-derived carbon ($p < 0.0001$) than from control cigarettes with coconut-shell-derived carbon, by an average of 25% with 30 mg polymer-derived carbon and around 50% with 56 mg." As taken from Nother K et al. 2016. Beiträge zur Tabakforschung International 27(2), 40–53. Available at https://doi.org/10.1515/cttr-2016-0007

6. Functional effects on

6.1. Broncho/pulmonary system

EPIDEMIOLOGY STUDIES/ /The objective was/ to investigate the risk of cancer and non-neoplastic respiratory diseases among workers who manufacture carbon electrodes, as this industry entails exposure to mixtures of polycyclic aromatic hydrocarbons. ... A historical cohort study was carried out of 1006 male workers employed for at least 1 year between 1945 and 1971 in a carbon (graphite) electrode production plant in central Italy, who were followed up for mortality between 1955 and 1996. The ratio of observed to expected deaths (standardised mortality ratios, SMRs) was computed from both national and (for the period 1964-96) regional age and period specific mortalities. A multivariate Poisson regression analysis was performed to investigate the relative risk (RR) of death according to duration of employment and time since first employment in the factory. ... A total of 424 workers had died, 538 were still alive, and 44 were lost to follow up. Mortalities from all causes, all cancers, and respiratory tract cancer were in line with the regional figure. An excess was found over the expected deaths from skin cancer including melanoma (SMR 3.16, 95% confidence interval (95% CI) 0.65 to 9.23) and from non-neoplastic respiratory diseases (SMR 1.58, 95% CI 1.16 to 2.11). Poisson regression analysis including age as a covariate showed an increased risk of dying from gastric cancer with increasing duration of employment, and an increase in the RR of dying from lung cancer and from non-neoplastic respiratory diseases with increasing time since first employment, although the linear trend was not significant. ... This study supports previous findings that working in the carbon electrode manufacturing industry may not increase the risk of dying from respiratory cancer.

/SIGNS AND SYMPTOMS/ ... INHALATION OF CARBON DUST ... CAN IMMEDIATELY GIVE RISE TO AN INCREASED MUCOCILIARY TRANSPORT ... & AIRWAY RESISTANCE MEDIATED

BY THE VAGUS. /CARBON DUST/ [Friberg, L., G.R. Nordberg, and V.B. Vouk. Handbook on the Toxicology of Metals. New York: Elsevier North Holland, 1979., p. 72] **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ The aim of this study was to evaluate the acute lung toxicity of intratracheally instilled single-wall carbon nanotubes (SWCNT) in rats. The lungs of rats were instilled either with 1 or 5 mg/kg of the following control or particle types: (1) SWCNT, (2) quartz particles (positive control), (3) carbonyl iron particles (negative control), (4) phosphate-buffered saline (PBS) + 1% Tween 80, or (5) graphite particles (lung tissue studies only). Following exposures, the lungs of PBS and particle-exposed rats were assessed using bronchoalveolar lavage (BAL) fluid biomarkers and cell proliferation methods, and by histopathological evaluation of lung tissue at 24 h, 1 week, 1 month, and 3 months postinstillation. Exposures to high-dose (5 mg/kg) SWCNT produced mortality in ~15% of the SWCNT-instilled rats within 24 h postinstillation. This mortality resulted from mechanical blockage of the upper airways by the instillate and was not due to inherent pulmonary toxicity of the instilled SWCNT particulate. Exposures to quartz particles produced significant increases versus controls in pulmonary inflammation, cytotoxicity, and lung cell parenchymal cell proliferation indices. Exposures to SWCNT produced transient inflammatory and cell injury effects. Results from the lung histopathology component of the study indicated that pulmonary exposures to quartz particles (5 mg/kg) produced dose-dependent inflammatory responses, concomitant with foamy alveolar macrophage accumulation and lung tissue thickening at the sites of normal particle deposition. Pulmonary exposures to carbonyl iron or graphite particles produced no significant adverse effects. Pulmonary exposures to SWCNT in rats produced a non-dose-dependent series of multifocal granulomas, which were evidence of a foreign tissue body reaction and were nonuniform in distribution and not progressive beyond 1 month postexposure (pe). The observation of SWCNT-induced multifocal granulomas is inconsistent with the following: (1) lack of lung toxicity by assessing lavage parameters, (2) lack of lung toxicity by measuring cell proliferation parameters, (3) an apparent lack of a dose response relationship, (4) nonuniform distribution of lesions, (5) the paradigm of dust-related lung toxicity effects, (6) possible regression of effects over time. In addition, the results of two recent exposure assessment studies indicate very low aerosol SWCNT exposures at the workplace. [Warheit DB et al; Toxicol Sci 77 (1): 117-25 (2004); Comment in: Toxicol Sci 77 (1): 3-5 (2004)] **PEER REVIEWED**

As taken from HSDB, 2009

“Male Sprague Dawley rats were exposed to carbon fibers 7 microns in diameter and 20 to 60 microns in length, for six hours a day and five days a week for up to 16 weeks at an average chamber concentration of 20 mg/m3. Rats were killed at 4, 8, 12, and 16 weeks of exposure and after a 32-week postexposure recovery period. A similar number of control rats exposed only to air were killed at the same times. Pulmonary function tests, conducted just prior to the animals' death, did not demonstrate any significant or consistent changes. The only pulmonary finding that could be causally related to the subchronic inhalation of carbon fibers was phagocytosis of the inhaled particles by alveolar macrophages. This physiologic response was not accompanied by any local reactive pulmonary inflammation or fibrosis.” As taken from Owen et al., (1986), J Occup Med. 1986 May;28(5):373-6., available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=3712116&query_hl=5&itool=pubmed_DocSum

“High levels of particulate matter in ambient air are associated with increased respiratory and cardiovascular health problems. It has been hypothesised that it is the ultrafine particle fraction (diameter <100 nm) that is largely responsible for these effects. To evaluate the associated mechanisms on a molecular level, the current authors applied an expression profiling approach. Healthy mice were exposed to either ultrafine carbon particles (UFCPs; mass concentration 380 microg x m(-3)) or filtered air for 4 and 24 h. Histology of the lungs did not indicate any pathomorphological changes after inhalation. Examination of the bronchoalveolar lavage fluid

revealed a small increase in polymorphonuclear cell number (ranging 0.6-1%) after UFCP inhalation, compared with clean air controls, suggesting a minor inflammatory response. However, DNA microarray profile analysis revealed a clearly biphasic response to particle exposure. As taken from André E, Eur Respir J. 2006 Aug; 28(2):275-85 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16641123&query_hl=9&itool=pubmed_docsum

“Increased levels of particulate air pollution are associated with increased respiratory and cardiovascular mortality and morbidity. Some epidemiologic and toxicologic research suggests ultrafine particles (UFPs) (< 100 nm) to be more harmful per unit mass than larger particles. Our study was aimed at a quantitative comparison of acute adverse effects of different types of carbonaceous UFPs at a dose range that causes a moderate inflammatory response in lungs. We used six different particle types (primary particle size 10-50 nm, specific surface area 30-800 m2/g, and organic content 1-20%): PrintexG, Printex90, flame soot particles with different organic content (SootL, SootH), spark-generated ultrafine carbon particles (ufCP), and the reference diesel exhaust particles (DEP) SRM1650a. Mice were instilled with 5, 20, and 50 microg of each particle type, and bronchoalveolar lavage was analyzed 24 hr after instillation for inflammatory cells and the level of proinflammatory cytokines. At respective mass-doses, particle-caused detrimental effects ranked in the following order: ufCP > SootL > or = SootH > Printex90 > PrintexG > DEP. Relating the inflammatory effects to the particle characteristics--organic content, primary particle size, or specific surface area--demonstrates the most obvious dose response for particle surface area. Our study suggests that the surface area measurement developed by Brunauer, Emmett, and Teller is a valuable reference unit for the assessment of causative health effects for carbonaceous UFPs. Additionally, we demonstrated the existence of a threshold for the particle surface area at an instilled dose of approximately 20 cm2, below which no acute proinflammatory responses could be detected in mice.” As taken from Stoeger et al., (2006), Environ Health Perspect. 2006 Mar;114(3):328-33, available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16507453&query_hl=9&itool=pubmed_docsum

“The hallmark geometric feature of single-walled carbon nanotubes (SWCNT) and carbon nanofibers (CNF) - high length to width ratio - makes them similar to a hazardous agent - asbestos. Very limited data are available concerning long-term effects of pulmonary exposure to SWCNT or CNF. Here we compared inflammatory, fibrogenic and genotoxic effects of CNF, SWCNT or asbestos in mice one year after pharyngeal aspiration. In addition, we compared pulmonary responses to SWCNT by bolus dosing through pharyngeal aspiration and inhalation 5h/day for 4 days, to evaluate the effect of dose rate. The aspiration studies showed that, these particles can be visualized in the lung at one year post-exposure, while some translocate to lymphatics. All these particles induced chronic bronchopneumonia and lymphadenitis, accompanied by pulmonary fibrosis. CNF and asbestos were found to promote the greatest degree of inflammation, followed by SWCNT, while SWCNT were the most fibrogenic of these three particles. Further, SWCNT induced cytogenetic alterations seen as micronuclei formation and nuclear protrusions in vivo. Importantly, inhalation exposure to SWCNT showed significantly greater inflammatory, fibrotic and genotoxic effects than bolus pharyngeal aspiration. Finally, SWCNT and CNF, but not asbestos exposures, increased the incidence of K-ras oncogene mutations in the lung….Overall, our data suggest that long-term pulmonary toxicity of SWCNT, CNF and asbestos - is defined not only by their chemical composition but also by the specific surface area and type of exposure.” As taken from Shvedova AA et al. 2014. J. Physiol. Lung Cell. Mol. Physiol. 306(2), L170-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24213921

“This article presents a regression-tree-based meta-analysis of rodent pulmonary toxicity studies of uncoated, nonfunctionalized carbon nanotube (CNT) exposure. The resulting analysis provides quantitative estimates of the contribution of CNT attributes (impurities, physical dimensions, and

aggregation) to pulmonary toxicity indicators in bronchoalveolar lavage fluid: neutrophil and macrophage count, and lactate dehydrogenase and total protein concentrations. The method employs classification and regression tree (CART) models, techniques that are relatively insensitive to data defects that impair other types of regression analysis: high dimensionality, nonlinearity, correlated variables, and significant quantities of missing values. Three types of analysis are presented: the RT, the random forest (RF), and a random-forest-based dose-response model. The RT shows the best single model supported by all the data and typically contains a small number of variables. The RF shows how much variance reduction is associated with every variable in the data set. The dose-response model is used to isolate the effects of CNT attributes from the CNT dose, showing the shift in the dose-response caused by the attribute across the measured range of CNT doses. It was found that the CNT attributes that contribute the most to pulmonary toxicity were metallic impurities (cobalt significantly increased observed toxicity, while other impurities had mixed effects), CNT length (negatively correlated with most toxicity indicators), CNT diameter (significantly positively associated with toxicity), and aggregate size (negatively correlated with cell damage indicators and positively correlated with immune response indicators). Increasing CNT N_2 -BET-specific surface area decreased toxicity indicators." As taken from Gernand JM & Casman EA. 2014. Risk Anal. 34(3), 583-97. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24024907

Aerosolized or aspirated manufactured carbon nanotubes have been shown to be cytotoxic, cause pulmonary lesions, and demonstrate immunomodulatory properties. CD-1 mice were used to assess pulmonary toxicity of helical carbon nanotubes (HCNTs) and alterations of the immune response to subsequent infection by Pseudomonas aeruginosa in mice. HCNTs provoked a mild inflammatory response following either a single exposure or 2X/week for three weeks (multiple exposures) but were not significantly toxic. Administering HCNTs 2X/week for three weeks resulted in pulmonary lesions including granulomas and goblet cell hyperplasia. Mice exposed to HCNTs and subsequently infected by P. aeruginosa demonstrated an enhanced inflammatory response to P. aeruginosa and phagocytosis by alveolar macrophages was inhibited. However, clearance of P. aeruginosa was not affected. HCNT exposed mice depleted of neutrophils were more effective in clearing P. aeruginosa compared to neutrophil-depleted control mice, accompanied by an influx of macrophages. Depletion of systemic macrophages resulted in slightly inhibited bacterial clearance by HCNT treated mice. Our data indicate that pulmonary exposure to HCNTs results in lesions similar to those caused by other nanotubes and pre-exposure to HCNTs inhibit alveolar macrophage phagocytosis of P. aeruginosa. However, clearance was not affected as exposure to HCNTs primed the immune system for an enhanced inflammatory response to pulmonary infection consisting of an influx of neutrophils and macrophages." As taken from Walling BE et al. 2013. PLoS One 8(11), e80283. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24324555

"Double-walled carbon nanotubes (DWCNT) are a rather new and unexplored variety of carbon nanotubes. Previously conducted studies established that exposure to a variety of carbon nanotubes produced lung inflammation and fibrosis in mice after pharyngeal aspiration. However, the bioactivity of double-walled carbon nanotubes (DWCNT) has not been determined. In this study, the hypothesis that DWCNT would induce pulmonary toxicity was explored by analyzing the pulmonary bioactivity of DWCNT. To test this hypothesis, C57Bl/6 mice were exposed to DWCNT by pharyngeal aspiration. Mice underwent whole-lung lavage (WLL) to assess pulmonary inflammation and injury, and lung tissue was examined histologically for development of pulmonary disease as a function of dose and time. The results showed that DWCNT exposure produced a dose-dependent increase in WLL polymorphonuclear leukocytes (PMN), indicating that DWCNT exposure initiated pulmonary inflammation. DWCNT exposure also produced a dose-dependent rise in lactate dehydrogenase (LDH) activity, as well as albumin levels, in WLL fluid, indicating that DWCNT exposure promoted cytotoxicity as well as decreases in the integrity of the blood-gas barrier in the lung, respectively. In addition, at 7 and 56 d postexposure, the presence of significant

alveolitis and fibrosis was noted in mice exposed to 40 µg/mouse DWCNT. In conclusion, this study provides insight into previously uninvestigated pulmonary bioactivity of DWCNT exposure. Data indicate that DWCNT exposure promotes inflammation, injury, and fibrosis in the lung." As taken from Sager TM et al. 2013. J. Toxicol. Environ. Health A. 76(15), 922-36. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24156695

"The aim of this study was to investigate the potential toxic mechanisms associated with multiwall carbon nanotubes (MWCNT) in normal mouse lung. A total of 100 µg of two types of MWCNT, namely, pristine MWCNT (PMWCNT) and acid-treated-MWCNT (TMWCNT), was administered to male C57BL/6 mice via intratracheal (IT) instillation for a period of 6 mo. Our results indicated that PMWCNT induced pulmonary autophagy accumulation and resulted in more potent tumorigenic effects compared to TMWCNT. Accordingly, MWCNT may exert differential toxicity attributed to various physicochemical properties. Data emphasize the need for careful regulation of production and use of CNT." As taken from Yu KN et al. 2013. J. Toxicol. Environ. Health A. 76(23), 1282-92. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24283420

"Carbon nanotubes (CNTs) represent promising vectors to facilitate cellular drug delivery and to overcome biological barriers, but some types may also elicit persistent pulmonary inflammation based on their fibre characteristics. Here, we show the pulmonary response to aqueous suspensions of block copolymer dispersed, double-walled carbon nanotubes (DWCNT, length 1-10 µm) in mice by bronchoalveolar lavage (BAL) analysis, and BAL and blood cytokine and lung antioxidant profiling. The intratracheally instilled dose of 50 µg DWCNT caused significant pulmonary inflammation that was not resolved during a 7-day observation period. Light microscopy investigation of the uptake of DWCNT agglomerates revealed no particle ingestion for granulocytes, but only for macrophages. Accumulating macrophage, multinucleated macrophage and lymphocyte numbers in the alveolar region further indicated ineffective resolution with chronification of the inflammation. The local inflammatory impairment of the lung was accompanied by pulmonary antioxidant depletion and haematological signs of systemic inflammation. While the observed inflammation during its acute phase was dominated by neutrophils and neutrophil recruiting cytokines, the contribution of macrophages and lymphocytes with related cytokines became more significant after day 3 of exposure. This study confirms that acute pulmonary toxicity can occur on exposure of high doses of DWCNT agglomerates and offers further insight for improved nanotube design parameters to avoid potential long-term toxicity." As taken from Tian F et al. 2013. Eur. J. Pharm. Biopharm. 84(2), 412-20. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23542608

"Carbon nanotubes (CNTs) find their extensive application as a promising material in medicine due to unique characteristics. However, such materials have been accompanied with potentially hazardous effects on human health. The toxicity of CNTs may vary depending on their structural characteristics, surface properties and chemical composition. To gain insight into the toxicity of CNTs in vivo and in vitro, we summarize contributing factors for the toxic effects of CNTs in this review. In addition, we elaborate on the toxic effects and mechanisms in target sites at systemic, organic, cellular, and biomacromolecule levels. Various issues are reported to be effected when exposed to CNTs including (1) blood circulation, (2) lymph circulation, (3) lung, (4) heart, (5) kidney, (6) spleen, (7) bone marrow, and (8) blood brain barrier. Though there have been published reports on thetoxic effects of CNTs to date, more studies will still be needed to gain full understanding of their potential toxicity and underlying mechanisms." As taken from Wang J et al. 2013a. Curr. Drug. Metab. 14(8), 891-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24016107

"Carbon nanotubes (CNTs) have been a subject of intensive research for a wide range of

applications. However, because of their extremely small size and light weight, CNTs are readily inhaled into human lungs resulting in increased rates of pulmonary disorders, most notably fibrosis. Several studies have demonstrated the fibrogenic effects of CNTs given their ability to translocate into the surrounding areas in the lung causing granulomatous lesions and interstitial and sub-pleural fibrosis. However, the mechanisms underlying the disease process remain obscure due to the lack of understanding of the cellular interactions and molecular targets involved. Interestingly, certain physicochemical properties of CNTs have been shown to affect their respiratory toxicity, thereby becoming significant determinants of fibrogenesis. CNT-induced fibrosis involves a multitude of cell types and is characterized by the early onset of inflammation, oxidative stress and accumulation of extracellular matrix. Increased reactive oxygen species activate various cytokine/growth factor signaling cascades resulting in increased expression of inflammatory and fibrotic genes. Profibrotic growth factors and cytokines contribute directly to fibroblast proliferation and collagen production. Given the role of multiple players during the pathogenesis of CNT-induced fibrosis, the objective of this review is to summarize the key findings and discuss major cellular and molecular events governing pulmonary fibrosis. We also discuss the physicochemical properties of CNTs and their effects on pulmonary toxicities as well as various biological factors contributing to the development of fibrosis." As taken from Manke A et al. 2013. Toxicol. Mech. Methods 23(3), 196-206. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23194015

"….This Account reviews the inhalation toxicity of manufactured nanomaterials and compares them with inhalation and intratracheal instillation studies of well-characterized fullerene and carbon nanotubes. In many reports, pulmonary inflammation and injury served as pulmonary endpoints for the inhalation toxicity. To assess pulmonary inflammation, we examined neutrophil and macrophage infiltration in the alveolar and/or interstitial space, and the expression of the neutrophil and/or monocyte chemokines. We also reported the release of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in the bronchoalveolar lavage fluid (BALF), the expression of oxidative stress-related genes characteristic of lung injury, and the presence of granulomatous lesion and pulmonary fibrosis. In the inhalation and intratracheal instillation studies of well-characterized fullerenes, exposure to fullerene did not induce pulmonary inflammation or transient inflammation. By contrast, in an inhalation study, a high concentration of multiwall carbon nanotubes (MWCNTs) and single-wall carbon nanotubes (SWCNTs) induced neutrophil inflammation or granulomatous formations in the lung, and intratracheal instillation of MWCNTs and SWCNTs induced persistent inflammation in the lung. Among the physicochemical properties of carbon nanotubes, the increased surface area is associated with inflammatory activity as measured by the increase in the rate of neutrophils measured in bronchoalveolar lavage fluid. Metal impurities such as iron and nickel enhanced the pulmonary toxicity of carbon nanotubes, and SWCNTs that included an amorphous carbon induced multifocal granulomas in the lung while purer SWCNTs did not. The aggregation state also affects pulmonary response: Exposure to well-dispersed carbon nanotubes led to the thickening of the alveolar wall and fewer granulomatous lesions in the lung, while agglomerated carbon nanotubes produced granulomatous inflammation….." As taken from Morimoto Y et al. 2013. Acc. Chem. Res. 46(3), 770-81. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22574947

