Disciplinarum Scientia. Série: Naturais e Tecnológicas, Santa Maria, v. 20, n. 3, p. 267-275, 2019.
Recebido em: 09.09.2019. Aprovado em: 30.10.2019.

ISSN 2176-462X

TOXICITY OF CARBON NANOTUBES ASSOCIATED WITH RESPIRATORY SYSTEM PROBLEMS: A LITERATURE REVIEW[1]

A TOXICIDADE DE NANOTUBOS DE CARBONO ASSOCIADA A PROBLEMAS PULMONARES: UMA REVISÃO BIBLIOGRÁFICA.

Katianne Wolf Krawczak[2], Jiames Nunes Barcellos[2], Ivana Zanella da Silva[3], Tiago Moreno Volkmer[3], Michele Rorato Sagrillo[3] e Aline Ourique[3]

ABSTRACT

Carbon nanotube is a type of nanomaterial that has gained prominence in nanotechnology due to its high electrical properties, high thermal conductivity, strength and stiffness and their wide applications in the fields of medicine, physics, chemistry and engineering. This type of nanomaterial is formed by carbon atoms and can be classified as single-walled nanotube or multi-walled nanotube, differentiating their applications. One of the major challenges presented by this type of nanomaterial is its relationship to the toxicity of exposed biological systems. Thus, the objective of this work is to perform a review regarding the studies performed on the toxicity of carbon nanotubes in the pulmonary system from 2010 to 2017. Studies have shown that there is a relationship between respiratory problems such as inflammatory airway process, cystic fibrosis, lung cancer and carbon nanotube toxicity. Even so, it is still suggested that more specific studies are needed to really prove this relationship between carbon nanotube toxicity and respiratory problems, considering that this toxicity is associated with the purity of these carbon nanotubes.

Keywords: Metallic Catalyst, Cystic Fibrosis, Inflammation, Metallic and Carbon Impurities, Nanotechnology.

RESUMO

Os nanotubos de carbono são um tipo de nanomaterial que vem ganhando destaque na nanotecnologia devido às suas altas propriedades elétricas, alta condutividade térmica, resistência e rigidez e suas amplas aplicações nas áreas de medicina, física, química e engenharia. Esse tipo de nanomaterial é formado por átomos de carbono e pode ser classificado como nanotubo de parede única ou nanotubo com paredes múltiplas, diferenciando suas aplicações. Um dos grandes desafios apresentados por esse tipo de nanomaterial é a sua relação com a toxicidade dos sistemas biológicos expostos. Sendo assim, o objetivo desse trabalho é realizar uma revisão entre os anos de 2010 - 2017 referente aos estudos realizados sobre a toxicidade dos nanotubos de carbono no sistema pulmonares. Estudos demonstraram que existe uma relação entre problemas respiratórios, como processo inflamatório das vias aéreas, fibrose cística, câncer de pulmão e a toxicidade causada por nanotubos de carbono. Mesmo assim, ainda é sugerido que sejam necessários estudos mais específicos para comprovar realmente essa relação da toxicidade dos nanotubos de carbono e problemas respiratórios, levando em consideração que essa toxicidade está associada ao grau de pureza desses nanotubos de carbono.

Palavras-chave: *Catalisador metálico, Fibrose Cística, Impurezas metálicas e Carbonáceas, Inflamação, Nanotecnologia.*

---

[1] Review article.

[2] Students at the Nanoscience Program - Universidade Franciscana. E-mail: katianne_94@hotmail.com; jiames_jnb@hotmail.com

[3] Advisors - Universidade Franciscana. E-mail: ivanazanella@gmail.com; tiagovolkmer@gmail.com; sagrillorm18@gmail.com; alineourique@gmail.com

INTRODUCTION

Nanotechnology is based on understanding and controlling matter with the ability to change material properties in sizes between 1 and 100 nanometers and thus create materials with different properties. This technology has been growing in the market and is being used in different areas, among them medicine, physics, chemistry, engineering (LOVE *et al.,* 2012). Different materials are used in the nanometer scale, among them are carbon nanotubes (NTCs), which are carbon atoms arranged in condensed aromatic rings, formed by cylinders wrapped in cylinders