"….In this Organization for Economic Cooperation and Development (OECD) 413 guideline inhalation study with VGCF-H carbon nanofibers (CNFs), rats were exposed to 0, 0.54, 2.5 or 25 mg/m(3) CNF for 13 weeks. The standard toxicology experimental design was supplemented with bronchoalveolar lavage (BAL) and respiratory cell proliferation (CP) endpoints. BAL fluid (BALF) recovery of inflammatory cells and mediators (i.e., BALF- lactate dehydrogenase [LDH], microprotein [MTP], and alkaline phosphatase [ALKP] levels) were increased only at 25 mg/m(3), 1 day after exposure. No differences versus control values in were measured at 0.54 or 2.5 mg/m(3) exposure concentrations for any BAL fluid endpoints. Approximately 90% (2.5 and 25 mg/m(3)) of the BAL-recovered macrophages contained CNF. CP indices at 25 mg/m(3) were increased in the airways, lung parenchyma, and subpleural regions, but no increases in CP versus controls were

measured at 0.54 or 2.5 mg/m(3). Based upon histopathology criteria, the NOAEL was set at 0.54 mg/m(3), because at 2.5 mg/m(3), "minimal cellular inflammation" of the airways/lung parenchyma was noted by the study pathologist; while the 25 mg/m(3) exposure concentration produced slight inflammation and occasional interstitial thickening. In contrast, none of the more sensitive pulmonary biomarkers such as BAL fluid inflammation/cytotoxicity biomarkers or CP turnover results at 2.5 mg/m(3) were different from air-exposed controls. Given the absence of convergence of the histopathological observations versus more quantitative measures at 2.5 mg/m(3), it is recommended that more comprehensive guidance measures be implemented for setting adverse effect levels in (nano)particulate, subchronic inhalation studies including a WOE approach for establishing no adverse effect levels; and a suggestion that some findings should be viewed as normal physiological adaptations (e.g., normal macrophage phagocytic responses-minimal inflammation) to long-term particulate inhalation exposures." As taken from Warheit DB et al. 2013. Toxicol. Pathol. 41(2), 387-94. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23242579

"To evaluate pulmonary toxicity of multi-walled carbon nanotubes (MWCNTs), F344 rats of both sexes were exposed by inhalation to 0.2, 1 or 5 mg/m(3) MWCNT aerosol for 6 h/day, 5 days/week for 2 weeks using a whole-body exposure system. At the end of the 2-week exposure period, one-half of the rats were necropsied, and at the end of an additional 4-week postexposure period, the remaining rats were necropsied. MWCNTs were deposited in the lungs of all MWCNT-exposed groups and mostly remained in the lungs throughout the 4-week postexposure period. Granulomatous changes in the lung were found in the rats exposed to 5 mg/m(3) MWCNTs, and these changes were slightly aggravated at the end of the 4-week postexposure period. In the bronchoalveolar lavage fluid (BALF), the numbers of neutrophils, percentages of bi- and multinucleated alveolar macrophages, levels of ALP activity and concentrations of total protein and albumin were elevated in the rats exposed to 1 and 5 mg/m(3) MWCNTs. At the end of the 4-week postexposure period, the values of the BALF parameters tended to remain elevated. In addition, goblet cell hyperplasias in the nasal cavity and nasopharynx were observed in the rats exposed to 1 and 5 mg/m(3) MWCNTs, but these lesions had largely regressed by the end of the postexposure period. Based on the histopathological and inflammatory changes, the no-observed-adverse-effect level (NOAEL) for inhalation of MWCNTs for 2 weeks was 0.2 mg/m(3)." As taken from Umeda Y et al. 2013. J. Toxicol. Pathol. 26(2), 131-40. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23914055

"For hazard assessment of multiwalled carbon nanotubes (MWCNTs), a 90-day inhalation toxicity study has been performed with Nanocyl NC 7000 in accordance with OECD 413 test guideline. MWCNTs produced no systemic toxicity. However, increased lung weights, multifocal granulomatous inflammation, diffuse histiocytic and neutrophilic infiltrates, and intra-alveolar lipoproteinosis were observed in lung and lung-associated lymph nodes at 0.5 and 2.5mg/m(3). Additional investigations of the lungs were performed, including special stains for examination of connective tissue, and electron microscopy was performed to determine the location of the MWCNTs. The alveolar walls revealed no increase of collagen fibers, whereas within the microgranulomas a slight increase of collagen fibers was observed. The pleura did not reveal any increase in collagen fibers. Only a slight increase in reticulin fibers in the alveolar walls in animals of the 0.5 and 2.5mg/m(3) concentration group was noted. In the 0.1mg/m(3) group, the only animal revealing minimal granulomas exhibited a minimal increase in collagen within the granuloma. No increase in reticulin was observed. Electron microscopy demonstrated entangled MWCNTs within alveolar macrophages. Occasionally electron dense particles/detritus were observed within membrane-bound vesicles (interpreted as phagosomes), which could represent degraded MWCNTs. If so, MWCNTs were degradable by alveolar macrophages and not persistent within the lung. Inhalation of MWCNTs caused granulomatous inflammation within the lung parenchyma but not the pleura in any of the concentration groups. Thus, there are some similarities to effects caused by inhaled asbestos, but the hallmark effects, namely pleural inflammation and/or fibrosis

leading to mesotheliomas, are absent." As taken from Treumann S et al. 2013. Toxicol. Sci. 134(1), 103-10. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23570993

"This study investigated the in vivo pulmonary toxicity of inhaled multi-walled carbon nanotubes (MWCNT). Mice-inhaled aerosolized MWCNT (10 mg/m³, 5 h/day) for 2, 4, 8 or 12 days. MWCNT lung burden was linearly related to exposure duration. MWCNT-induced pulmonary inflammation was assessed by determining whole lung lavage (WLL) polymorphonuclear leukocytes (PMN). Lung cytotoxicity was assessed by WLL fluid LDH activities. WLL fluid albumin concentrations were determined as a marker of alveolar air-blood barrier integrity. These parameters significantly increased in MWCNT-exposed mice versus controls and were dose-dependent. Histopathologic alterations identified in the lung included (1) bronciolocentric inflammation, (2) bronchiolar epithelial hyperplasia and hypertrophy, (3) fibrosis, (4) vascular changes and (5) rare pleural penetration. MWCNT translocated to the lymph node where the deep paracortex was expanded after 8 or 12 days. Acute inhalation of MWCNT induced dose-dependent pulmonary inflammation and damage with rapid development of pulmonary fibrosis, and also demonstrated that MWCNT can reach the pleura after inhalation exposure." As taken from Porter DW et al. 2013. Nanotoxicology 7, 1179-94. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22881873

"….The present study was designed to seek a simple, effective, and oxidative stress-based biomarker system used for screening toxicity of nanomaterials. Nano-ferroso-ferric oxide (nano-Fe3O4), nano-silicon dioxide (nano-SiO2), and single-walled carbon nanotubes (SWCNTs) were dispersed in corn oil and characterized using transmission electron microscopy (TEM). Rats were exposed to the three nanomaterials by intratracheal instillation once every 2 days for 5 weeks. We investigated their lung oxidative and inflammatory damage by bronchoalveolar lavage fluid (BALF) detection and comparative proteomics by lung tissue. Two-dimensional electrophoresis (2-DE) of proteins isolated from the lung tissue, followed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry, was performed. In the present study, we chose to detect lactate dehydrogenase, total antioxidant capacity, superoxide dismutase, and malondialdehyde as the biomarker system for screening the oxidative stress of nanomaterials and IL-6 as the inflammatory biomarker in BALF. Proteomics analysis revealed 17 differentially expressed proteins compared with the control group: nine were upregulated and eight were downregulated. Our results indicated that exposure by intratracheal instillation to any of the three typical nanomaterials may cause lung damage through oxidative damage and/or an inflammatory reaction." As taken from Lin Z et al. 2013. Nanoscale Res. Lett. 8(1), 521. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24321467

"NIOSH systematically reviewed 54 laboratory animal studies, many of which indicated that CNT/CNF could cause adverse pulmonary effects including inflammation (44/54), granulomas (27/54), and pulmonary fibrosis (25/54).

The estimated risk of developing early-stage (slight or mild) lung effects over a working lifetime if exposed to CNT at the analytical limit of quantification (NIOSH Method 5040) of 1 µg/m3 (8-hr time-weighted average [TWA] as respirable elemental carbon) is approximately 0.5% to 16% (upper confidence limit estimates) (Table A–8). In addition, the working lifetime equivalent estimates of the animal no observed adverse effect level (NOAEL) of CNT or CNF were also near 1 µg/m3 (8-hr TWA).

The concern about worker exposure to CNT or CNF arises from the results of recent laboratory animal studies with CNT and CNF. Short-term and subchronic studies in rats and mice have shown qualitatively consistent noncancerous adverse lung effects including pulmonary inflammation, granulomas, and fibrosis with inhalation, intratracheal instillation, or pharyngeal aspiration of several types of CNT (single or multiwall; purified or unpurified). These early-stage, noncancerous adverse lung effects in animals include: (1) the early onset and persistence of pulmonary fibrosis in CNT-

exposed mice [Shvedova et al. 2005, 2008; Porter et al. 2010; Mercer et al. 2011], (2) an equal or greater potency of CNT compared with other inhaled particles known to be hazardous (e.g., crystalline silica, asbestos) in causing pulmonary inflammation and fibrosis [Lam et al. 2004; Shvedova et al. 2005; Muller et al. 2005], and (3) reduced lung clearance in mice or rats exposed to relatively low-mass concentrations of CNT [Mercer et al. 2009; Pauluhn 2010a]. Findings of acute pulmonary inflammation and interstitial fibrosis have also been observed in mice exposed to CNF [Murray et al. 2012]. The extent to which these animal data may predict clinically significant lung effects in workers is not known. However, NIOSH considers these animal study findings of pulmonary inflammation, granulomas, and fibrosis associated with exposure to CNT and CNF to be relevant to human health risk assessment because similar lung effects have been observed in workers in dusty jobs [Rom and Markowitz 2006; Hubbs et al. 2011].

…in experimental animal studies, both unpurified and purified (low metal content) CNT are associated with early onset and persistent pulmonary fibrosis and other adverse lung effects [Lam et al. 2004; Shvedova et al. 2005; 2008]. Other studies indicate that differences in physical-chemical properties, including functionalization or bio-modification, may alter the lung retention and biological responses [Kagan et al. 2010; Osmond-McLeod et al. 2011; Pauluhn 2010a; Oyabu et al. 2011]. Although a number of different types of CNT and CNF have been evaluated, uncertainty exists on the generalizability of the current animal findings to new CNT and CNF.

Studies in mice exposed to multi-walled carbon nanotubes (MWCNT) have shown the migration of MWCNT from the pulmonary alveoli to the intrapleural space [Hubbs et al. 2009; Porter et al. 2010; Mercer et al. 2010]. The intrapleural space is the same site in which malignant mesothelioma can develop due to asbestos exposure. Intraperitoneal injection of CNT in mice has resulted in inflammation from long MWCNT (> 5 μm in length), but not short MWCNT (< 1 μm in length) or tangled CNT [Poland et al. 2008; Takagi et al. 2008; Muller et al. 2009; Murphy et al. 2011]. In rats administered CNT by peritoneal injection, the pleural inflammation and mesothelioma were related to the thin diameter and rigid structure of MWCNT [Nagai et al. 2011]. In a study of rats administered MWCNT or crocidolite by intrapulmonary spraying, exposure to either material produced inflammation in the lungs and pleural cavity in addition to mesothelial proliferative lesions [Xu et al. 2012].

NIOSH considers the pulmonary responses of inflammation and fibrosis observed in short-term and subchronic studies in animals to be relevant to humans, as inflammatory and fibrotic effects are also observed in occupational lung diseases associated with workplace exposures to other inhaled particles and fibers. Uncertainties include the extent to which these lung effects in animals are associated with functional deficits and whether similar effects would be clinically significant among workers. However, these fibrotic lung effects observed in some of the animal studies developed early (e.g., 28 days after exposure) in response to relatively low-mass lung doses, and also persisted or progressed after the end of exposure [Shvedova et al. 2005, 2008; Ma-Hock et al. 2009; Pauluhn 2010a; Porter et al. 2010; Mercer et al. 2011; DeLorme et al. 2012; Murray et al. 2012]. Given the relevance of these types of lung effects to humans, the REL was derived using the published subchronic and short-term animal studies with dose-response data of early stage fibrotic and inflammatory lung responses to CNT exposure."

As taken from NIOSH, 2013.

"Toxicity of engineered nanomaterials is associated with their inherent properties, both physical and chemical. Recent studies have shown that exposure to multi-walled carbon nanotubes (MWCNTs) promotes tumors and tumor-associated pathologies and lead to carcinogenesis in model *in vivo* systems. Here in we examined the potential of purified MWCNTs used at occupationally relevant exposure doses for particles not otherwise regulated to affect human lung epithelial cells. The uptake of the purified MWCNTs was evaluated using fluorescence activated cell sorting (FACS), while the effects on cell fate were assessed using 2- (4-iodophenyl) - 3- (4-nitrophenyl) - 5-(2, 4-disulfophenyl) -2H-tetrazolium salt colorimetric assay, cell cycle and nanoindentation. Our results showed that exposure to MWCNTs reduced cell metabolic activity and induced cell cycle arrest. Our analysis further emphasized that MWCNTs-induced cellular fate results from multiple types of interactions that could be analyzed by means of intracellular biomechanical changes and are pivotal

in understanding the underlying MWCNTs-induced cell transformation." As taken from Dong C et al. 2014. Environ. Sci. Nano. 1(6), 95-603. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/25485116.

"Recent studies indicate that the brain is a target for toxic carbonaceous nanoparticles present in ambient air. It has been proposed that the neurotoxic effects of such particles are driven by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase mediated generation of reactive oxygen species (ROS) in activated microglia. In the present study, we have evaluated the effects of short term (4h) nose-only inhalation exposure to carbon NP (CNP) in the brains and lungs of C57BL/6J mice and in p47(phox-/-) mice that lack a functional NADPH oxidase. It was shown that the lungs of the p47(phox-/-) mice are less responsive to CNP inhalation than lungs of the corresponding C57BL/6J control animals. Lung tissue mRNA expression of the oxidative stress/DNA damage response genes 8-oxoguanine glycosylase (OGG1) and apurinic/apyrimidinic endonuclease 1 (APE1) were induced by CNP exposure in C57BL/6J but not in the p47(phox-/-) mice. In contrast, the expression of these genes, as well as Tumor Necrosis Factor-α (TNFα), Cyclooxygenase-2 (COX-2) and Heme Oxygenase-1 (HO-1) was not altered in the olfactory bulb, cerebellum or remaining brain tissue part of either mouse background. This indicates that neuroinflammation was not induced by this exposure. CNP inhalation for 4h or for 4h on three consecutive days also did not affect brain tissue protein expression of interleukin (IL)-1β, while a clear significant difference in constitutive expression level of this pro-inflammatory cytokine was found between C57BL/6J and p47(phox-/-) mice. In conclusion, short-term inhalation exposure to pure carbon nanoparticles can trigger mild p47(phox) dependent oxidative stress responses in the lungs of mice whereas in their brains at the same exposure levels signs of oxidative stress and inflammation remain absent. The possible role of p47(phox) in the neuro-inflammatory effects of nanoparticles in vivo remains to be clarified." As taken from van Berlo D et al. 2014. Neurotoxicology 43, 65-72. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24792328.

"Human Health Assessment

….. Hazards related to substances used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS). However, based on the available information on structurally related nanomaterials, the substance may cause respiratory toxicity, ….. following oral and inhalation exposure. ….."

As taken from Environment Canada, 2015

"Carbon nanotubes (CNTs) are rapidly emerging as high-priority occupational toxicants. CNT powders contain fibrous particles that aerosolize readily in places of manufacture and handling, posing an inhalation risk for workers. Studies using animal models indicate that lung exposure to CNTs causes prolonged inflammatory responses and diffuse alveolar injury. The mechanisms governing CNT-induced lung inflammation are not fully understood but have been suggested to involve alveolar macrophages (AMs). In the current study, we sought to systematically assess the effector role of AMs in vivo in the induction of lung inflammatory responses to CNT exposures and investigate their cell type-specific mechanisms. Multi-wall CNTs characterized for various physicochemical attributes were used as the CNT type. Using an AM-specific depletion and repopulation approach in a mouse model, we unambiguously demonstrated that AMs are major effector cells necessary for the in vivo elaboration of CNT-induced lung inflammation. We further investigated in vitro AM responses and identified molecular targets which proved critical to pro-inflammatory responses in this model, namely MyD88 as well as MAPKs and Ca(2+)/CamKII. We further demonstrated that MyD88 inhibition in donor AMs abrogated their capacity to reconstitute CNT-induced inflammation when adoptively transferred into AM-depleted mice. Taken together, this is the first in vivo demonstration that AMs act as critical effector cell types in CNT-induced lung inflammation and that MyD88 is required for this in vivo effector function. AMs and their cell type-specific mechanisms may therefore represent potential targets for future therapeutic intervention of CNT-related lung injury." As taken from Frank EA et al. 2015. Toxicol. Appl. Pharmacol. 288(3), 322-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26272622

“An in vitro model resembling the respiratory epithelium was used to investigate the biological response to laboratory-made pristine and functionalised multi-walled carbon nanotubes (pMWCNT and MWCNT-COOH). Cell uptake was analysed by MWCNT-COOH, FITC labelled and the effect of internalisation was evaluated on the endocytic apparatus, mitochondrial compartment and DNA integrity. In the dose range 12.5-100μgml(-1), cytotoxicity and ROS generation were assayed, evaluating the role of iron (the catalyst used in MWCNTs synthesis). We observed a correlation between MWCNTs uptake and lysosomal dysfunction and an inverse relationship between these two parameters and cell viability (P<0.01). In particular, pristine-MWCNT caused a time- and dose-dependent ROS increase and higher levels of lipid hydroperoxides compared to the controls. Mitochondrial impairment was observed. Conversely to the functionalised MWCNT, higher micronuclei (MNi) frequency was detected in mono- and binucleate pMWCNT-treated cells, underlining an aneugenic effect due to mechanical damage. Based on the physical and chemical features of MWCNTs, several toxicological pathways could be activated in respiratory epithelium upon their inhalation. The biological impacts of nano-needles were imputable to their efficient and very fast uptake and to the resulting mechanical damages in cell compartments. Lysosomal dysfunction was able to trigger further toxic effects.” As taken from Visalli G et al. 2015. Toxicol. In Vitro 29(2), 352-62. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25499066

“There is a current interest in reducing the in vivo toxicity testing of nanomaterials in animals by increasing toxicity testing using in vitro cellular assays; however, toxicological results are seldom concordant between in vivo and in vitro models. This study compared global multi-walled carbon nanotube (MWCNT)-induced gene expression from human lung epithelial and microvascular endothelial cells in monoculture and coculture with gene expression from mouse lungs exposed to MWCNT. Using a cutoff of 10% false discovery rate and 1.5 fold change, we determined that there were more concordant genes (gene expression both up- or downregulated in vivo and in vitro) expressed in both cell types in coculture than in monoculture. When reduced to only those genes involved in inflammation and fibrosis, known outcomes of in vivo MWCNT exposure, there were more disease-related concordant genes expressed in coculture than monoculture. Additionally, different cellular signaling pathways are activated in response to MWCNT dependent upon culturing conditions. As coculture gene expression better correlated with in vivo gene expression, we suggest that cellular cocultures may offer enhanced in vitro models for nanoparticle risk assessment and the reduction of in vivo toxicological testing.” As taken from Snyder-Talkington BN et al. 2015. Toxicology 328, 66-74. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25511174

“Carbon nanotubes (CNTs) are rapidly emerging as high-priority occupational toxicants. CNT powders contain fibrous particles that aerosolize readily in places of manufacture and handling, posing an inhalation risk for workers. Studies using animal models indicate that lung exposure to CNTs causes prolonged inflammatory responses and diffuse alveolar injury. The mechanisms governing CNT-induced lung inflammation are not fully understood but have been suggested to involve alveolar macrophages (AMs). In the current study, we sought to systematically assess the effector role of AMs in vivo in the induction of lung inflammatory responses to CNT exposures and investigate their cell type-specific mechanisms. Multi-wall CNTs characterized for various physicochemical attributes were used as the CNT type. Using an AM-specific depletion and repopulation approach in a mouse model, we unambiguously demonstrated that AMs are major effector cells necessary for the in vivo elaboration of CNT-induced lung inflammation. We further investigated in vitro AM responses and identified molecular targets which proved critical to pro-inflammatory responses in this model, namely MyD88 as well as MAPKs and Ca(2+)/CamKII. We further demonstrated that MyD88 inhibition in donor AMs abrogated their capacity to reconstitute CNT-induced inflammation when adoptively transferred into AM-depleted mice. Taken together, this is the first in vivo demonstration that AMs act as critical effector cell types in CNT-induced lung inflammation and that MyD88 is required for this in vivo effector function. AMs and their cell type-specific mechanisms may therefore represent potential targets for future therapeutic intervention of CNT-related lung injury.” As taken from Frank EA et al. 2015. Toxicol. Appl. Pharmacol. 288(3), 322-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26272622

“We summarized the findings of in vivo toxicity studies of single-walled carbon nanotubes (SWCNTs) in laboratory animals. ….. Injected SWCNTs were distributed throughout most of the organs including the brain, mainly retained in the lungs, liver, and spleen, and eliminated through the kidney and bile duct. Orally administered SWCNTs are suggested to be absorbed from the gastrointestinal tract to the blood circulation in mice and rats. ….. Overall, the available data provides initial information on SWCNT toxicity. To further clarify their toxicity and risk assessment, studies should be conducted using well-characterized SWCNTs, standard protocols, and the relevant route and doses of human exposure.” As taken from Ema M et al. 2016. Regul. Toxicol. Pharmacol. 74, 42-63. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26619783

6.2. Cardiovascular system

“To measure the inflammatory and autonomic responses of healthy humans and patients with coronary artery disease to controlled concentrations of two specific components of vehicle derived air pollution, carbon particles and sulphur dioxide (SO2). METHODS: Placebo controlled, double blind, random order human challenge study examining the effects of carbon particles (50 microg/m3) and SO2 (200 parts per billion (ppb)) on heart rate variability (HRV) and circulating markers of inflammation and coagulation in healthy volunteers and patients with stable angina. RESULTS: In healthy volunteers, markers of cardiac vagal control did not fall in response to particle exposure but, compared with the response to air, increased transiently immediately after exposure (root mean square of successive RR interval differences (RMSSD) 15 (5) ms with carbon particles and 4 (3) ms) with air, $p < 0.05$). SO2 exposure resulted in no immediate change but a significant reduction in HRV markers of cardiac vagal control at four hours (RMSSD -2 (3.6) ms with air, -7 (2.7) ms with SO2, $p < 0.05$). No such changes were seen in patients with stable angina. Neither pollutant caused any change in markers of inflammation or coagulation at zero, four, or 24 hours. CONCLUSION: In healthy volunteers, short term exposure to pure carbon particles does not cause adverse effects on HRV or a systemic inflammatory response. The adverse effects of vehicle derived particulates are likely to be caused by more reactive species found on the particle surface. SO2 exposure does, however, reduce cardiac vagal control, a response that would be expected to increase susceptibility to ventricular arrhythmia.” As taken from Routeledge et al., (2006), Heart. 2006 Feb;92(2):220-7, available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15923279&query_hl=26&itool=pubmed_docsum

“While environmental particles are associated with mortality and morbidity related to pulmonary and cardiovascular (CV) disease, the mechanisms involved in CV health effects are not known. Changes in systemic clotting factors have been associated with pulmonary inflammation. We hypothesized that inhaled ultrafine particles result in an inflammatory response which may stimulate systemic clotting factor release. Adult male Wistar rats were exposed to either fine or ultrafine carbon black (CB) for 7 h. The attained total suspended particle concentrations were 1.66 mg/m(3) for ultrafine CB and 1.40 mg/m(3) for fine CB. Particle concentration of ultrafine particles was more than 10 times greater than that of fine particles and the count median aerodynamic diameter averaged 114 nm for the ultrafine and 268 nm for the fine carbon particles. Data were collected immediately, 16 and 48 h following exposure. Only ultrafine CB caused an increase in total bronchoalveolar lavage (BAL) leukocytes, whereas both fine (2-fold) and ultrafine (4-fold) carbon particles caused an increase in BAL neutrophils at 16 h postexposure. Exposure to the ultrafine, but not fine, carbon was also associated with significant increases in the total numbers of blood leukocytes. Plasma fibrinogen, factor VII and von Willebrand factor (vWF) were unaffected by particle treatments as was plasma Trolox equivalent antioxidant status (TEAC). Macrophage inflammatory protein-2 mRNA was significantly increased in BAL cells 48 h following exposure to ultrafine CB. The data show that there is a small but consistent significant proinflammatory effect of this exposure to ultrafine particles that is greater than the effect of the same exposure to fine CB.” As taken from Gilmour et al., (2004), Toxicol Appl Pharmacol. 2004 Feb 15;195(1):35-44, available

at
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=14962503&query_hl=26&itool=pubmed_docsum

“Ever increasing use of engineered carbon nanoparticles in nanopharmacology for selective imaging, sensor or drug delivery systems has increased the potential for blood platelet–nanoparticle interactions. We studied the effects of engineered and combustion-derived carbon nanoparticles on human platelet aggregation in vitro and rat vascular thrombosis in vivo. Multiplewall (MWNT), singlewall (SWNT) nanotubes, C60 fullerenes (C60CS) and mixed carbon nanoparticles (MCN) (0.2–300 mgml1) were investigated. Nanoparticles were compared with standard urban particulate matter (SRM1648, average size 1.4 mm). Platelet function was studied using lumi aggregometry, phase-contrast, immunofluorescence and transmission electron microscopy, flow cytometry, zymography and pharmacological inhibitors of platelet aggregation. Vascular thrombosis was induced by ferric chloride and the rate of thrombosis was measured, in the presence of carbon particles, with an ultrasonic flow probe. Carbon particles, except C60CS, stimulated platelet aggregation (MCNN>SWNT>MWNT>SRM1648) and accelerated the rate of vascular thrombosis in rat carotid arteries with a similar rank order of efficacy. All particles resulted in upregulation of GPIIb/IIIa in platelets. In contrast, particles differentially affected the release of platelet granules, as well as the activity of thromboxane-, ADP, matrix metalloproteinase- and protein kinase C-dependent pathways of aggregation. Furthermore, particle-induced aggregation was inhibited by prostacyclin and S-nitroso-glutathione, but not by aspirin. Thus, some carbon nanoparticles and microparticles have the ability to activate platelets and enhance vascular thrombosis. These observations are of importance for the pharmacological use of carbon nanoparticles and pathology of urban particulate matter” (Radomski et al., 2005. British Journal of Pharmacology 146, 882–893). As taken from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1751219/pdf/146-0706386a.pdf

“Sachar and Saxena (Sachar and Saxena 2011) administered single doses (100 µg/animal) of either SWCNTs or acid functionalized SWCNTs (AF-SWCNTs) to inbred Swiss and C57BL76 female mice (6–12 week old, weighing 20-25 g; number per group not reported) by either intratracheal instillation, intravenous (i.v.) or intra-peritoneal (i.p.) injections, or orally by gavage. The acid functionalized (AF)-SWCNTs were surface oxidized by a mixture of nitric and sulphuric acid under pressure at elevated temperature. The carboxylic acid moieties formed were derivatised by a fluorophor for imaging purposes, and were intensively purified to remove excess fluorescent dye. The particle size distribution and surface charge was not indicated. A transient decrease was observed in the number of erythrocytes and levels of blood haemoglobin (from 3 to 48 hours but not after 72 hours) after i.v. injection and to a lesser extent after i.p. injections of AF-SWCNTs as compared to SWCNTs. Administration of AF-SWCNTs through oral gavage and the i.p. route did not reduce erythrocyte count (haemoglobin was apparently not measured for these routes of as no information is given in the paper).”

As taken from Binderup et al. 2013.

“Epidemiologic and toxicologic studies were carried out in concert to provide complementary insights into the compositional features of ambient particulate matter (PM*) that produce cardiovascular effects. In the epidemiologic studies, we made use of cohort data from two ongoing studies--the Multi-Ethnic Study of Atherosclerosis (MESA) and the Women's Health Initiative--Observational Study (WHI-OS)--to investigate subclinical markers of atherosclerosis and clinical cardiovascular events. In the toxicologic study, we used the apolipoprotein E null (ApoE(-/-)) hypercholesterolemic mouse model to assess cardiovascular effects of inhalation exposure to various atmospheres containing laboratory-generated pollutants. In the epidemiologic studies, individual-level residential concentrations of fine PM, that is, PM with an aerodynamic diameter of 2.5 microm or smaller (PM2.5), PM2.5 components (primarily elemental carbon [EC] and organic carbon [OC], silicon, and sulfur but also sulfate, nitrate, nickel, vanadium, and copper), and

the gaseous pollutants sulfur dioxide and nitrogen dioxide were estimated using spatiotemporal modeling and other exposure estimation approaches. In the MESA cohort data, evidence for associations with increased carotid intima-media thickness (CIMT) was found to be strongest for PM2.5, OC, and sulfur, as well as for copper in more limited analyses; the evidence for this was found to be weaker for silicon, EC, and the other components and gases. Similarly, in the WHI-OS cohort data, evidence for associations with incidence of cardiovascular mortality and cardiovascular events was found to be good for OC and sulfur, respectively, and for PM2.5; the evidence for this was found to be weaker for EC and silicon. Source apportionment based on extensive monitoring data in the six cities in the MESA analyses indicated that OC represented secondary formation processes as well as primary gasoline and biomass emissions, that sulfur represented largely secondary inorganic aerosols, and that copper represented brake dust and diesel emissions. In the toxicologic study, hypercholesterolemic mice were exposed for 50 days to atmospheres containing mixed vehicular engine emissions (MVE) consisting of mixed gasoline and diesel engine exhaust or to MVE-derived gases only (MVEG). Mice were also exposed to atmospheres containing sulfate, nitrate, or road dust, either alone or mixed with MVE or MVEG. Sulfate alone or in combination with MVE was associated with increased aortic reactivity. All exposures to atmospheres containing MVE (including a combination of MVE with other PM) were associated with increases in plasma and aortic oxidative stress; exposures to atmospheres containing only sulfate or nitrate were not. Exposure to MVE and to MVEG combinations except those containing road dust resulted in increased monocyte/macrophage sequestration in aortic plaque (a measure of plaque inflammation). Exposure to all atmospheres except those containing nitrate was associated with enhanced aortic vasoconstriction. Exposure to the MVEG was an independent driver of lipid peroxidation, matrix metalloproteinase (MMP) activation, and vascular inflammation. The epidemiologic and toxicologic study designs were intended to complement each other. The epidemiologic studies provided evidence in real-world human settings, and the toxicologic study directly assessed the biologic effects of various pollutant mixtures (in a way that is not possible in epidemiologic studies) by examining endpoints that probably underlie the subclinical and clinical cardiovascular endpoints examined in the epidemiologic studies. The epidemiologic studies were not suited to determining whether the observed associations were caused by direct effects of individual pollutants or by the mixtures in which individual pollutants are found. These studies were consistent in finding that OC and sulfate had the strongest evidence for associations with the cardiovascular disease endpoints, with much weaker evidence for EC and silicon. Both OC and sulfate reflected a large secondary aerosol component. Results from the toxicologic study indicated, for the most part, that MVE and mixtures of MVE and MVEG with other PM pollutants were important in producing the toxic cardiovascular effects found in the study. Further work on the effects of pollutant mixtures and secondary aerosols should allow better understanding of the pollution components and sources most responsible for the adverse cardiovascular effects of air pollution exposure." As taken from Vedal S et al. 2013. Resp. Rep. Health Eff. Inst. 178, 5-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24377210

"Carbon nanotubes (CNTs) find their extensive application as a promising material in medicine due to unique characteristics. However, such materials have been accompanied with potentially hazardous effects on human health. The toxicity of CNTs may vary depending on their structural characteristics, surface properties and chemical composition. To gain insight into the toxicity of CNTs in vivo and in vitro, we summarize contributing factors for the toxic effects of CNTs in this review. In addition, we elaborate on the toxic effects and mechanisms in target sites at systemic, organic, cellular, and biomacromolecule levels. Various issues are reported to be effected when exposed to CNTs including (1) blood circulation, (2) lymph circulation, (3) lung, (4) heart, (5) kidney, (6) spleen, (7) bone marrow, and (8) blood brain barrier. Though there have been published reports on thetoxic effects of CNTs to date, more studies will still be needed to gain full understanding of their potential toxicity and underlying mechanisms." As taken from Wang J et al. 2013a. Curr. Drug. Metab. 14(8), 891-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24016107

Pulmonary exposure to CNT has also produced systemic responses including an increase in inflammatory mediators in the blood, as well as oxidant stress in aortic tissue and increase plaque formation in an atherosclerotic mouse model [Li et al. 2007; Erdely et al. 2009]. Pulmonary exposure to MWCNT also depresses the ability of coronary arterioles to respond to dilators [Stapleton et al. 2011]. These cardiovascular effects may be due to neurogenic signals from sensory irritant receptors in the lung. Mechanisms, such as inflammatory signals or neurogenic pathways causing these systemic responses, are under investigation.

As taken from NIOSH, 2013.

“We summarized the findings of in vivo toxicity studies of single-walled carbon nanotubes (SWCNTs) in laboratory animals. ….. Airway exposure to SWCNTs also induced cardiovascular diseases in mice. ….. Overall, the available data provides initial information on SWCNT toxicity. To further clarify their toxicity and risk assessment, studies should be conducted using well-characterized SWCNTs, standard protocols, and the relevant route and doses of human exposure.” As taken from Ema M et al. 2016. Regul. Toxicol. Pharmacol. 74, 42-63. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26619783

“Human Health Assessment

….. Hazards related to substances used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS). However, based on the available information on structurally related nanomaterials, the substance may cause …. cardiovascular toxicity …. following oral and inhalation exposure…….”

As taken from Environment Canada, 2015

6.3. Nervous system

“Recent observations have demonstrated that nanomaterials may be toxic to human tissue. While the ability of nano-scaled particulate matter is known to cause a range of problems in respiratory system, recent observations suggest that the nervous system may be vulnerable as well. In the current paper we asked whether exposure of primary neuronal cell cultures to nanoparticles might compromise regenerative axon growth. Regenerative response was triggered by performing a conditioning lesion of sciatic nerve five days prior to collection of dorsal root ganglia (DRG). DRG neurons were plated at a low density and incubated with multi-walled carbon nanotubes (MWCNTs) (0.1-10 μg/ml in 10% of surfactant in saline) overnight. The experiments showed that exposure of DRG cultures to MWCNT significantly impaired regenerative axonogenesis without concomitant cell death. These results indicate that MWNCTs may have detrimental effect on nerve regeneration and may potentially trigger axonal pathology” (Wu et al., 2012. Neuroscience Letters 507, 72-77). As taken from http://www.ncbi.nlm.nih.gov/pubmed/22172934

“This study was to investigate the neurotoxicity of multi-walled carbon nanotube (MWCNT) by measuring neuronal excitability in rat hippocampal neurons and exploring the underlying mechanism. Whole cell patch-clamp technique was used. Action potential properties and the pattern of repetitive firing rate were assessed. Our data showed that spike half-width and repetitive firing rate were significantly increased in a concentration-dependent manner. Furthermore, voltage-activated potassium currents were recorded. It was found that MWCNT produced a concentration-dependent inhibition in amplitudes of I(A) and I(K). In addition, MWCNT had effect on the activation kinetics of I(A) and I(K) with V(h) being shifted to the negative potential at high concentration, while I(A) inactivation curve was considerably shifted to the hyperpolarize potential with V(h) being increased. However, no effect was found on the recovery from inactivation of I(A). The results suggest that MWCNT increases the excitability of hippocampal CA1 neurons by inhibiting voltage-gated potassium current.” As taken from Chen T et al. 2013a. Toxicol. Lett. 217(2), 121-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23274715

“The assay of the toxic effects of carbon nanotubes (CNTs) on human health is a stringent need in view of their expected increasing exploitation in industrial and biomedical applications. Most studies so far have been focused on lung toxicity, as the respiratory tract is the main entry of airborne particulate, but there is also recent evidence on the existence of toxic effects of multiwalled carbon nanotubes (MWCNTs) on neuronal and neuroendocrine cells (Belyanskaya et al., 2009; Xu et al., 2009; Gavello et al., 2012). Commercial MWCNTs often contain large amounts of metals deriving from the catalyst used during their synthesis. Since metals, particularly iron, may contribute to the toxicity of MWCNTs, we compared here the effects of two short MWCNTs samples (<5μm length), differing only in their iron content (0.5 versus 0.05% w/w) on the secretory responses of neurotransmitters in mouse chromaffin cells. We found that both iron-rich (MWCNT+Fe) and iron-deprived (MWCNT-Fe) samples enter chromaffin cells after 24h exposure, even though incorporation was attenuated in the latter case (40% versus 78% of cells). As a consequence of MWCNT+Fe or MWCNT-Fe exposure (50-263μg/ml, 24h), catecholamine secretion of chromaffin cells is drastically impaired because of the decreased Ca(2+)-dependence of exocytosis, reduced size of ready-releasable pool and lowered rate of vesicle release. On the contrary, both MWCNTs were ineffective in changing the kinetics of neurotransmitter release of single chromaffin granules and their quantal content. Overall, our data indicate that both MWCNT samples dramatically impair secretion in chromaffin cells, thus uncovering a true depressive action of CNTs mainly associated to their structure and degree of aggregation. This cellular "loss-of-function" is only partially attenuated in iron-deprived samples, suggesting a minor role of iron impurities on MWCNTs toxicity in chromaffin cells exocytosis.” As taken from Gavello D et al. 2013. Neurotoxicology 39, 84-94. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23999117

“We evaluated local inflammatory activity of oxidized multiwalled carbon nanotubes in rat experimental models of acute inflammation (paw edema and hyperalgesia) by analyzing their toxicity in non-mesoendothelial tissues. Subcutaneous injection of the nanotubes induced paw edema, that was maximal in the first 2 h after administration at 0.1 mg/kg (43.25 +/- 3.8 AUC) and 1 mg/kg (30.1 +/- 1.8 AUC) compared to saline (18.32 +/- 02.05 AUC). The histopathological analysis showed acute inflammation characterized by vasodilatation, edema formation, neutrophil infiltrate and tissue damage. The nanotubes also elicited hyperalgesic response, seen by the increase of animal paw withdrawal that was maximal in the first 3 hours. The data obtained at the 3rd h was: 75 +/- 9.3% (0.01 mg/kg), 58 +/- 8.3% (0.1 mg/kg) and 53 +/- 6.69% (1 mg/kg) in relation with saline (28 +/- 3.5%). In conclusion, the oxidized multiwalled carbon nanotubes elicit inflammatory and hyperalgesic effects associated to severe tissue damage in rats.” As taken from Pinto NV et al. 2013. J. Nanosci. Nanotechnol. 13(8), 5276-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23882754

“Carbon nanotubes (CNTs) have become an intriguing and promising biomaterial platform for the regeneration and functional recovery of damaged nerve tissues. The unique electrical, structural and mechanical properties, diversity of available surface chemistry and cell-penetrating ability of CNTs have made them useful implantable matrices or carriers for the delivery of therapeutic molecules. Although there are still challenges being faced in the clinical applications of CNTs mainly due to their toxicity, many studies to overcome this issue have been published. Modification of CNTs with chemical groups to ensure their dissolution in aqueous media is one possible solution. Functionalization of CNTs with biologically relevant and effective molecules (biofunctionalization) is also a promising strategy to provide better biocompatibility and selectivity for neural regeneration. Here, we review recent advances in the use of CNTs to promote neural regeneration.” As taken from Hwang JY et al. 2013. Nanoscale 5(2), 487-97. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23223857

“Recent studies indicate that the brain is a target for toxic carbonaceous nanoparticles present in ambient air. It has been proposed that the neurotoxic effects of such particles are driven by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase mediated generation of reactive oxygen species (ROS) in activated microglia. In the present study, we have evaluated the effects of short term (4h) nose-only inhalation exposure to carbon NP (CNP) in the brains and lungs of C57BL/6J mice and in p47(phox-/-) mice that lack a functional NADPH oxidase. It was shown that the lungs of the p47(phox-/-) mice are less responsive to CNP inhalation than lungs of the corresponding C57BL/6J control animals. Lung tissue mRNA expression of the oxidative stress/DNA damage response genes 8-oxoguanine glycosylase (OGG1) and apurinic/apyrimidinic endonuclease 1 (APE1) were induced by CNP exposure in C57BL/6J but not in the p47(phox-/-) mice. In contrast, the expression of these genes, as well as Tumor Necrosis Factor-α (TNFα), Cyclooxygenase-2 (COX-2) and Heme Oxygenase-1 (HO-1) was not altered in the olfactory bulb, cerebellum or remaining brain tissue part of either mouse background. This indicates that neuroinflammation was not induced by this exposure. CNP inhalation for 4h or for 4h on three consecutive days also did not affect brain tissue protein expression of interleukin (IL)-1β, while a clear significant difference in constitutive expression level of this pro-inflammatory cytokine was found between C57BL/6J and p47(phox-/-) mice. In conclusion, short-term inhalation exposure to pure carbon nanoparticles can trigger mild p47(phox) dependent oxidative stress responses in the lungs of mice whereas in their brains at the same exposure levels signs of oxidative stress and inflammation remain absent. The possible role of p47(phox) in the neuro-inflammatory effects of nanoparticles in vivo remains to be clarified.” As taken from van Berlo D et al. 2014. Neurotoxicology 43, 65-72. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24792328.

“Multi-walled carbon nanotubes (MWCNTs) have shown potential applications in many fields, especially in the field of biomedicine. Several studies have reported that MWCNTs induce apoptosis and oxidative damage in nerve cells during in vitro experiments. However, there are few studies focused on the neurotoxicity of MWCNTs used in vivo. Many studies have reported that autophagy, a cellular stress response to degrade damaged cell components, can be activated by diverse nanoparticles. In this study, we investigated the neurotoxic effects of MWCNTs on hippocampal synaptic plasticity and spatial cognition in rats. Then, we used an inhibitor of autophagy called chloroquine (CQ) to examine whether autophagy plays an important role in hippocampal synaptic plasticity, since this was damaged by MWCNTs. In this study, adult male Wister rats were randomly divided into three groups: a control group, a group treated with MWCNTs (2.5mg/kg/day) and a group treated with MWCNTs+CQ (20mg/kg/day). After two-weeks of intraperitoneal (i.p.) injections, rats were subjected to the Morris water maze (MWM) test, and the long-term potentiation (LTP) and other biochemical parameters were determined. Results showed that MWCNTs could induce cognitive deficits, histopathological alteration and changes of autophagy level (increased the ratio of LC3 II /LC3 I and the expression of Beclin-1). Furthermore, we found that CQ could suppress MWCNTs-induced autophagic flux and partly rescue the synapse deficits, which occurred with the down-regulation of NR2B (a subunit of NMDA receptor) and synaptophysin (SYP) in the hippocampus. Our results suggest that MWCNTs could induce cognitive deficits in vivo via the increased autophagic levels, and provide a potential strategy to avoid the adverse effects of MWCNTs.” As taken from Gao J et al. 2015. Toxicology 337, 21-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26327526

6.4. Other organ systems, dependent on the properties of the substance

“The management of hyperphosphataemia remains a challenge in people with CKD, particularly those requiring dialysis. In this study, Wang et al demonstrate the potential efficacy of activated charcoal at lowering phosphate and PTH levels. ABSTRACT: Aim: Hyperphosphatemia is almost inevitable in end stage renal disease (ESRD) patients and is associated with increased morbidity and mortality. In this study we examined whether oral activated charcoal (oAC) reduces serum phosphate level in hemodialysis patients. Methods: This was an open-label, prospective,

uncontrolled study. One hundred and thirty-five hemodialysis patients were included in this study, with cessation of treatment with any phosphate binders during a 2-week washout period. Patients with serum phosphate levels greater than 5.5mg/dl during the washout period were included for treatment with oAC. oAC was started at a dose of 600mg three times per day with meals, and was administered for 24 weeks. oAC dose was titrated up during the 24-week period to achieve phosphate control(3.5-5.5mg/dl). A second 2-week washout period followed the end of oAC treatment. Results: In the 114 patients who successfully completed the trial, the mean dose of activated charcoal was 3190±806mg/day. oAC reduced mean phosphate levels to below 5.5mg/dl, with mean decreases of 2.60 ± 0.11 mg/dl($p<0.01$), and 103(90.4%) of the patients reached the phosphate target. After the second washout period the phosphate levels increased to 7.50 ± 1.03 mg/dl ($p<0.01$). Serum intact parathyroid hormone (iPTH) levels declined from 338.75±147.77 pg/ml to 276.51±127.82 pg/ml ($p<0.05$) during the study. oAC had no influence on serum prealbumin, total cholesterol, triglycerides, serum ferritin, haemoglobin or platelet levels, and the levels of 1,25-dihydroxyvitamin D were stable during the study. Conclusion: In this open-label uncontrolled study, oAC effectively controls hyperphosphatemia and hyperparathyroidism in hemodialysis patients. the safety and efficacy of oAC need to be assessed in a randomized controlled trial" (Wang et al. 2012. Nephrology (Carlton) 17(7), 616-20). As taken from http://www.ncbi.nlm.nih.gov/pubmed/22697887

"In the course of severe pathological conditions, such as acute liver failure and sepsis, toxic metabolites and mediators of inflammation are released into the patient's circulation. One option for the supportive treatment of these conditions is plasmapheresis, in which plasma, after being separated from the cellular components of the blood, is cleansed by adsorption of harmful molecules on polymers or activated carbon. In this work, the adsorption characteristics of activated carbon beads with levels of activation ranging from 0 to 86% were assessed for both hydrophobic compounds accumulating in liver failure (bilirubin, cholic acid, phenol and tryptophan) and cytokines (tumor necrosis factor α and interleukin-6). Progressive activation resulted in significant gradual reduction of both bulk density and mean particle size, in an increase in the specific surface area, and to changes in pore size distribution with progressive broadening of micropores. These structural changes went hand in hand with enhanced adsorption of small adsorbates, such as IL-6 and cholic acid and, to a lesser extent, also of large molecules, such as TNF-α" (Tripisciano et al. 2011. Biomacromolecules 12, 3733-3740). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21842874

"Carbon nanotubes (CNTs) have been used in orthopaedic applications because of their exceptional mechanical properties. However, the influence of CNTs on the behaviour of bone-forming cells and on the ability of these cells to respond to growth factors, such as bone morphogenetic proteins (BMPs), remains poorly known. Therefore, in the present study, single-walled CNTs (SWCNTs) were synthesised using an induction thermal plasma process and purified using a multistep procedure. The impact of these purified SWCNTs on the Smad activation, cell proliferation and differentiation, with or without BMP-2 and BMP-9 (1.92 nM), was also studied using western blot, mitochondrial enzymatic activity, TUNEL, RT-PCR and alkaline phosphatase activity analyses. Pre-treatment of MC3T3-E1 preosteoblasts with SWCNTs accelerated the Smad1/5/8 activation, induced by both BMP-2 and BMP-9, within 15 min. It also slightly affected their proliferation at 48 h without apoptosis. Interestingly, at 72 h, BMP-9 favoured the differentiation of MC3T3-E1 preosteoblasts pretreated with SWCNTs to a larger extent than BMP-2 did. Therefore, the combination of BMP-9 with SWCNTs appears to be a promising avenue for bone applications." As taken from Alinejad Y et al. 2013. J. Biomed. Nanotechnol. 9(11), 1904-13. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24059089?dopt=AbstractPlus

"Carbon nanotubes (CNTs) find their extensive application as a promising material in medicine due to unique characteristics. However, such materials have been accompanied with potentially hazardous effects on human health. The toxicity of CNTs may vary depending on their structural characteristics, surface properties and chemical composition. To gain insight into the toxicity of CNTs in vivo and in vitro, we summarize contributing factors for the toxic effects of CNTs in this

review. In addition, we elaborate on the toxic effects and mechanisms in target sites at systemic, organic, cellular, and biomacromolecule levels. Various issues are reported to be effected when exposed to CNTs including (1) blood circulation, (2) lymph circulation, (3) lung, (4) heart, (5) kidney, (6) spleen, (7) bone marrow, and (8) blood brain barrier. Though there have been published reports on thetoxic effects of CNTs to date, more studies will still be needed to gain full understanding of their potential toxicity and underlying mechanisms." As taken from Wang J et al. 2013a. Curr. Drug. Metab. 14(8), 891-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24016107

"Awasthi and co-workers (Awasthi et al. 2013) administered male Swiss albino mice (N=6/group) single doses of 0 (vehicle control, distilled water), 60, or 100 mg/kg bw) of MWCNTs and studied hepatotoxicity on post dosing days 7, 14, 21 and 28 using liver SOD and CAT activity and microscopic examination as end-points. The tested MWCNTs, which were synthesised by chemical vapour deposition (CVD) technique, were purified and washed to remove metallic and carbonaceous impurities. Their size range was determined by SEM as 20–30 nm and length of 5–50 μm. The testing suspensions were made by physical mixing and ultrasonication of surface-oxidised material, but any further data on characterization or aggregation was missing. Slight hepatotoxicity was reported at both dose levels, however, no incidences of the lesions were presented to enable comparison with the control group and support their relation to the treatment."

As taken from Binderup et al. 2013.

For medical purposes activated charcoal is administered orally in a therapy for acute diarrhoea and, due to its ability to adsorb many chemicals and drugs, also for the treatment of acute oral poisonings. Adsorption characteristics can be influenced by the charcoal's particle size, thus different responses may be obtained with different preparations (Martindale, 2011; Ph. Eur. Comment., 2009). At therapeutic dose levels activated charcoal has the potential to reduce the absorption of other drugs from the gastrointestinal tract and thus reduce their efficacy (Martindale, 2011; Ph. Eur. Comment., 2009) (EFSA, 2012b)

"Activated charcoal (AC) is a sorbent that has been shown to remove urinary toxins like urea and indoxyl sulfate. Here, the influence of AC on kidney function of rats with experimental chronic renal failure (CRF) is investigated. CRF was induced in rats by feeding adenine (0.75%) for four weeks. As an intervention, AC was added to the feed at concentrations of 10%, 15% or 20%. Adenine treatment impaired kidney function: it lowered creatinine clearance and increased plasma concentrations of creatinine, urea, neutrophil gelatinase-associated lipocalin and vanin-1. Furthermore, it raised plasma concentrations of the uremic toxins indoxyl sulfate, phosphate and uric acid. Renal morphology was severely damaged and histopathological markers of inflammation and fibrosis were especially increased. In renal homogenates, antioxidant indices, including superoxide dismutase and catalase activity, total antioxidant capacity and reduced glutathione were adversely affected. Most of these changes were significantly ameliorated by dietary administration of AC at a concentration of 20%, while effects induced by lower doses of dietary AC on adenine nephrotoxicity were not statistically significant. The results suggest that charcoal is a useful sorbent agent in dietary adenine-induced CRF in rats and that its usability as a nephroprotective agent in human kidney disease should be studied". As taken from Ali BH et al. 2014. Food Chem. Toxicol. 65, 321-8. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24412558.

"The effects of multi-walled carbon nanotubes (MWCNTs) exposure have garnered great interest in the field of public health, due to the high aspect ratio of MWCNTs. Because of worldwide increases in obesity prevalence, nonalcoholic fatty liver disease (NAFLD) is now the most common prevalent liver disease and is considered to be a component of metabolic syndrome, which is a cluster of disorders that also includes dyslipidemia, diabetes mellitus, arteriosclerosis, and hypertension. Exposure to MWCNTs is known to be a risk factor for lung and cardiovascular diseases, but its

effect on NAFLD is unknown. In this study, we investigated the effects of intratracheal exposure of two different types of MWCNTs, namely, pristine multi-walled carbon nanotubes (PMWCNTs) and acid-treated multi-walled carbon nanotubes (TMWCNTs), on liver pathogenesis. Direct instillation of a test material into the lungs has been employed as a quantitatively reliable alternative method of inhalation exposure. The 10% weight loss dose was assessed in three months of subchronic study and is defined here as the maximum tolerated dose (MTD) of PMWCNTs and TMWCNTs; by this metric, MTD for a 1-year exposure of MWCNTs was determined to be 0.1 mg/mouse. Mice exposed to PMWCNTs and TMWCNTs for one year developed a nonalcoholic steatohepatitis (NASH)-like phenotype, characterized by inflammation, hepatic steatosis, and fibrosis. Furthermore, PMWCNTs induced a more severe NASH-like phenotype than TMWCNTs, which was related to consistent up-regulation of interleukin (IL)-6 and plasminogen activator inhibitor (PAI)-1. Impaired cholesterol homeostasis, overexpression of NF-κBp65, and suppression of peroxisome proliferator-activated receptor gamma (PPARγ) in the liver were also observed." As taken from Kim JE et al. 2015b. Nanotoxicology 9(5), 613-623. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25265201

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

No data available to us at this time.

9. Heated/vapor emissions toxicity

No data available to us at this time.

10. Ecotoxicity

10.1. Environmental fate

Environmental Abiotic Degradation:

... is rapidly oxidized to carbon dioxide ... /which enters/ into animals and plants by photosynthesis and metabolism. /(14)C/
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 2006.., p. 293] **PEER REVIEWED**

As taken from HSDB, 2009

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that carbon is persistent in the environment.

Data accessed January 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

"Carbon nanotubes (CNT) have numerous industrial applications and may be released to the environment. In the aquatic environment, pristine or functionalized CNT have different dispersion behavior, potentially leading to different risks of exposure along the water column. Data included in

this review indicate that CNT do not cross biological barriers readily." As taken from Jackson P et al. 2013. Chem. Cent. J. 7(1), 154. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24034413

"With the large amount production and application of engineering carbon nanomaterials, their potential ecological risk has attracted extensive attention. The degradation and transformation of the carbon nanomaterials in the environment directly affect the fates and eco-toxicity of the nanomaterials in the environment, and the research of the degradation and transformation processes of the nanomaterials in the environment is the key link for the determination of the environmental capacity of the nanomaterials and for the evaluation of the nanomaterials life cycle in the environment. This paper briefly introduced the chemical transformation, microbial degradation, and photodegradation of the major engineering carbonnanomaterials (carbon nanotubes and fullerene) in the environment, and summarized the environmental and structural factors affecting the degradation of the nanomaterials and the related intrinsic mechanisms. The shortcomings of the related researches and the directions of the future research were also put forward." As taken from Yue FN et al. 2013. Ying Yong Sheng Tai Xue Bao. 24(2), 589-96. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23705409

"The batch equilibrium approach was used to examine the influence of multi-walled carbon nanotubes (MWNTs) on the sorption behaviors of polyaromatic hydrocarbons (PAHs) in soil. To the knowledge of the authors, this is the first study of PAH sorption to MWNTs in real natural soil systems. The sorption behavior of three PAHs (naphthalene, fluorene, and phenanthrene) in the presence of commercially available MWNTs in two natural soils (a sandy loam and a silt loam) and Ottawa sand was evaluated. Adsorption of PAHs by MWNTs in this study was three orders of magnitude higher than that of natural soils. Sorption coefficients of PAHs (Kd and Koc) were unchanged in the presence of 2 mg g(-1) MWNTs in soil ($p > 0.05$). A micro-mechanics approach, termed 'the rule of mixtures' was used for predicting PAH sorption behaviors in mixtures based on sorption coefficients derived from single sorbents. The equation, $KT = KM\alpha + KN(1 - \alpha)$ (K, sorption coefficients, Kd or Koc), predicted sorption coefficients in a mixture based on mixture component sorption coefficients and mass fractions. Data presented in this study could be used to fill data gaps related to the environmental fate of carbon nanotubes in soil." As taken from Li S et al. 2013. Environ. Sci. Process Impacts 15(6), 1130-6. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23591941

"Carbon nanotubes (CNTs) are exciting new materials that have been intensively researched and are becoming increasingly used in consumer products. With rapid growth in production and use of CNTs in many applications, there is the potential for emissions to the environment and thus research is needed to assess the risks associated with CNTs in the environment. Here we show that commercial CNTs differ in their stability, photoactivity, metal leachate, and toxicity to freshwater algae. The behavior between raw and purified variants of the CNTs differs considerably; for example purified CNTs are generally more photoactive, producing singlet oxygen and superoxide, while raw CNTs show little or no photoactivity. Residual metal catalysts differ based on synthesis method used to prepare CNTs and thus may be comprised of elements with varying degrees oftoxic potential. Influenced by pH and other constituents of the natural waters, our work shows that metals can leach out from all the commercial CNTs studied, even purified versions, albeit at different levels in many natural waters. As much as 10% of the total residual nickel leached from a purified CNT after 72 h. Aqueous concentrations of molybdenum leached from a different purified CNT were nearly 0.060 mg L(-1) after 72 h. With little sample preparation, CNTs are dispersible in most freshwaters and stable for several days. Not all tested CNTs were toxic; for those CNTs that did inducetoxicity we show that photoactivity, not metal leaching, contributes to the toxicity of commercial CNTs to freshwater algae, with growth rates significantly reduced by as much as 200%." As taken from Bennett SW et al. 2013. Water Res. 47(12), 4074-85. PubMed, 2014

available at http://www.ncbi.nlm.nih.gov/pubmed/23591109

"The quality of water is continuously deteriorating due to its increasing toxic threat to humans and the environment. It is imperative to perform treatment of wastewater in order to remove pollutants and to get good quality water. Carbon materials like porous carbon, carbon nanotubes and fullerene have been extensively used for advanced treatment of wastewaters. In recent years, carbon nanomaterials have become promising adsorbents for water treatment. This review attempts to compile relevant knowledge about the adsorption activities of porous carbon, carbonnanotubes and fullerene related to various organic and inorganic pollutants from aqueous solutions. A detailed description of the preparation and treatment methods of porous carbon, carbon nanotubes and fullerene along with relevant applications and regeneration is also included." As taken from Gupta VK & Saleh TA. 2013. Environ. Sci. Pollut. Res. Int. 20(5), 2828-43. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23430732

10.2. Aquatic toxicity

Toxicity to Microorganisms e.g. Bacteria

Remark: Peat based steam activated carbons, lignite based steam activated carbons and wood based chemical activated carbons were found not to be toxic to **waste–water bacteria.** An EC50 value for respiration inhibition could not be determined. Tests by RCC Notox B.V. The Netherlands.

Source: CHEMVIRON CARBON BRUXELLES

As taken from IUCLID Dataset (2000), Carbon (7440-44-0)

"Amendment of contaminated sediment with activated carbon (AC) is a remediation technique that has demonstrated its ability to reduce aqueous concentrations of hydrophobic organic compounds. The application of AC, however, requires information on possible ecological effects, especially effects on benthic species. Here, we provide data on the effects of AC addition on locomotion, ventilation, sediment avoidance, mortality, and growth of two benthic species, Gammarus pulex and Asellus aquaticus , in clean versus polycyclic aromatic hydrocarbon (PAH) contaminated sediment. Exposure to PAH was quantified using 76 µm polyoxymethylene passive samplers. In clean sediment, AC amendment caused no behavioral effects on both species after 3-5 days exposure, no effect on the survival of A. aquaticus , moderate effect on the survival of G. pulex (LC(50) = 3.1% AC), and no effects on growth. In contrast, no survivors were detected in PAH contaminated sediment without AC. Addition of 1% AC, however, resulted in a substantial reduction of water exposure concentration and increased survival of G. pulex and A. aquaticus by 30 and 100% in 8 days and 5 and 50% after 28 days exposure, respectively. We conclude that AC addition leads to substantial improvement of habitat quality in contaminated sediments and outweighs ecological side effects" (Kupryianchyk et al., 2011. Environmental Science and Technology 45, 8567-8574). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21846106

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that carbon is not inherently toxic to aquatic organisms.

Data accessed January 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

Record for carbon:

Spec. Sci. Name	Exp. Type	Media Type	Resp. Site	Endpoint	Trend	Effect	Conc (Standardized)	Stat. Signif.

Spec. Common Name	Chem. Anal.	Loc	Obs. Dur. (Days)	BCF	Eff %	Effect Meas.	Appl. Rate		Sig. Level
Isochrysis Haptophyte	AQUA U	LAB	1 d			ABND	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	5 d		>40-<50/	LADH	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	10 d		>30-<40/	ACPH	A	ug/L	<=0.01
Navicula Diatom	AQUA U	LAB	5 d			ABND	A)0	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	2 d			ABND	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	1 d			ABND	A	ug/L	<=0.01
Navicula Diatom	AQUA U	LAB	10 d		>50-<60/	LADH	A	ug/L	<=0.01
Navicula Diatom	AQUA U	LAB	1 d		>20-<30/	ACPH	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	5 d		>30-<40/	ACPH	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	9 d			ABND	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	6 d			ABND	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	0.083 d			ABND	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	4 d			ABND	A	ug/L	<=0.01
Navicula Diatom	AQUA U	LAB	8 d			ABND	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	3 d			ABND	A)0	ug/L	<=0.05
Isochrysis	AQUA	SW				ROR	A		ASIC

Haptophyte	U	LAB	9 d			ABND	A	ug/L	<=0.05
Isochrysis Haptophyte	AQUA U	LAB	1 d		>0-<40/	LADH	A	ug/L	<=0.01
Isochrysis Haptophyte	AQUA U	LAB	7 d			ABND	A	ug/L	<=0.01
Isochrysis Haptophyte	AQUA U	LAB	5 d			ABND	A	ug/L	<=0.05
Isochrysis Haptophyte	AQUA U	LAB	0.083 d			ABND	A	ug/L	<=0.01
Isochrysis Haptophyte	AQUA U	LAB	10 d		>20-<25/	ACPH	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	7 d			ABND	A	ug/L	<=0.01
Isochrysis Haptophyte	AQUA U	LAB	6 d			ABND	A 00	ug/L	<=0.05
Isochrysis Haptophyte	AQUA U	LAB	1 d		>10-<15/	ACPH	A	ug/L	<=0.05
Isochrysis Haptophyte	AQUA U	LAB	5 d		>40-<60/	LADH	A	ug/L	<=0.01
Isochrysis Haptophyte	AQUA U	LAB	10 d		>40-<60/	LADH	A	ug/L	<=0.01
Isochrysis Haptophyte	AQUA U	LAB	4 d			ABND	A	ug/L	<=0.05
Isochrysis Haptophyte	AQUA U	LAB	8 d			ABND	A	ug/L	<=0.05
Isochrysis Haptophyte	AQUA U	LAB	2 d			ABND	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	5 d			ABND	A	ug/L	<=0.05
Isochrysis Haptophyte	AQUA U	LAB	6 d			ABND	A	ug/L	<=0.05
Navicula Diatom	AQUA U	LAB	3 d			ABND	A	ug/L	<=0.05

Isochrysis [illegible] Haptophyte	AQUA [illegible] U	[illegible] LAB	3 d		[illegible]	[illegible] ABND	A (50000-1000000) [illegible]	[illegible] <=0.05
Isochrysis [illegible] Haptophyte	AQUA [illegible] U	[illegible] LAB	(0.083 - 9) d		[illegible]	[illegible] CCHG/	A (50000-1000000) [illegible]	
Isochrysis [illegible] Haptophyte	AQUA [illegible] U	[illegible] LAB	5 d		[illegible] >15-<20/	[illegible] ACPH	A [illegible] ug/L	[illegible] <=0.05
Navicula [illegible] Diatom	AQUA [illegible] U	[illegible] LAB	1 d		[illegible] >30-<40/	[illegible] LADH	A (50000-1000000) [illegible]	[illegible] <=0.01
Navicula [illegible] Diatom	AQUA [illegible] U	[illegible] LAB	(0.083 - 9) d		[illegible]	[illegible] CCHG/	A (50000-1000000) [illegible]	
Danio rerio Zebra Danio	IJ U	FW LAB	0.99	NOEC	NEF 0/	MOR MORT	F 2 ng/org	ANOSIG <0.05
Danio rerio Zebra Danio	IJ U	FW LAB	14	NOEC	INC >20-<40/	MOR MORT	F 2 ng/org	ANOSIG <0.05
Danio rerio Zebra Danio	IJ U	FW LAB	28	NOEC	INC >20-<40/	MOR MORT	F 2 ng/org	ANOSIG <0.05
Danio rerio Zebra Danio	IJ U	FW LAB	56	NOEC	INC >20-<40/	MOR MORT	F 2 ng/org	ANOSIG <0.05
Danio rerio Zebra Danio	IJ U	FW LAB	0.99	NR-ZERO	NEF 0	MOR MORT	F 2 ng/org	
Danio rerio Zebra Danio	IJ U	FW LAB	MUL/ (0.115 - 0.323)		INC	HIS GHIS/	F 2 ng/org	
Danio rerio Zebra Danio	IJ U	FW LAB	<= 0.99		NEF	DVP NORM	F 2 ng/org	
Danio rerio Zebra Danio	IJ U	FW LAB	WO (0 - 0.99)		INC	IMM GIMM/	F 2 ng/org	
Danio rerio Zebra Danio	IJ U	FW LAB	WO/ (0 - 2.99)		INC	ACC RSDE	F 2 ng/org	
Cyprinus carpio	E	FW	GI		NEF	HIS	F 10000 ug/L	

Common Carp	U	FIELDA	120			GHIS		

As taken from EPA ECOTOX database.

"….The objective of this study was to optimize NIRF-based imaging and quantitation methods for tracking and quantifying SWCNTs in an aquatic vertebrate model in conjunction with assessing toxicological endpoints. Fathead minnows (Pimephales promelas) were exposed by single gavage to SWCNTs and their distribution was tracked using a custom NIRF imaging system for 7 days. No overt toxicity was observed in any of the SWCNT treated fish; however, histopathology observations from gastrointestinal (GI) tissue revealed edema within the submucosa and altered mucous cell morphology. NIRF images showed strong SWCNT-derived fluorescence signals in whole fish and excised intestinal tissues. Fluorescence was not detected in other tissues examined, indicating that no appreciable intestinal absorption occurred. SWCNTs were quantified in intestinal tissues using a NIRF spectroscopic method revealing values that were consistent with the pattern of fluorescence observed with NIRF imaging….." As taken from Bisesi JH et al. 2014. Environ. Sci. Technol. 48(3), 1973-83. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24383993

"Fish behaviours are often considered to be sensitive endpoints of waterborne contaminants, but little attention has been given to engineered nanomaterials. The present study aimed to determine the locomotor and social behaviours of rainbow trout (Oncorhynchus mykiss) during waterborne exposure to single-walled carbon nanotubes (SWCNTs), and to ascertain the physiological basis for any observed effects. Dispersed stock suspensions of SWCNTs were prepared by stirring in sodium dodecyl sulphate (SDS), an anionic surfactant, on an equal w/w basis. Trout were exposed to control (no SWCNT or SDS), 0.25mgL(-1) SDS (dispersant control), or 0.25mgL(-1) of SWCNT for 10 days. Video tracking analysis of spontaneous locomotion of individual fish revealed no significant effects of SWCNT on mean velocity when active, total distance moved, or the distribution of swimming speeds. Hepatic glycogen levels were also unaffected. Fish exposed to SWCNTs retained competitive fitness when compelled to compete in energetically costly aggressive interactions with fish from both control groups. Assessment of the respiratory physiology of the fish revealed no significant changes in ventilation rate or gill injuries. Haematocrit and haemoglobin concentrations in the blood were unaffected by SWCNT exposure; and the absence of changes in the red and white pulp of the spleen excluded a compensatory haematopoietic response to protect the circulation. Despite some minor histological changes in the kidneys of fish exposed to SWCNT compared to controls, plasma ion concentrations and tissue electrolytes were largely unaffected. Direct neurotoxicity of SWCNT was unlikely with the brains showing mostly normal histology, and with no effects on acetylcholinesterase or Na(+)/K(+)-ATPase activities in whole brain homogenates. The minimal effects of waterborne exposure to SWCNT observed in this study are in contrast to our previous report of SWCNT toxicity in trout, suggesting that details of the dispersion method and co-exposure concentration of the dispersing agent may alter toxicity." As taken from Boyle D et. al. 2014. Aquat. Toxicol. 146, 154-64. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24308918

"The potential toxic effects of carboxylated (COOH) single-walled carbon nanotubes (SWNTs) were investigated on the cell growth and viability of two reference (Silicibacter pomeroyi, Oceanospirillum beijerinckii) and two environmental (Vibrio splendidus, Vibrio gigantis) Gram-negative marine bacterial strains. Bacterial cells were exposed to six concentrations of SWNT-COOH, during different incubation times. Our results revealed different sensitivity levels of marine bacterial strains toward SWNT-COOH exposure. A bactericidal effect of SWNT-COOH has been observed only for Vibrio species, with cell loss viability estimated to 86% for V. gigantis and 98% for V. splendidus exposed to 100µgmL(-1) of SWNT-COOH during 2h. For both Vibrio strains, dead cells were well individualized and no aggregate formation was observed after SWNT-COOH treatment. The toxic effect of SWNT-COOH on O. beijerinckii cells displayed time dependence, with a longer exposure time reducing their specific growth rate by a factor of 1.2. No significant effect of SWNT-

COOH concentration or incubation time had been demonstrated on both growth ability and viability of S. pomeroyi, suggesting a stronger resistance capacity of this strain to carbon nanotubes. The analysis of the relative expression of some functional genes involved in stress responses, using the real-time reverse transcriptase PCR, suggests that the cell membrane damage is not the main toxicity mechanism by which SWNT-COOH interacts with marine bacterial strains. Overall, our results show that SWNT-COOH present a strain dependent toxic effect to marine bacteria and that membrane damage is not the main toxicity mechanism of SWNT in these bacteria." As taken from Berdjeb L et al. 2013. Aquat. Toxicol. 144-145, 230-41. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24184842

"Carbon nanotubes (CNT) have numerous industrial applications and may be released to the environment. In the aquatic environment, pristine or functionalized CNT have different dispersion behavior, potentially leading to different risks of exposure along the water column. Data included in this review indicate that CNT do not cross biological barriers readily. When internalized, only a minimal fraction of CNT translocate into organism body compartments. The reported CNT toxicity depends on exposure conditions, model organism, CNT-type, dispersion state and concentration. In the ecotoxicological tests, the aquatic organisms were generally found to be more sensitive than terrestrial organisms. Invertebrates were more sensitive than vertebrates. Single-walled CNT were found to be more toxic than double-/multi-walled CNT. Generally, the effect concentrations documented in literature were above current modeled average environmental concentrations. Measurement data are needed for estimation of environmental no-effect concentrations. Future studies with benchmark materials are needed to generate comparable results. Studies have to include better characterization of the starting materials, of the dispersions and of the biological fate, to obtain better knowledge of the exposure/effect relationships." As taken from Jackson P et al. 2013. Chem. Cent. J. 7(1), 154. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24034413

"The present study explored the ecotoxicology of single-walled carbon nanotubes (SWCNTs) and their likely interaction with dissolved metals, with a focus on the effect of in vivo exposure in marine mussels. Any nano-scale effects were negated by the tendency of uncoated SWCNTs to agglomerate in water, particularly with high ionic strength as is the case in estuarine and full-strength seawater…..For the first time, the authors describe a potentiating toxicological effect, expressed as DNA strand breaks obtained using the comet assay, on divalent metals afforded by negatively charged SWCNT agglomerates in seawater at concentrations as low as 5 $\mu g\ L^{-1}$. This is supported by the observation that SWCNTs alone were only toxic at concentrations ≥100 $\mu g\ L^{-1}$ and that the SWCNT-induced DNA damage was correlated with oxidative stress only in the absence of metals. If these laboratory experiments are confirmed in the natural environment, the present results will have implications for the understanding of the role of carbon nanotubes in environmental metal dynamics, toxicology, and consequently, regulatory requirements." As taken from Al-Shaeri M et al. 2013. Environ. Toxicol. Chem. 32(12), 2701-10. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23982896

"There are currently over ninety products incorporating carbon nanomaterials (CNMs) on the market today for a variety of applications. Modifications in core structure and surface chemistry of manufactured nanomaterials are used to optimize nanomaterials for specific uses. However, there is a notable lack of information on how core structure and surface chemistry may alter toxicity in low-level, chronic exposures. This paper examines the effects of twelve CNMs that differ in their core structure and surface chemistry to Daphnia magna over a 21-day chronic exposure. Overall, nanomaterials with a carbon nanotube core were more toxic to daphnids than fullerenes, with the one exception of fullerenes with a gamma-cyclodextrin surface chemistry. Acute mortality was not a good predictor of chronic effects as none of the CNMs induced toxicity at tested concentrations after 48 h, yet chronic assays indicated significant differences in mortality, reproduction, and growth realized after 21 days. Our results indicate that (1) acute exposure assays do not accurately

describe the impact of CNMs to biological systems, (2) chronic exposures provide valuable information that indicates the potential for different modes of action for nanomaterials of differing chemistries, and (3) core structure and surface chemistry both influence particletoxicity." As taken from Arndt DA et al. 2013. Environ. Sci. Technol. 47(16), 9444-52. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23862695

"With the development and application of carbon nanotubes (CNTs), the potential hazards of CNTs to biological systems and the environment are getting more and more attention. This review evaluated the effects of physicochemical properties of CNTs on toxicity and summarized the advances on the mechanism of CNTs toxicity. We also proposed the possible hazards associated with CNTs and harmful effects resulting from exposure of aquatic animals, bacteria and higher plants to CNTs *in vitro* and *in vivo*. The current knowledge and gaps on CNTs were outlined as a potential problem for the environment and human health. The current research gaps on CNTs toxicity were identified and the further studying focus was proposed, too. This essay concluded with a set of recommendations for the advancement of understanding of the role of CNTs and future challenges in environmental and ecotoxicological research." As taken from Du J et al. 2013. Environ. Toxicol. Pharmacol. 36(2), 451-62. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23770455

"Carbon nanotubes (CNTs) are exciting new materials that have been intensively researched and are becoming increasingly used in consumer products. With rapid growth in production and use of CNTs in many applications, there is the potential for emissions to the environment and thus research is needed to assess the risks associated with CNTs in the environment. Here we show that commercial CNTs differ in their stability, photoactivity, metal leachate, and toxicity to freshwater algae. The behavior between raw and purified variants of the CNTs differs considerably; for example purified CNTs are generally more photoactive, producing singlet oxygen and superoxide, while raw CNTs show little or no photoactivity. Residual metal catalysts differ based on synthesis method used to prepare CNTs and thus may be comprised of elements with varying degrees of toxic potential. Influenced by pH and other constituents of the natural waters, our work shows that metals can leach out from all the commercial CNTs studied, even purified versions, albeit at different levels in many natural waters. As much as 10% of the total residual nickel leached from a purified CNT after 72 h. Aqueous concentrations of molybdenum leached from a different purified CNT were nearly 0.060 mg L(-1) after 72 h. With little sample preparation, CNTs are dispersible in most freshwaters and stable for several days. Not all tested CNTs were toxic; for those CNTs that did induce toxicity we show that photoactivity, not metal leaching, contributes to the toxicity of commercial CNTs to freshwater algae, with growth rates significantly reduced by as much as 200%." As taken from Bennett SW et al. 2013. Water Res. 47(12), 4074-85. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23591109

"With the rapid increase of carbon nanotube (CNT) applications, there are considerable concerns of their inevitable releases into the aquatic environments. CNTs may interact with and further influence the fate and transport of other pollutants such as toxic metals. In the present study, non-covalent and nontoxic dispersant polyvinyl pyrrolidone (PVP) was used to provide a relatively stable test solution for CNTs. The dissolved uptake rate constant (ku) and the dietary assimilation efficiency (AE) of cadmium (Cd) and zinc (Zn) were then quantified in a freshwater zooplankton Daphnia magna in the presence of different CNTs (without functionalized - single-walled nanotubes-SWNTs, multi-walled nanotubes-MWNTs, and with functionalized - F-SWNTs, F-MWNTs, containing oxygen functional groups at the defect sites of CNTs) concentrations. We demonstrated that different CNTs exposures led to distinctive metal accumulation patterns. Non-functionalized CNTs significantly decreased the metal uptake rate from the dissolved phase, possibly because of their effects on the physiological activity of animals. In contrast, the F-CNTs (F-SWNTs and F-MWNTs) adsorbed the metals and increased the metal accumulation in daphnids in a concentration-dependent manner,

due to the ingestion of F-CNTs associated metals. The AEs of metals in D. magna were elevated by CNTs physical blocking of the animal guts. Our present study showed that CNTs could serve as a new pathway for metal accumulation. This raised a new environmental problem of CNTs since they may induce the accumulation of toxic metals from the dietary exposure." As taken from Yu ZG & Wang WX. 2013. Water Res. 47(12), 4179-87. PubMed, 204 available at http://www.ncbi.nlm.nih.gov/pubmed/23582308

"In this study the freshwater zebrafish (Danio rerio) was exposed to two kinds of carbon NM, single-wall carbon nanotubes (SWCNT) and fullerenol [C60(OH)18-22(OK4)] to analyze oxidative stress responses on fish brain. Adult zebrafish (mean mass: 0.52±0.01g) were submitted to intraperitoneal injections of SWCNT suspension and fullerenol solution (30mg/kg of fish), receiving one or two doses with a time interval of 24h. Results showed that total antioxidant capacity was lowered in brains of fish exposed 24h to fullerenol when compared to those from SWCNT treatment ($p<0.05$). After 48h, fullerenol induced higher expression of both catalytic and regulatory subunits of enzyme glutamate cysteine ligase when compared to control group ($p<0.05$), indicating an antioxidant behavior. In vitro assays showed a dual effect of SWCNT, since a pro-oxidant behavior was observed at low concentrations (0.1 and 1.0mg/L) and an antioxidant one at the highest concentration (10.0mg/L). Few biological responses were altered by this NM: decrease in total antioxidant capacity and induction of the expression of the transcription factor Nrf2 when compared to control group." As taken from da Rocha AM et al. 2013. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 165(4), 460-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23542748

"Black carbon (BC) has a strong affinity for hydrophobic organic compounds (HOCs), and it is a potential material to control HOCs pollution in aquatic ecosystems. Here, flow cytometry (FCM) was used to evaluate the ecotoxicological effect of fly ash, rice-straw ash, and their acid-demineralised products on the growth of Microcystis aeruginosa. It was found that the BCs had little negative effect on cyanobacteria, when the content of BCs was not above 1mgml(-1). However, higher doses of BCs (>2mgml(-1)) had an obvious negative effect on cell density and esterase activity, especially for BCs with acid treatment, which greatly inhibited cell density caused by its high adsorptivity for cyanobacteria. The BCs had little impact on the fluorescence intensity, only with a slight stimulation in later period, so the fluorescence intensity was a less sensitive indicator than cell density and esterase activity. Considering ecotoxicological effect of BCs on the algae, the application concentration of BCs for HOCs pollution control as in situ remediation material would better not exceed 1mgml(-1)." As taken from Lou L et al. 2013. Ecotoxicol. Environ. Saf. 92, 51-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23522529

10.3. Sediment toxicity

"Sediment amendment with activated carbon (AC) is a promising technique for in situ sediment remediation. To date it is not clear whether this technique sufficiently reduces sediment-to-water fluxes of sediment-bound hydrophobic organic chemicals (HOCs) in the presence of bioturbators. Here, we report polychlorobiphenyl (PCB) pore water concentrations, fluxes, mass transfer coefficients, and survival data of two benthic species, for four treatments: no AC addition (control), powdered AC addition, granular AC addition and addition and subsequent removal of GAC (sediment stripping). AC addition decreased mass fluxes but increased apparent mass transfer coefficients because of dissolved organic carbon (DOC) facilitated transport across the benthic boundary layer (BBL). In turn, DOC concentrations depended on bioturbator activity which was high for the PAC tolerant species Asellus aquaticus and low for AC sensitive species Lumbriculus variegatus. A dual BBL resistance model combining AC effects on gradients, DOC facilitated transport and biodiffusion was evaluated against the data and showed how the type of resistance differs with treatment and chemical hydrophobicity. Data and simulations illustrate the complex interplay between AC and contaminant toxicity to benthic organisms and how differences in species

tolerance affect mass fluxes from sediment to the water column." As taken from Kupryianchyk D et al. 2013. Environ. Sci. Technol. 47(10), 5092-100. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23590290

"As the use of single-walled carbon nanotubes (SWNTs) increases over time, so does the potential for environmental release. This research aimed to determine the toxicity, bioavailability, and bioaccumulation of SWNTs in marine benthic organisms at the base of the food chain. The toxicity of SWNTs was tested in a whole sediment exposure with the amphipod Ampelisca abdita and the mysid Americamysis bahia. In addition, SWNTs were amended to sediment and/or food matrices to determine their bioavailability and bioaccumulation through these routes in A. abdita, A. bahia, and the estuarine amphipod Leptocheirus plumulosus. No significant mortality to any species via sediment or food matrices was observed at concentrations up to 100 ppm. A novel near-infrared fluorescence spectroscopic method was utilized to measure and characterize the body burdens of pristine SWNTs in nondepurated and depurated organisms. We did not detect SWNTs in depurated organisms but quantified them in nondepurated A. abdita fed SWNT-amended algae. After a 28-d exposure to [(14) C]SWNT-amended sediment (100 μg/g) and algae (100 μg/g), [(14) C]SWNT was detected in depurated and nondepurated L. plumulosus amphipods at 0.50 μg/g and 5.38 μg/g, respectively. The results indicate that SWNTs are bioaccessible to marine benthic organisms but do not appear to accumulate or cause toxicity." As taken from Parks AN et al. 2013. Environ. Toxicol. Chem. 32(6), 1270-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23404747

10.4. Terrestrial toxicity

BIRDS and MAMMALS/ ... Several non-target organisms, including burrowing owls, may inhabit the burrows of target pests ... . Due to the potential risk to non-target organisms, the EPA is currently developing more extensive labeling regarding timing of application and observation of signs indicating the presence or absence of target and non-target organisms. These instructions will be explicit concerning actions users must take before applying the product. [USEPA/Office of Pesticide Programs; Reregistration Eligibility Decision Document - Carbon and Carbon Dioxide p.12 (September 1991). Available from, as of July 19, 2008: http://www.epa.gov/pesticides/reregistration/status.htm] **PEER REVIEWED**

As taken from HSDB, 2009

"Premise of the study: Single-walled carbon nanotubes (SWCNTs) have many unique structural and mechanical properties. Their potential applications, especially in biomedical engineering and medical chemistry, have been increasing in recent years, but the toxicological impact of nanoparticles has rarely been studied in plants. • Methods: We exposed Arabidopsis and rice leaf protoplasts to SWCNTs and examined cell viability, DNA damage, reactive oxygen species generation, and related gene expression. We also tested the effects of nanoparticles on Arabidopsis leaves after injecting a SWCNT solution. EM-TUNEL (electron-microscopic terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) and a cerium chloride staining method were used. • Key results: SWCNTs caused adverse cellular responses including cell aggregation, chromatin condensation along with a TUNEL-positive reaction, plasma membrane deposition, and H(2)O(2) accumulation. The effect of SWCNTs on the survival of cells was dose dependent, with 25 μg/mL inducing 25% cell death in 6 h. In contrast, activated carbon, which is not a nano-sized carbon particle, did not induce cell death even 24 h after treatments. The data indicated that the nano-size of the particle is a critical factor for toxicity. Moreover, endocytosis-like structures with cerium chloride deposits formed after SWCNT treatment, suggesting a possible pathway for nanoparticles to traverse the cell membrane. • Conclusions: Consequently, SWCNTs have an adverse effect on protoplasts and leaves through oxidative stress, leading to a certain

amount of programmed cell death. Although nanomaterials have great advantages in many respects, the benefits and side effects still need to be assessed carefully" (Shen et al., 2010. American Journal of Botany 97, 1602-1609). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21616795

"….The potential impact of single-walled carbon nanotubes (SWCNTs) was evaluated using Caenorhabditis elegans (C. elegans) as a toxicological animal model. SWCNTs are extremely hydrophobic to form large agglomerates in aqueous solutions. Highly soluble amide-modified SWCNTs (a-SWCNTs) were therefore used in the present study so that the exact impact of SWCNTs could be studied. No significant toxicity was observed in C. elegans due to the amide modification. a-SWCNTs were efficiently taken up by worms and caused acute toxicity, including retarded growth, shortened lifespan and defective embryogenesis. The resulting toxicity was reversible since C. elegans could recover from a-SWCNT-induced toxicity once the exposure terminates. Chronic exposure to low doses of a-SWCNTs during all development stages could also cause a toxic accumulation in C. elegans. Genome-wide gene expression analysis was performed to investigate the toxic molecular mechanisms. Functional genomic analysis and molecular biology validation suggest that defective endocytosis, the decreased activity of the citrate cycle and the reduced nuclear translocation of DAF-16 transcription factor play key roles in inducing the observed a-SWCNT toxicity in worms….." As taken from Chen PH et al. 2013b. Biomaterials 34(22), 5661-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23623425?dopt=AbstractPlus

"With the development and application of carbon nanotubes (CNTs), the potential hazards of CNTs to biological systems and the environment are getting more and more attention. This review evaluated the effects of physicochemical properties of CNTs on toxicity and summarized the advances on the mechanism of CNTs toxicity. We also proposed the possible hazards associated with CNTs and harmful effects resulting from exposure of aquatic animals, bacteria and higher plants to CNTs *in vitro* and *in vivo*. The current knowledge and gaps on CNTs were outlined as a potential problem for the environment and human health. The current research gaps on CNTs toxicity were identified and the further studying focus was proposed, too. This essay concluded with a set of recommendations for the advancement of understanding of the role of CNTs and future challenges in environmental and ecotoxicological research." As taken from Du J et al. 2013. Environ. Toxicol. Pharmacol. 36(2), 451-62. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23770455

"The high surface area of multi-walled carbon nanotubes (MWCNTs) tends to adsorb a large variety of toxic chemicals, which may enhance the toxicityof both MWCNTs and chemicals to organisms. In order to evaluate the combined toxicity of nonylphenol (NP) and MWCNTs to the earthworm Eisenia fetida in soil, artificial soil systems containing distilled water, 0.1 g kg(-1) MWCNTs, 1 g kg(-1) MWCNTs, 1 g kg(-1) MWCNTs absorbed 5 mg kg(-1) NP, and 10 mg kg(-1) NP alone were prepared and exposed to earthworms for 7 days. Antioxidative responses, and activities of cellulase, Na(+), K(+)-ATPase and acetylcholinesterase (TChE) as well as DNA damage were chosen as toxicological endpoints. The results showed that 1 g kg(-1) MWCNTs adsorbed 5 mg kg(-1) NP from the soil which caused much more adverse effects on the earthworms than each chemical alone, evident from the responses of cellulase, Na(+), K(+)-ATPase and comet assay. This study indicated that MWCNTs facilitated the bioavailability of NP to the earthworm and increased the harmful effects of NP." As taken from Hu C et al. 2013. Environ. Sci. Process Impacts 15(11), 2125-30. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24104387

"Carbon nanotubes (CNT) have numerous industrial applications and may be released to the environment. In the aquatic environment, pristine or functionalized CNT have different dispersion behavior, potentially leading to different risks of exposure along the water column. Data included in this review indicate that CNT do not cross biological barriers readily. When internalized, only a

minimal fraction of CNT translocate into organism body compartments. The reported CNT toxicity depends on exposure conditions, model organism, CNT-type, dispersion state and concentration. In the ecotoxicological tests, the aquatic organisms were generally found to be more sensitive than terrestrial organisms. Invertebrates were more sensitive than vertebrates. Single-walled CNT were found to be more toxic than double-/multi-walled CNT. Generally, the effect concentrations documented in literature were above current modeled average environmental concentrations. Measurement data are needed for estimation of environmental no-effect concentrations. Future studies with benchmark materials are needed to generate comparable results. Studies have to include better characterization of the starting materials, of the dispersions and of the biological fate, to obtain better knowledge of the exposure/effect relationships." As taken from Jackson P et al. 2013. Chem. Cent. J. 7(1), 154. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24034413

"The inconsistent impact of nanomaterials on different plant species has been reported, but little is known about this effect at the cellular and genetic levels. Here we report that single-walled carbon nanotubes (SWCNTs) accelerate maize seminal root growth, but display little effect on the primary root growth. In contrast, root hair growth inhibition by SWCNTs is observed. Further gene transcription analysis shows that SWCNTs could increase the expression of seminal root associated genes whereas decrease root hair associated gene expression. Their effect is on both tissue and gene selectiveness since both enhanced and inhibited gene expression and tissue growth are observed during root development. Microscopy images reveal the distribution of SWCNTs inside the root and mainly in the intercellular space in cortex tissues. We also find that SWCNT-treatment dynamically and selectively induces the up-regulation of epigenetic modification enzyme genes, leading to global deacetylation of histone H3, similar to the response of plants to other stress. Our results suggest that the nanoparticle-root cell interaction could cause the change in gene expression, and consequently affect relative root growth and development." As taken from Yan S et al. 2013. J. Hazard. Mater. 246-247, 110-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23291336

"Nanomaterials such as single-walled carbon nanotubes (SWCNTs) may enter the soil environment with unknown consequences resulting from the development of nanotechnology for a variety of applications. We determined the effects of SWCNTs on soil enzyme activity and microbial biomass through a 3-week incubation of urban soils treated with different concentrations of SWCNTs ranging from 0 to 1000 μg g(-1) soil. The activities of cellobiohydrolase, β-1,4-glucosidase, β-1,4-xylosidase, β-1,4-N-acetylglucosaminidase, L-leucine aminopeptidase, and acid phosphatase and microbial biomass were measured in soils treated with powder and suspended forms of SWCNTs. SWCNTs of concentrations at 300-1000 μg g(-1) soil significantly lowered activities of most enzymes and microbial biomass. It is noteworthy that the SWCNTs showed similar effects to that of multi-walled carbon nanotubes (MWCNTs), but at a concentration approximately 5 times lower; we suggest that this is mainly due to the higher surface area of SWCNTs than that of MWCNTs. Indeed, our results show that surface area of CNTs has significant negative relationship with relative enzyme activity and biomass, which suggests that greater microorganism-CNT interactions could increase the negative effect of CNTs on microorganisms. Current work may contribute to the preparation of a regulatory guideline for the release of CNTs to the soil environment." As taken from Jin L et al. 2013. Ecotoxicol. Environ. Saf. 88, 9-15. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23218497

"Culture-dependent and -independent methods were employed to determine the impact of carboxyl-functionalized single-walled carbon nanotubes (SWNTs) on fungal and bacterial soil microbial communities. Soil samples were exposed to 0 (control), 250, and 500 μg of SWNTs per gram of soil. Aliquots of soil were sampled for up to 14 days for culture-dependent analyses, namely, plate count agar and bacterial community level physiological profiles, and culture-independent analyses,

namely, quantitative real-time polymerase chain reaction (qPCR), mutliplex-terminal restriction fragment length polymorphism (M-TRFLP), and clone libraries. Results from culture-independent and -dependent methods show that the bacterial soil community is transiently affected by the presence of SWNTs. The major impact of SWNTs on bacterial community was observed after 3 days of exposure, but the bacterial community completely recovered after 14 days. However, no recovery of the fungal community was observed for the duration of the experiment. Physiological and DNA microbial community analyses suggest that fungi and bacteria involved in carbon and phosphorus biogeochemical cycles can be adversely affected by the presence of SWNTs. This study suggests that high concentrations of SWNTs can have widely varying effects on microbial communities and biogeochemical cycling of nutrients in soils." As taken from Rodrigues DF et al. 2013. Environ. Sci. Technol. 47(1), 625-33. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23205469

"This study evaluated the impacts of multiwalled carbon nanotubes (MWNTs) on microbial community composition and functioning in a sandy loam soil over 90 d. We used test concentrations in the range of lower MWNT concentrations (10mg/kg) to extremely high MWNT concentrations (10,000 mg/kg) as a worst case scenario. We observed no effects of MWNTs on soil respiration, enzymatic activities, and microbial community composition at 10, 100 and 1,000 mg/kg. However, increases in fungal fatty acid methyl ester markers were observed at the highest treatment. In addition, pyrosequencing demonstrated a decreased abundance of some bacterial genera like Derxia, Holophaga, Opitutus and Waddlia at the highest treatment while bacterial genera that are considered potential degraders of recalcitrant contaminants (such as polycyclic aromatic hydrocarbons) like Rhodococcus, Cellulomonas, Nocardioides and Pseudomonas increased. These results suggest a shift in soil microbial community composition to more tolerant microbial populations in the presence of extremely high MWNT concentrations. It is unlikely that the change observed at 10,000 mg/kg is due to metal or carbon impurities as the MWNTs used in this study were of high purity. Given the need for wide-ranging data for regulation and risk assessment of nanomaterials, this study provides valuable data." As taken from Shrestha B et al. 2013. J. Hazard. Mater. 261, 188-97. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23921182

Record for carbon:

Spec. Sci. Name Spec. Common Name	Resp. Site Exp. Dur. (Days)	Media Type Test Loc.	Exp. Type Chem. Anal.	Dose # Res. Sample Unit	Endpoint BAF/BCF	Effect Effect Meas.	Signif. Sig. Level	Dose Dose Stat. Meth.
Chen caerulescens Blue Goose	4	NONE FIELDN	EN U	2	LOEL	AVO CHEM	ASIG <0.05	A 3.4 AI kg/ha
Stipa [illegible]ilata Doublejointed Speargrass	21 d	LAB	U			GERM	<0.01	F 200/ (NR/- kg/ha
[illegible]ta pepo Vegetable Marrow	5 d	LAB	U			LGTH	<0.05	A 1000/ (NR/- mg/L
[illegible]ta pepo Vegetable Marrow	15 d	LAB	U			BMAS	<0.05	A 1000/ (NR/- mg/L

[illegible]ta pepo Vegetable Marrow	12 d	LAB	U			GERM	<0.05	F 1000/ (NR/- mg/L
Themeda [illegible] Kangaroo Grass	21 d	LAB	U			GERM	<0.01	F 200/ (NR/- kg/ha
Bothriochloa [illegible] Bluestem	21 d	LAB	U			GERM	<0.01	F 200/ (NR/- kg/ha
[illegible]ia sp. Oatgrass	21 d	LAB	U			GERM	<0.01	F 200/ (NR/- kg/ha

As taken from EPA ECOTOX database.

“With the aim of investigating the effects of carbonaceous sorbent amendment on plant health and end point contaminant bioavailability, plant experiments were set up to grow maize (Zea mays) in soil contaminated with polycyclic aromatic hydrocarbons (PAHs) and metals. Maize and pine derived biochars, as well as a commercial grade activated carbon, were used as amendments. Plant growth characteristics, such as chlorophyll content and shoot to root biomass, improved with sorbent amendment to varying extents and contaminant uptake to shoots was consistently reduced in amended soils. By further defining the conditions in which sorbent amended soils successfully reduce contaminant bioavailability and improve plant growth, this work will inform field scale remediation efforts.” As taken from Brennan A et al. 2014. Environ. Pollut. 193, 79-87. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/25014015.

“Carbonaceous amendments reduce PAH dissolved concentrations (Cfree), limiting their uptake and toxicity. A soil contaminated with PAHs was mixed with activated carbon (AC), charcoal or compost and planted with radish (Raphanus sativus L.), and Cfree, chemical activities and diffusive uptake of the PAHs measured over 2 months. For AC, Cfree and diffusive uptake were decreased by up to 94% compared to the unamended soil within one week. In addition, the sum chemical activity of the PAHs remained below the threshold for baseline toxicity. In contrast, charcoal and compost only led to modest reductions in Cfree and diffusive uptake, with sum chemical activities that could potentially result in baseline toxicity being observed. Furthermore, both Cfree and diffusive uptake were lower in the planted compared to unplanted soils. Therefore, only AC successfully reduced PAH acute toxicity in the soil, but plant-promoted microbial degradation may also play an important role in PAH attenuation.” As taken from Marchal G et al. 2014. Environ. Pollut. 188, 124-31. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24583710

“Activated carbon (AC), biochar from wheat straw (BCS), and biochar from willow (BCW) were added to the soils sampled from areas of strong anthropogenic influence at doses of 0.5%, 1%, 2.5%, or 5% (w/w) and incubated for 2 mo. At the end of this period, the toxicity of the soils was measured. The effect of AC and biochars on the toxicity of the soils varied based on soil, type of amendment, dose, and test organism. For most of the parameters tested, the highest effectiveness of AC in terms of reduction of toxicity was observed in soil POPI (from bitumen processing plant area). In the case of the remaining soils, after the addition of AC varied results were observed, in which a reduction or an increase of toxicity, relative to the control soil, occurred. As in the case of AC, biochars also caused a significant reduction of phytotoxicity of soil POPI. In soils KB (from coking plant area, industrial waste deposit) and KOK (from coking plant area, coking battery), the reduction or increase of toxicity depended on biochar dose. Compared with the biochars, the effectiveness of AC in the reduction of toxicity depended also on soil, type of amendment, dose, and test organism. Generally, the AC was more effective than biochars in relation to mortality and reproduction of Folsomia candida (in all soils) and for reduction of luminescence inhibition of Vibrio fischeri (in POPI soil).” As taken from Koltowski M et al. 2016. Environ. Toxicol. Chem. 35(5), 1321-8. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26378767

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that the bioaccumulative potential of carbon in the environment has not been determined.

Data accessed January 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

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13. Last audited

May 2018

I U C L I D

D a t a s e t

Existing Chemical	Substance ID: 7440–44–0
CAS No.	7440–44–0
EINECS Name	carbon
EINECS No.	231–153–3
Molecular Weight	12
Molecular Formula	C

Dataset created by: EUROPEAN COMMISSION – European Chemicals Bureau

This dossier is a compilation based on data reported by the European Chemicals Industry following 'Council Regulation (EEC) No. 793/93 on the Evaluation and Control of the Risks of Existing Substances'. All (non–confidential) information from the single datasets, submitted in the IUCLID/HEDSET format by individual companies, was integrated to create this document.

The data have not undergone any evaluation by the European Commission.

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1.0.1 OECD and Company Information

Name: ACQUENYMCO
Street: DEI GIOVI, 6
Town: 20032 CORMANO
Country: Italy
Phone: 02/6150621
Telefax: 02/66301278
Cedex: 20032

Name: CAMEL CHEMICALS
Street: P. PICASSO, 9
Town: 20060 POZZUOLO MARTESANA
Country: Italy
Phone: 0295357161
Telefax: 0295358271

Name: CECA SA
Street: 12, Pleace de l'Iris
Town: F–92062 Paris–La Defence 2
Country: France
Phone: +33 1 47969311
Telefax: +33 1 47969233
Cedex: 54

Name: CHEMVIRON CARBON
Street: BOULEVARD DE LA WOLUWE 60
Town: 1200 BRUXELLES
Country: Belgium
Phone: 32.2.7730211
Telefax: 32.2.7709394

Name: MARE S.p.A.
Street: Via Verdi, 3
Town: 20010 Ossona/Fraz. Asmonte (MI)
Country: Italy
Phone: 02 903261
Telefax: 02 90380474

Name: NORIT N.V.
Street: Nijverheidsweg Noord 72
Town: 3812 PM Amersfoort
Country: Netherlands
Phone: +31–33–648911
Telefax: +31–33–648911
Telex: 79040

Name: Süd-Chemie AG
Street: Lenbachplatz 6
Town: 80333 München
Country: Germany
Phone: 0 89/5110-0
Telefax: 0 89/5110-375

Name: UOP Ltd.
Street: Liongate Ladymead
Town: GU1 1AT Guildford, Surrey
Country: United Kingdom
Phone: +44/1483 304 848
Telefax: +44/1483 304 863

1.0.2 Location of Production Site

-

1.0.3 Identity of Recipients

-

1.1 General Substance Information

Substance type: element
Physical status: solid

Substance type: inorganic
Physical status: solid

Substance type: natural substance
Physical status: solid

Substance type: organic
Physical status: solid

1.1.1 Spectra

-

1.2 Synonyms

Activated carbon
Source: UOP Ltd. Guildford, Surrey

activated coal, activated charcoal, active carbon
Remark: Please note that another CAS no and EINECS no exist for thiscarbon, following is also used in the industry:
CAS no 64365-11-3
EINECS no 264-846-4
Source: NORIT N.V. Amersfoort

Activated coal, activated charcoal, active carbon
Remark: Please note that another cas no and einecs no exist for this carbon, following is also used in the industry :
CAS no. 64365-11-3
EINECS no. 264-846-4
Source: CHEMVIRON CARBON BRUXELLES

ACTIVATED COAL, ACTIVATED CHARCOAL, ACTIVE CARBON
Remark: Please note that another cas no and einecs no exist for thiscarbon, following is also used in the industry:
CAS no. 64365-11-3
EINECS no. 264-846-4
Source: CECA SA Paris-La Defence 2

ADSORBENTE
Source: ACQUENYMCO CORMANO
CAMEL CHEMICALS POZZUOLO MARTESANA

CARBONE ATTIVO
Source: ACQUENYMCO CORMANO
CAMEL CHEMICALS POZZUOLO MARTESANA

CHEMISORB
Source: MARE S.p.A. Ossona/Fraz. Asmonte (MI)

WATERCARB
Source: MARE S.p.A. Ossona/Fraz. Asmonte (MI)

1.3 Impurities

-

1.4 Additives

-

1.5 Quantity

Quantity 50 000 - 100 000 tonnes

1.6.1 Labelling

-

1.6.2 Classification

-

1.7 Use Pattern

Type: type
Category: Non dispersive use

Type: industrial
Category: Basic industry: basic chemicals

Type: industrial
Category: Fuel industry

Type: industrial
Category: Paints, lacquers and varnishes industry

Type: industrial
Category: other

Type: use
Category: Absorbents and adsorbents

1.7.1 Technology Production/Use

-

1.8 Occupational Exposure Limit Values

Type of limit: MAC (NL)
Limit value: 2 mg/m3
Remark: There is no MAC value for activated carbon; the given value is applicable to inconvenient dust
Source: NORIT N.V. Amersfoort

Type of limit: MAK (DE)
Limit value: 6 mg/m3
Remark: There is no MAC value for activated carbon. The given value is applicable to inconvenient dust with a respirable quartz content of over 1 w/w %.
Source: CHEMVIRON CARBON BRUXELLES

Type of limit: MAK (DE)
Limit value: 6 mg/m3
Remark: There is no MAC value for activated carbon. Tha given value is applicable to inconvenient dust with a respirable quartz content of over 1 w/w %.
Source: CECA SA Paris-La Defence 2

1.9 Source of Exposure

-

1.10.1 Recommendations/Precautionary Measures

-

1.10.2 Emergency Measures

-

1.11 Packaging

-

1.12 Possib. of Rendering Subst. Harmless

-

1.13 Statements Concerning Waste

-

1.14.1 Water Pollution

-

1.14.2 Major Accident Hazards

-

1.14.3 Air Pollution

-

1.15 Additional Remarks

Remark: Not dangerous for transport.
Source: MARE S.p.A. Ossona/Fraz. Asmonte (MI)

1.16 Last Literature Search

-

1.17 Reviews

-

1.18 Listings e.g. Chemical Inventories

-

2.1 Melting Point

Value: >= 3500 degree C
Decomposition: no
Sublimation: no
Method: other
GLP: yes
Source: ACQUENYMCO CORMANO

2.2 Boiling Point

Value: ca. 4000 degree C
Decomposition: no
Method: other
GLP: yes
Source: ACQUENYMCO CORMANO

2.3 Density

Type: bulk density
Value: .25 – .75 kg/m3 at 20 degree C
Method: other
Source: CHEMVIRON CARBON BRUXELLES

Type: relative density
Value: = 250 – 600 kg/m3 at 25 degree C
Method: other
GLP: yes
Source: ACQUENYMCO CORMANO

2.3.1 Granulometry

–

2.4 Vapour Pressure

Value:
Remark: NON APPLICABILE.
Source: ACQUENYMCO CORMANO

2.5 Partition Coefficient

log Pow:
Method:
Year:
Remark: NON APPLICABILE
Source: ACQUENYMCO CORMANO

2.6.1 Water Solubility

Qualitative: not soluble
Source: CHEMVIRON CARBON BRUXELLES

Remark: NON APPLICABILE
Source: ACQUENYMCO CORMANO

2.6.2 Surface Tension

-

2.7 Flash Point

Value:
Type:
Method:
Year:
Remark: NON APPLICABILE
Source: ACQUENYMCO CORMANO

2.8 Auto Flammability

Value: > 400 degree C
Method: other
GLP: yes
Source: ACQUENYMCO CORMANO

Value: 300 degree C
Remark: Ignition point in air is 300-500 degree C.
Source: CHEMVIRON CARBON BRUXELLES

2.9 Flammability

Result: non flammable
Source: CHEMVIRON CARBON BRUXELLES

Result: non flammable
Remark: NON INFIAMMABILE
Source: ACQUENYMCO CORMANO

2.10 Explosive Properties

Result: no data
Remark: NON ESPLOSIVO; NUBI DI POLVERE POSSONO CREARE IN PARTICOLARISITUAZIONI, CONDIZIONI DI ESPLOSIVITA'
Source: ACQUENYMCO CORMANO

Result: not explosive
Source: CHEMVIRON CARBON BRUXELLES

2.11 Oxidizing Properties

Result: no oxidizing properties
Source: CHEMVIRON CARBON BRUXELLES

Result: no oxidizing properties
Remark: NON APPLICABILE
Source: ACQUENYMCO CORMANO

2.12 Additional Remarks

Remark: IL CARBONE ATTIVO NON E’ CONSIDERATO PRODOTTO PERICOLOSO E TROVA LARGHI IMPIEGHI NELLA POTABILIZZAZIONE DELLE ACQUE O NEI PROCESSI ALIMENTARI
Source: ACQUENYMCO CORMANO

3.1.1 Photodegradation

–

3.1.2 Stability in Water

–

3.1.3 Stability in Soil

–

3.2 Monitoring Data (Environment)

–

3.3.1 Transport between Environmental Compartments

–

3.3.2 Distribution

–

3.4 Mode of Degradation in Actual Use

Remark: IL PRODOTTO E’ STABILE ALLE CONDIZIONI NORMALI DI IMPIEGO
Source: ACQUENYMCO CORMANO

3.5 Biodegradation

–

3.6 BOD5, COD or BOD5/COD Ratio

B O D 5

Method: ISO 5815 "Water quality – Determination of biochemical oxygen demand after 5 days (BOD5) – Dilution and seeding method"
BOD5: ca. 2 mgO2/l

C O D

Method: ISO DP 6060 "Water quality – Determination of the chemical oxygen demand"
COD: 2000 mg/g substance

Source: CHEMVIRON CARBON BRUXELLES

3.7 Bioaccumulation

–

3.8 Additional Remarks

Source: ACQUENYMCO CORMANO

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

-

4.2 Acute Toxicity to Aquatic Invertebrates

-

4.3 Toxicity to Aquatic Plants e.g. Algae

-

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:
Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Peat based steam activated carbons, lignite based steam activated carbons and wood based chemical activated carbons were found not to be toxic to waste-water bacteria.
An EC50 value for respiration inhibition could not be determined.
Tests by RCC Notox B.V. The Netherlands.
Source: CHEMVIRON CARBON BRUXELLES

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

-

4.5.2 Chronic Toxicity to Aquatic Invertebrates

-

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

–

4.6.2 Toxicity to Terrestrial Plants

–

4.6.3 Toxicity to other Non–Mamm. Terrestrial Species

–

4.7 Biological Effects Monitoring

–

4.8 Biotransformation and Kinetics

–

4.9 Additional Remarks

–

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Sex:
Number of Animals:
Vehicle:
Value: > 10000 mg/kg bw
Method: other
Year: 1979 **GLP:**
Test substance:
Remark: Determination for toxic substances – Test conducted in 1979 by the American Agency FHSA.
Activated carbon is not an oral toxic substance.
Determination for toxic substances, tests conducted in 1977 by CIVO TNO, the Netherlands.
Source: CHEMVIRON CARBON BRUXELLES

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Sex:
Number of Animals:
Vehicle:
Exposure time:
Value: > 64.4 mg/l
Method: other
Year: 1979 **GLP:**
Test substance:
Remark: Determination for toxic substances. Tests conducted in 1979 by the American Agency FHSA.
Source: CHEMVIRON CARBON BRUXELLES

5.1.3 Acute Dermal Toxicity

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: Activated carbon is not a primary skin irritant.
Source: CHEMVIRON CARBON BRUXELLES

5.1.4 Acute Toxicity, other Routes

–

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species:
Concentration:

Exposure:
Exposure Time:
Number of Animals:
PDII:
Result:
EC classificat.:
Method:
Year: **GLP:**
Test substance:
Remark: No data – None irritating
Source: CHEMVIRON CARBON BRUXELLES

5.2.2 Eye Irritation

Species: other
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result:
EC classificat.: not irritating
Method:
Year: **GLP:**
Test substance:
Remark: LA POLVERE DI CARBONE ATTIVO PUO’ PROVOCARE LIEVE IRRITAZIONE DEGLI OCCHI.
Source: ACQUENYMCO CORMANO

Species:
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result:
EC classificat.:
Method:
Year: **GLP:**
Test substance:
Remark: No data – None irritating
Source: CHEMVIRON CARBON BRUXELLES

5.3 Sensitization

Type:
Species:
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: **GLP:**
Test substance:
Remark: No data – Not sensitizing
Source: CHEMVIRON CARBON BRUXELLES

5.4 Repeated Dose Toxicity

-

5.5 Genetic Toxicity 'in Vitro'

-

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

-

5.8 Toxicity to Reproduction

-

5.9 Developmental Toxicity/Teratogenicity

-

5.10 Other Relevant Information

-

5.11 Experience with Human Exposure

Source: CHEMVIRON CARBON BRUXELLES

(1)

(1) Wet activated carbon removes oxygen from air causing severe hazard to workers inside carbon vessels and closed or confined spaces. Before entering such an area, sampling and work procedures for low oxygen levels should be taken to ensure ample oxygen availability.

7.1 Risk Assessment

-

European Food Safety Authority

EFSA Journal 2014;12(2):3571

SCIENTIFIC OPINION

Scientific Opinion on the safety assessment of the active substances, sodium erythorbate, sodium carbonate, sodium bicarbonate, iron sulphate, activated carbon, cellulose, calcium hydroxide, calcium chloride and water, for use as active system in food contact materials[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 6 May 2014, replaces the earlier version published on 12 February 2014*.

ABSTRACT

This scientific opinion of EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids deals with the safety assessment of the active substances sodium erythorbate, sodium carbonate, sodium bicarbonate, iron sulfate, activated carbon, cellulose, calcium hydroxide, calcium chloride and water, used in mixture which is packed into sachets for absorbing oxygen/carbon dioxide emitting from/into the headspace surrounding packed food. All substances of this formulation have been evaluated and approved for use as additives in plastic food contact materials or as food additives. No migration of calcium, iron and sodium ions was detected. No volatile organic compounds other than carbon dioxide were detected at the limit of detection of 0.5 µg/l. The CEF Panel concluded that the use of the substances sodium erythorbate, sodium carbonate, sodium bicarbonate, iron sulfate, activated carbon, cellulose, calcium hydroxide, calcium chloride and water does not raise a safety concern when used in oxygen absorber/carbon dioxide emitter systems, in sachets that prevent the physical release of their contents into the food. The sachets are to be placed in the headspace of the packaging and as such may come into occasional contact with the food, e.g. during handling. The sachet should not come into direct contact with liquid foods or foods that have and external aqueous liquid phase on the surface (liquid or exudates).

[1] On request from the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes, France, Question No EFSA-Q-2011-00240, adopted on 29 January 2014.

[2] Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Claudia Bolognesi, Leon Brimer, Laurence Castle, Alessandro Di Domenico, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Martine Kolf-Clauw, Catherine Leclercq (until July 2013), Jean-Claude Lhuguenot (until November 2012), Wim Mennes, Maria Rosaria Milana, Maria de Fátima Tavares Poças, Iona Pratt, Kettil Svensson, Fidel Toldrá and Detlef Wölfle. Correspondence: cef@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Food Contact Materials: Mona-Lise Binderup, Laurence Castle, Riccardo Crebelli, Roland Franz, Nathalie Gontard, Ragna Bogen Hetland, Eugenia Lampi, Maria Rosaria Milana, Maria de Fátima Tavares Poças, Philippe Saillard, Kettil Svensson and Detlef Wölfle for the preparatory work on this scientific opinion.

* Minor changes of an editorial nature were made. The changes do not affect the contents of this report. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request, as a version showing all the changes made.

Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzyme, Flavourings and Processing Aids), 2014. Scientific Opinion on the safety assessment of the active substances, sodium erythorbate, sodium carbonate, sodium bicarbonate, iron sulphate, activated carbon, cellulose, calcium hydroxide, calcium chloride and water, for use as active system in food contact materials. EFSA Journal 2014;12(2):3571, 11 pp. doi:10.2903/j.efsa.2014.3571

Available online: www.efsa.europa.eu/efsajournal

KEY WORDS

sodium erythorbate, sodium carbonate, sodium bicarbonate, food contact materials, safety assessment, evaluation

SUMMARY

According to the Commission Regulation (EC) No 450/2009 of the Commission of European Communities of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, substances responsible for the active or intelligent function need first to be evaluated by the EFSA before their inclusion into a positive Community list. The procedure of the evaluation and the tasks of EFSA are described in the Regulation (EC) No. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

In the context of this evaluation procedure, following a request from the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes , France, the EFSA Panel on Food Contact Materials, Enzymes and Processing aids (CEF) was asked to deliver an opinion on the safety of a mixture comprising sodium erythorbate (CAS 6381-77-7 and FCM Substance No 1042), sodium carbonate (CAS No 497-19-8 and FCM Substance No 21), sodium bicarbonate (CAS No 144-55-8 and FCM Substance No 21), iron sulphate (CAS No 7782-63-0 and FCM Substance No 511), activated carbon (CAS No 7440-44-0, FCM Substance No 984), cellulose (CAS No 9004-34-6 and FCM Substance No 553), calcium hydroxide (CAS No 1305-62-0 and FCM Substance No 394), calcium chloride (CAS No 10043-52-4 and FCM Substance No 585) and water (CAS No 7732-18-5, FCM Substance No 515), for use as oxygen absorber and carbon dioxide emitter. The mixture is intended to be placed in a sachet made from perforated polyethylene terephtalate (PET)/cellulosic non woven (NT)polypropylene (PP) material. The dossier was submitted by the applicant, Atmosphère Contrôle SAS (ATCO), France.

The active ingredient responsible for the oxygen absorbing function is sodium erythorbate, which reacts with the oxygen present in the primary packaging. The carbon dioxide emitting function is fulfilled by the presence of sodium carbonate or sodium bicarbonate. All the other substances are used to provide adequate media to facilitate both reactions. This oxygen absorber/carbon dioxide emitter system is intended to be used in various applications, such as meat and meat products, precooked dishes, delicatessen, cheese, bakery, cakes, pastry products. These foods are generally stored at +4 °C. Shelf-lives vary from several days to several weeks.

All starting substances have been evaluated and approved for use as additives in plastic food contact materials or as food additives. Activated carbon was not evaluated as such, but it meets the specifications for activated charcoal, which is authorised as additive for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) i.e. same purity requirements as for Vegetable Carbon (E 153) set out by Regulation (EC) No 1333/2008 with the exception of ash content which may be up to 10 %.

Specific migration of calcium, iron and sodium were determined under realistic conditions, in minced meat, in contact with one sachet for 7 days, at 5 °C. By comparing the average content of calcium, iron and sodium naturally present in minced meat, with the corresponding concentrations measured in minced meat in direct contact with sachets, no significant migration of the ions present in the sachet is expected.

Potential byproducts linked to the use of the oxygen absorber/carbon dioxide emitter system were investigated. No volatile organic compounds other than carbon dioxide were detected at the limit of detection of 0.5 µg/l.

Based on the level of migration and the intended uses (no direct contact with food), no toxicity studies on the formulation and migrants were required. The use the oxygen absorber/carbon dioxide emitter formulation is toxicologically acceptable.

Therefore, the CEF Panel concluded that the use of the substances sodium erythorbate, iron sulfate, activated carbon, cellulose, calcium hydroxide, sodium carbonate, sodium bicarbonate, calcium

chloride and water does not raise a safety concern when used in oxygen absorber/carbon dioxide emitter systems, in sachets that prevent the physical release of their contents into the food. The sachets are to be placed in the headspace of the packaging and as such may come into occasional contact with the food, e.g. during handling. The sachet should not come into direct contact with liquid foods or foods that have and external aqueous liquid phase on the surface (liquid or exudates).

Activated carbon should in addition comply with the same purity requirements as for Vegetable Carbon (E 153) set out by Regulation (EC) No 1333/2008 with exception of ash content which can be up to 10 % (w/w).

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BACKGROUND AS PROVIDED BY THE LEGISLATION

Regulation (EC) No 450/2009[4] of the Commission of European Communities is a specific measure that lays down specific rules for active and intelligent materials and articles intended for contact with foodstuffs in addition to the general requirements established in Regulation (EC) No 1935/2004[5] of the European Parliament and of the Council on materials and articles intended to come into contact with food. Active materials and articles are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food. In the context of this evaluation procedure, the CEF Panel received a request from a competent Member State Authority for safety evaluation of three mixtures of substances following the corresponding applications from the industry.

The substance(s) responsible for the active and/or intelligent function of the material should be included in a positive list by the Commission following a safety evaluation by EFSA according to the procedure described in the abovementioned regulations.

According to this procedure the industry submits applications to the Member States competent Authorities which in their turn transmit the applications to EFSA for evaluation. The application is supported by a technical dossier submitted by the industry following the EFSA guidelines on "submission of a dossier for safety evaluation by EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food" (EFSA, 2009).

In this context, EFSA received an application from the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes, France, requesting the evaluation of a mixture comprising iron sulfate, activated carbon, sodium erythorbate, cellulose, calcium hydroxide, sodium carbonate, sodium bicarbonate, calcium chloride solution and water, for use as use as oxygen scavenger and carbon dioxide emitter.

TERMS OF REFERENCE AS PROVIDED BY THE LEGISLATION

EFSA is required to carry out a risk assessment on the risks originating from the migration into food of the substances activated carbon, sodium erythorbate, iron sulfate, cellulose, calcium hydroxide, sodium carbonate, sodium bicarbonate, calcium chloride solution, and water, used in oxygen absorbing systems in food contact materials, and deliver a scientific opinion, according to the Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food.

The opinion of EFSA will be considered by the Commission for adoption of a Community list of authorised substances where according to the Regulation (EC) No 450/2009 there will be specified:

(a) the identity of the substance(s);
(b) the function of the substance(s);
(c) the reference number;
(d) if necessary, the conditions of use of the substance(s) or component;
(e) if necessary, restrictions and/or specifications of use of the substance(s);
(f) if necessary, conditions of use of the material or article to which the substance or component is added or into which it is incorporated.

[4] Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. OJ L 135, 30.5.2009, p. 3–11

[5] Regulation (EC) No 1935/2004 of the European parliament and of the council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ L 338, 13.11.2004, p. 4–17

ASSESSMENT

1. Introduction

The European Food Safety Authority was asked by the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes, France to evaluate the safety of a mixture comprising sodium erythorbate (CAS 6381-77-7 and FCM Substance No 1042), sodium carbonate (CAS No 497-19-8 and FCM Substance No 21), sodium bicarbonate (CAS No 144-55-8 and FCM Substance No 21), iron sulphate (CAS No 7782-63-0 and FCM Substance No 511), activated carbon (CAS No 7440-44-0, FCM Substance No 984), cellulose (CAS No 9004-34-6 and FCM Substance No 553), calcium hydroxide (CAS No 1305-62-0 and FCM Substance No 394), calcium chloride (CAS No 10043-52-4 and FCM Substance No 585) and water (CAS No 7732-18-5, FCM Substance No 515). The request has been registered in the EFSA's register of received questions under the number EFSA-Q-2011-00240. The dossier was submitted by the applicant, Atmosphère Contrôle SAS (ATCO), France.

2. General information

According to the applicant, the active mixture constituting the oxygen absorber and carbon dioxide emitter system is a powder comprising sodium erythorbate, sodium carbonate, sodium bicarbonate, iron sulphate, activated carbon, cellulose, calcium hydroxide, calcium chloride and water. It is introduced into multilayer sachet made from polyethylene terephtalate (PET)/cellulosic non-woven/polypropylene (PP) material, and heat sealed after filling. Both PET and PP are perforated prior lamination to allow gas exchanges.

According to the applicant, sachets containing the active mixture, with a weight of active formulation per unit of the sachet surface of 6 g/dm², are introduced in food packaging to scavenge oxygen and to produce carbon dioxide. Sachets should be placed in the headspace (to allow air circulation) of the packaging. Nevertheless, unintended occasional contact with dry or other solid foods cannot be excluded. The sachets must not be put in direct contact with acid food (pH < 4.5), with liquid foods (i.e. dressings, soups, beverages) or foods with external aqueous liquid fraction (liquids or exudates) to avoid inhibition of the oxygen absorption.

This oxygen absorber/carbon dioxide emitter system is intended to be used in various applications, such as meat and meat products, precooked dishes, delicatessen, cheese, bakery, cakes, pastry products. These foods are generally stored at +4 °C. Shelf-lives vary from several days to several weeks.

The active mixture has not been evaluated by the SCF or EFSA in the past. However, all the substances constituting the mixture (activated carbon, sodium erythorbate, iron sulfate, cellulose, calcium hydroxide, sodium carbonate, sodium bicarbonate, calcium chloride solution) are authorised either for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011[6]) or food additives (Regulation (EC) No 1333/2008[7]) as follows:

- Sodium erythorbate is authorised as a food additive (E316), with the lowest limit of 500 mg/kg for cured meat products and preserved meat products. Iron sulphate is authorised as sulphuric acid, salts (FCM Substance No 511), as additive for plastic materials and articles in contact with foods, with no restriction.

[6] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food
OJ L 12. 15.1.2011, p. 1-89

[7] Regulation (EC) No 1333/2008 of the European Parliament and the Council of 16 December 2008 on food additives, OJ L 354.31.12.2008, p.16-33

- Carbonic acid salts is authorised as additive for plastic materials and articles in contact with foods, with no specific restriction (FCM Substance No 21); it is also authorised as a food additive (E 500) so its sodium salts are also authorised.
- Activated carbon has been evaluated for use as additive for plastic materials and articles in contact with foods (EFSA, 2004) and for use in oxygen scavenger mixtures (EFSA CEF Panel, 2012). For use in oxygen scavenger mixtures placed in sachets which would prevent the physical release of their contents into the food and placed in the headspace of the packaging or when used in direct contact with dry foods, the CEF Panel concluded that activated carbon does not raise safety concern if it complies with the same purity requirements as for Vegetable Carbon (E 153) set out by Regulation (EC) No 1333/2008 with exception of ash content which can be up to 10 % (w/w). (FCM Substance No 984)
- Cellulose is authorised as additive for plastic materials and articles in contact with foods, with no specific restriction (FCM Substance No 553).
- Calcium hydroxide is authorised as an additive for plastic materials and articles in contact with foods with no specific migration restriction (FCM Substance No 394). It is also listed in Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of Food Additives (specific maximum level: *quantum satis*) (E 526)[8].
- Hydrochloric acid is authorised as additive for plastic materials and articles in contact with foods, with no specific restriction (FCM Substance No 507).
- Calcium chloride is an authorised food additive (E509).
- Water is authorised as additive or monomer for plastic materials and articles in contact with foods, with no specific restrictions. The water specifications must be in compliance with Directive 98/83/EC (FCM Substance No 51).

3. Data available in the dossier used for this evaluation

The studies submitted for evaluation followed the EFSA guidelines on submission of a dossier for safety evaluation by EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food (EFSA, 2009).

Non-toxicity data:

- Data on identity
- Data on physical and chemical properties
- Data on manufacturing process
- Data on function, intended use and authorisation
- Data on overall and specific migration
  Screening on potential volatile byproducts

Toxicity data:

- Bacterial gene mutation tests on overall migration solution
- *In vitro* micronucleus test on overall migration solution

[8] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354.31.12.2008, p.16-33

4. Evaluation

4.1. Non-toxicological data

The active powder, used for oxygen scavenging and carbon dioxide emission, comprises sodium erythorbate, iron sulphate, activated carbon, cellulose, calcium hydroxide, sodium carbonate or bicarbonate, calcium chloride solution and water. The active ingredient responsible of the oxygen absorber function is sodium erythorbate which reacts with oxygen, removing the oxygen from the primary packaging. Sodium carbonate/bicarbonate is involved into the carbon dioxide release into the packaging headspace. The other chemicals are used to provide adequate media to facilitate both reactions.

Overall and specific migration tests for iron, sodium, calcium, as well as a screening of volatile byproducts were performed. Measurements were done by total immersion and under more realistic conditions (minced meat). The Panel considered that experiments by total immersion of sachets are not representative of the intended conditions of use and reported only the results from experiments carried out under more realistic conditions.

Specific migration of calcium, iron and sodium were determined in minced meat in contact with one sachet for 7 days, at 5 °C. The average content of calcium, iron and sodium naturally present in minced meat, without contact with sachets, was respectively: 88 ±20 mg/kg food; 32 ±6 mg/kg food and 609 ±92 mg/kg food. The corresponding concentrations measured in minced meat in direct contact with sachets were respectively 73 ± 16 mg/kg for calcium; 24 ± 3mg/kg for iron and 616 ± 71 mg/kg for sodium. Based on these values, no significant migration of the ions present in the sachet is expected.

The release of volatile byproducts was analysed by placing seven sachets in one liter sealed plastic bag containing air at 23 °C. Samples have been collected after 30 min, 102 min and 1 week. No volatile organic compounds other than carbon dioxide were detected at the limit of detection of 0.5 µg/l.

4.2. Toxicological data

All ingredients of the oxygen absorber/carbon dioxide emitter formulation have been evaluated and approved for use in food contact materials, without specific restriction limits, or as food additives. Activated carbon was not evaluated as such, but it meets the specifications for activated charcoal, which is authorized as additive for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) i.e. same purity requirements as for Vegetable Carbon (E 153) set out by Regulation (EC) No 1333/2008 with the exception of ash content which may be up to 10 %.

All ingredients of the active formulation are expected to be stable in normal storage and handling conditions. Moreover, the oxygen absorber/carbon dioxide emitter formulation is not intended for direct contact with liquid food or food with external liquid fraction, so no migration of non volatile species is expected. No migration of volatile byproducts was detected. Thus no toxicity studies on the formulation and migrants are required.

Nevertheless, two limited *in vitro* genotoxicity studies, namely a gene mutation test in bacteria and an *in vitro* micronucleus tests, were performed on migration solutions obtained under extreme conditions (10 days at 40 °C, by immersion). The tests were negative.

The Panel concluded that under the intended conditions of use the oxygen absorber/carbon dioxide emitter formulation is toxicologically acceptable.

CONCLUSIONS

Having considered the above-mentioned data, the CEF Panel concluded that the use of the substances sodium erythorbate, sodium carbonate, sodium bicarbonate, iron sulphate, activated carbon, cellulose, calcium hydroxide, calcium chloride and water does not raise a safety concern when used in oxygen absorber/carbon dioxide emitter systems, in sachets that prevent the physical release of their contents into the food. The sachets are to be placed in the headspace of the packaging and as such may come into occasional contact with the food, e.g. during handling. The sachet should not come into direct contact with liquid foods or foods that have and external aqueous liquid phase on the surface (liquid or exudates).

Activated carbon should in addition comply with the same purity requirements as for Vegetable Carbon (E 153) set out by Regulation (EC) No 1333/2008 with exception of ash content which can be up to 10 % (w/w).

DOCUMENTATION PROVIDED TO EFSA

1. Dossier referenced: 03/3434 Dated: 07/03/2011. Submitted by the applicant, Atmosphère Control SAS (ATCO).

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EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013c. Scientific Opinion on the safety assessment of the active substances iron, iron oxides, sodium chloride and calcium hydroxide for use in food contact materials. EFSA Journal 2013;11(10):3387, 9 pp. doi:10.2903/j.efsa.2013.3387

SCF (Scientific Committee of Food), 1990, First series of food additives of various technological functions, Report 25th Series http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_25.pdf.

GLOSSARY AND ABBREVIATIONS

Overall migration: The sum of the amounts of volatile and non volatile substances, except water, released from a food contact material or article into food or food simulant

Specific migration: The amount of a specific substance released from a food contact material or article into food or food stimulant

bw	body weight
CAS	Chemical Abstracts Service
CEF	Scientific Panel on food contact materials, enzymes, flavourings and processing aids
EU	European Union
EC	European Commission
EFSA	European Food Safety Authority
FCM	Food Contact Materials
Mw	Weight average molecular weight
PET	Poly(ethylene terephthalate)
REF No	Reference Number
SCF	Scientific committee on food
SML	Specific Migration Limit
w/w	Weight by weight

European Food Safety Authority

EFSA Journal 2014;12(5):3649

SCIENTIFIC OPINION

Scientific Opinion on the safety assessment of the active substances iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride, for use as active system in food contact materials[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids deals with the safety assessment of the active substances iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride, used in mixture which is packed into labels, for absorbing oxygen from the headspace surrounding packed food. All substances of this formulation have been evaluated and approved for use as additives in plastic food contact materials or as food supplements. Migration of substances from the labels and formation and release of volatile constituents are not expected under the intended conditions of use. The CEF Panel concluded that the use of substances iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride does not raise a safety concern when used in oxygen absorbers in labels, which prevent the physical release of their content into the food. When placed in the headspace of the packaging or when used in direct contact with foods, the labels should not intentionally or unintentionally come into direct contact with liquid foods or foods that have an external aqueous phase on the surface such as sliced fruits.

KEY WORDS

iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid sodium salt crosslinked, calcium chloride, safety assessment

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[1] On request from the Bundesamt für Verbraucherschultz und Lebensmittelsicherheit, Germany, Question No EFSA-Q-2011-00241, adopted on 10 April 2014.

[2] Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Claudia Bolognesi, Leon Brimer, Laurence Castle, Alessandro Di Domenico, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Martine Kolf-Clauw, Catherine Leclercq (until July 2013), Jean-Claude Lhuguenot (until November 2012), Wim Mennes, Maria Rosaria Milana, Maria de Fátima Poças, Iona Pratt †, Kettil Svensson, Fidel Toldrá and Detlef Wölfle. Correspondence: cef@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Food Contact Materials: Mona-Lise Binderup, Laurence Castle, Riccardo Crebelli, Alessandro Di Domenico, Roland Franz, Nathalie Gontard, Ragna Bogen Hetland, Martine Kolf-Clauw, Eugenia Lampi, Maria Rosaria Milana, Maria de Fátima Poças, Philippe Saillard, Kettil Svensson and Detlef Wölfle for the preparatory work on this scientific opinion.

† Deceased

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SUMMARY

According to the Commission Regulation (EC) No 450/2009 of the Commission of European Communities of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, substances responsible for the active or intelligent function need first to be evaluated by EFSA before their inclusion into a positive Community list. The procedure of the evaluation and the tasks of EFSA are described in the Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

In the context of this evaluation procedure, following a request from the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes, France, the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEF) was asked to deliver a scientific opinion on a mixture comprising iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride, for use as oxygen absorber in labels. Dossier was submitted on behalf of Atmosphère Control SAS, France.

According to the applicant, the substances constituting the oxygen absorber systems are mixed together and the active formulation is deposited on a multilayer film made from porous polyethylene terephthalate (PET) / nonwoven spunbonded high density polyethylene (HDPE) and covered by a multilayer film polyethylene terephthalate (PET) / polyethylene (PE). Both films are heat-sealed on 4 sides. Labels are stuck inside the packaging. The active ingredient responsible of the oxygen absorber function is iron which reacts with oxygen, removing the oxygen from the primary packaging. The other chemicals are used to provide adequate media to facilitate the reaction. The labels containing the oxygen absorber system can be used for various foods such as processed-meat products, precooked dishes, delicatessen, cheese, bakery, cakes and pastry products. These foods are generally stored at +4 °C. Shelf-lives vary from several days to several months.

The Panel concluded that under the intended conditions of use where there is no contact with liquid food or foods that have an external aqueous phase on the surface, the constituents of the mixture will not migrate because they are not volatile.

Considering the nature of these ingredients and their mode of action, the formation and release of volatile byproducts is not expected.

The CEF Panel concluded that the use of substances iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride does not raise a safety concern when used in oxygen absorbers in labels, which prevent the physical release of their content into the food. When placed in the headspace of the packaging or when used in direct contact with foods, the labels should not intentionally or unintentionally come into direct contact with liquid foods or foods that have an external aqueous phase on the surface such as sliced fruits.

Activated carbon should in addition comply with the same purity requirements as for Vegetable Carbon (E 153) set out by Commission Regulation (EU) No 231/2012 with exception of ash content which can be up to 10 % (w/w).

Iron is a natural constituent of foods. Iron compounds are also used as food additives, nutrient sources and for other purposes. The Commission may wish to take note of this if setting a restriction for iron.

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BACKGROUND AS PROVIDED BY THE LEGISLATION

Regulation (EC) No 450/2009[4] of the Commission of European Communities is a specific measure that lays down specific rules for active and intelligent materials and articles intended for contact with foodstuffs in addition to the general requirements established in Regulation (EC) No 1935/2004[5] of the European Parliament and of the Council on materials and articles intended to come into contact with food. Active materials and articles are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food.

The substance(s) responsible for the active and/or intelligent function of the material should be included in a positive list by the Commission following a safety evaluation by EFSA according to the procedure described in the above mentioned regulations.

According to this procedure the industry submits applications to the Member States competent Authorities which transmit the applications to EFSA for evaluation. The application is supported by a technical dossier submitted by the industry following the EFSA guidelines on "submission of a dossier for safety evaluation by the EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food" (EFSA, 2009).

In this context, EFSA received an application from the Direction Generale De la Concurrence de la Consommation et de la Repression des Fraudes, France, requesting the evaluation of a mixture comprising iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride, for use as an oxygen absorbers in labels.

TERMS OF REFERENCE AS PROVIDED BY THE LEGISLATION

According to Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food EFSA is asked to carry out an assessment of the risks related to the intended use of the substances and to deliver a scientific opinion.

[4] Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. OJ L 135, 30.5.2009, pp.3–11

[5] Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ L 338, 13.11.2004, pp. 4–17

ASSESSMENT

1. Introduction

The European Food Safety Authority was asked by the Direction Generale De la Concurrence de la Consommation et de la Repression des Fraudes, France to evaluate the safety of a mixture comprising iron powder (CAS No 7439-89-6, FCM Substance No 983), activated carbon (CAS No 7440-44-0, FCM Substance No 713), calcined kaolin (CAS No 92704-41-1, FCM Substance No 753), sodium chloride (CAS No 7647-14-5, FCM Substance No 985), polyacrylic acid, sodium salt, crosslinked (FCM substance No 1015) and calcium chloride (CAS 10043-52-4, FCM Substance No 585), for use as an oxygen absorber in labels. The request has been registered in the EFSA's register of questions under EFSA-Q-2011-00241. The dossier was submitted by the applicant, Atmosphère Contrôle SAS (ATCO), France.

2. General information

According to the applicant, the substances constituting the oxygen absorber systems (iron powder activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride) are mixed together and the active formulation is a powder. The active formulation is deposited on a multilayer film made from porous polyethylene terephthalate (PET) / nonwoven spunbonded high density polyethylene (HDPE) and covered by a multilayer film polyethylene terephthalate (PET) / polyethylene (PE). Both films are heat-sealed on 4 sides. Labels are stuck inside the packaging.

According to the applicant, labels containing the oxygen absorber system can be used for various foods such as processed-meat products, precooked dishes, delicatessen, cheese, bakery, cakes and pastry products. These foods are generally stored at +4 °C. Shelf-lives vary from several days to several months.

According to the applicant, the oxygen absorber system needs a humid atmosphere (Aw > 0.8) to activate chemical reactions but must not be put in contact with liquids or acidic food (pH < 4.5) or entirely covered by food as the system loses its performance.

The mixture has not been evaluated by SCF or EFSA in the past. However, all substances constituting the oxygen absorber systems are either authorised for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011[6]) and as food supplements (Regulation EC No 1170/2009[7]) or evaluated before, as follows:

- Iron powder is authorised as an additive for plastic materials and articles in contact with foods with a specific restriction of 48 mg iron/kg food based on a Provisional Maximum TDI (PMTDI) of 0.8 mg/kg bw set by JECFA/WHO (1983) and agreed by the SCF (1990) (FCM Substance No 983). The EFSA NDA Panel considered that data available are insufficient to establish a tolerable upper intake level for iron (EFSA, 2004a).

- Calcined kaolin is authorized as an additive for plastic materials and articles in contact with foods with no specific restriction (FCM Substance No 753).

- Sodium chloride is authorised as a food supplement with no specific restriction.

[6] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food OJ L 12. 15.1.2011, p. 1-89

[7] Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements (Text with EEA relevance). OJ L 314, 01/12/2009, p. 36–42

- Calcium chloride is authorised as an additive for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restriction (FCM Substance No 585).

- Activated carbon has been evaluated for use as additive for plastic materials and articles in contact with foods (EFSA, 2004b) and for use in oxygen scavenger mixtures (EFSA CEF Panel, 2012). For use in oxygen scavenger mixtures placed in sachets which would prevent the physical release of their contents into the food and placed in the headspace of the packaging or when used in direct contact with dry foods, the CEF Panel concluded that activated carbon does not raise safety concern if it complies with the same purity requirements as for Vegetable Carbon (E 153) set out by Commission Regulation (EU) No 231/2012[8] with exception of ash content which can be up to 10 % (w/w) (FCM Substance No 713).

- Polyacrylic acid, sodium salt, crosslinked, has been evaluated for use as a liquid absorber (EFSA CEF Panel, 2014). The CEF Panel concluded that the use of the substance does not raise a safety concern when used in absorbent pads in the packaging of fresh or frozen meat, poultry, and seafood as well as fresh fruits and vegetables. The absorbent pads must be used only under conditions in which the liquid absorption capacity is not exceeded and direct contact between the substance and the food is excluded.

3. Data available in the dossier used for this evaluation

The studies submitted for evaluation followed the EFSA guidelines on submission of a dossier for safety evaluation by the EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food (EFSA, 2009).

Non-toxicity data:

- Data on identity
- Data on physical and chemical properties
- Data on manufacturing process
- Data on function, intended use and authorisation
- Data on overall and specific migrations

Toxicity data:

- Gene mutations in bacteria on global migrants
- *In vitro* micronucleus test on global migrants

4. Evaluation

4.1. Non-toxicological data

The active powder used as an oxygen absorber, comprises iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride. The weight of powder mixture used and the design of each label depend on the final application and the needed capacity of oxygen absorption, the highest use of the active powder is up 12.4 g/kg packaged food. The active ingredient responsible for the oxygen absorber function is iron which reacts with oxygen, removing the oxygen from the primary packaging. The other chemicals are used to provide adequate media to facilitate the reaction.

Overall and specific migration were measured by total immersion of labels, with the highest weight of active formulation per unit of the sachet surface, up to 5.2 g/dm². Overall migration was determined in

[8] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council (Text with EEA relevance).OJ L 83, 22.3.2012, pp. 1-280

3 % acetic acid, distilled water and 95 % ethanol (each for 10 days, at 40 °C) and into isooctane (2 days at 20 °C) whereas specific migrations were performed only in 3 % acetic acid and distilled water under same contact conditions.

Due to the design of labels (perforated material) and the nature of the active principle, labels must not be placed in contact with a liquid fraction. Consequently, experiments by total immersion of labels are not appropriate. However the results submitted by the applicant have been summarised here for information.

The overall migration can be up to 3100 mg/kg in 3 % acetic acid, 478 mg/kg in water, 595 mg/kg in 95 % ethanol and 207 mg/kg in isooctane.

The specific migration of iron in 3 % acetic acid was up to 75 mg/kg, whereas there was no detectable migration (below 0.033 mg/kg) into water.

The specific migration of silicon, sodium, calcium in water was experimentally determined. Corresponding calculated migration of kaolin, sodium chloride and calcium chloride was respectively up to 0.4 mg/kg, 182 mg/kg and 2.1 mg/kg.

Under the intended conditions of use where there is no contact with liquid food or foods that have an external aqueous phase on the surface, these constituents will not migrate because they are not volatile.

Considering the nature of these ingredients and their mode of action, the formation and release of volatile byproducts is not expected.

4.2. Toxicological data

This oxygen absorber formulation is not intended for direct contact with liquid foods or foods that have an external aqueous phase on the surface or exudates. Therefore no migration of the constituents is expected. All ingredients of the oxygen absorber formulation have been evaluated and approved for use in food contact materials, and are expected to be stable in normal storage and handling conditions. Thus no toxicity studies on the formulation are required.

Nevertheless, two limited *in vitro* genotoxicity studies, namely a gene mutation test in bacteria and an *in vitro* micronucleus tests were performed on global migrants obtained under harsh conditions (10 days at 40 °C, by immersion in water), not representative of real conditions of use. In both tests no evidence of genotoxicity was observed.

The Panel concluded that under the intended conditions of use, the oxygen absorber formulation is toxicologically acceptable.

CONCLUSIONS

The CEF Panel concluded that the use of substances iron powder, activated carbon, calcined kaolin, sodium chloride, polyacrylic acid, sodium salt, crosslinked and calcium chloride, does not raise a safety concern when used in oxygen absorbers in labels, which prevent the physical release of their content into the food. When placed in the headspace of the packaging or when used in direct contact with foods, the labels should not intentionally or unintentionally come into direct contact with liquid foods or foods that have an external aqueous phase on the surface such as sliced fruits.

Activated carbon should in addition comply with the same purity requirements as for Vegetable Carbon (E 153) set out by Commission Regulation (EU) No 231/2012 with exception of ash content which can be up to 10 % (w/w).

Iron is a natural constituent of foods. Iron compounds are also used as food additives, nutrient sources and for other purposes. The Commission may wish to take note of this if setting a restriction for iron.

DOCUMENTATION PROVIDED TO EFSA

1. ATCO OS/DE. March 2011. Submitted by Atmosphère Contrôle SAS (ATCO), France.

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EFSA (European Food Safety Authority), 2004b. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the Commission related to a 5th list of substances for food contact materials. The EFSA Journal 2004, 109, 1-26.

EFSA (European Food Safety Authority), 2009. Guidelines on submission of a dossier for safety evaluation by the EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food. The EFSA Journal (2009) 1208, 10-1.

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012. Scientific Opinion on the safety evaluation of the active substances, activated carbon, water, iron powder, kaolin calcined, sulphur and sodium chloride for use as active component in food contact materials. EFSA Journal 2012;10(3):2643, 12 pp. doi:10.2903/j.efsa.2012.2643

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on safety assessment of the active substance, polyacrylic acid, sodium salt, crosslinked, for use in active food contact materials. EFSA Journal 2014;12(5):3648, 9 pp. doi:10.2903/j.efsa.2014.3648.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1983. Evaluation of certain additives and contaminants. 27th report. WHO Techn. Report Series, No 696;

http://whqlibdoc.who.int/trs/WHO_TRS_696.pdf

SCF (Scientific Committee of Food), 1990. First series of food additives of various technological functions, Report 25th Series

http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_25.pdf

ABBREVIATIONS

CAS Chemical Abstracts Service

CEF Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids

EC European Commission

FAO Food and Agriculture Organization of the United Nations

FCM Food Contact Materials

EFSA European Food Safety Authority

EU European Union

HDPE High Density Polyethylene

JECFA The Joint FAO/WHO Expert Committee on Food Additiv

NDA Panel on Dietetic Products, Nutrition and Allergies

PE Polyethylene

PET Polyethylene terephthalate

PMTDI Provisional Maximum Tolerable Daily Intake

SCF Scientific Committee on Food

WHO World Health Organization

SAFETY DATA SHEET

Creation Date 03-May-2011 **Revision Date** 11-Apr-2018 **Revision Number** 5

1. Identification

Product Name **Carbon, Activated**

Cat No. : **C272-212; C272-500**

CAS-No 7440-44-0
Synonyms Black Pearls; Charcoal Black; Graphite Nautural

Recommended Use Laboratory chemicals.
Uses advised against Food, drug, pesticide or biocidal product use

Details of the supplier of the safety data sheet

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. Hazard(s) identification

Classification
This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Specific target organ toxicity - (repeated exposure) Target Organs - Lungs.	Category 2

Label Elements

Signal Word
Warning

Hazard Statements
May cause damage to organs through prolonged or repeated exposure

Precautionary Statements
Prevention
Do not breathe dust/fume/gas/mist/vapors/spray

Wear protective gloves/protective clothing/eye protection/face protection
Response
Get medical attention/advice if you feel unwell
Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)
None identified

3. Composition/Information on Ingredients

Component	CAS-No	Weight %
Activated charcoal	7440-44-0	100

4. First-aid measures

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Move to fresh air. If not breathing, give artificial respiration. If symptoms persist, call a physician.

Ingestion Drink plenty of water. Do not induce vomiting. Get medical attention if symptoms occur.

Most important symptoms and effects No information available.

Notes to Physician Treat symptomatically

5. Fire-fighting measures

Unsuitable Extinguishing Media No information available

Flash Point No information available
Method - No information available

Autoignition Temperature 452 °C
Explosion Limits
Upper No data available
Lower No data available
Sensitivity to Mechanical Impact No information available
Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
Keep product and empty container away from heat and sources of ignition.

Hazardous Combustion Products
Carbon oxides
Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

NFPA

Health	Flammability	Instability	Physical hazards
1	1	0	N/A

6. Accidental release measures

Personal Precautions Ensure adequate ventilation. Avoid dust formation. Avoid contact with the skin and the eyes. Use personal protective equipment.

Environmental Precautions Should not be released into the environment.

Methods for Containment and Clean Up Sweep up and shovel into suitable containers for disposal. Avoid dust formation. Provide adequate ventilation.

7. Handling and storage

Handling Avoid contact with skin and eyes. Avoid dust formation. Ensure adequate ventilation. Avoid breathing dust/fume/gas/mist/vapors/spray.

Storage Keep containers tightly closed in a cool, well-ventilated place.

8. Exposure controls / personal protection

Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH	Mexico OEL (TWA)
Activated charcoal				TWA: 2 mg/m³

Engineering Measures None under normal use conditions.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection No protective equipment is needed under normal use conditions.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Solid
Appearance	Black
Odor	Odorless
Odor Threshold	No information available
pH	No information available
Melting Point/Range	3652 °C
Boiling Point/Range	No information available
Flash Point	No information available
Evaporation Rate	Not applicable
Flammability (solid,gas)	No information available
Flammability or explosive limits	
Upper	No data available
Lower	No data available
Vapor Pressure	1 mmHg @ 3586 °C
Vapor Density	Not applicable
Specific Gravity	1.8 - 2.1
Solubility	Insoluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	452 °C
Decomposition Temperature	No information available
Viscosity	Not applicable

Molecular Formula	C
Molecular Weight	12

10. Stability and reactivity

Reactive Hazard None known, based on information available

Stability Stable under normal conditions.

Conditions to Avoid Avoid dust formation. Protect from moisture. Excess heat.

Incompatible Materials Incompatible Materials, Strong oxidizing agents

Hazardous Decomposition Products Carbon oxides

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Activated charcoal	LD50 > 10000 mg/kg (Rat)	Not listed	Not listed

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation May cause skin and eye irritation

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS-No	IARC	NTP	ACGIH	OSHA	Mexico
Activated charcoal	7440-44-0	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects No information available

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure None known

STOT - repeated exposure Lungs

Aspiration hazard No information available

Symptoms / effects,both acute and delayed No information available

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity

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Persistence and Degradability No information available

Bioaccumulation/ Accumulation No information available.

Mobility No information available.

13. Disposal considerations

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT Not regulated
TDG Not regulated
IATA Not regulated
IMDG/IMO Not regulated

15. Regulatory information

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	IECSC	KECL
Activated charcoal	X	X	-	231-153-3	-		X	-	X	X	X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313 Not applicable

SARA 311/312 Hazard Categories See section 2 for more information

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA Occupational Safety and Health Administration
Not applicable

CERCLA Not applicable

California Proposition 65 This product does not contain any Proposition 65 chemicals

U.S. State Right-to-Know Regulations

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Activated charcoal	-	-	-	-	X

U.S. Department of Transportation

Reportable Quantity (RQ): N
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

16. Other information

Prepared By
Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

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Revision Summary This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Disclaimer
The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text

End of SDS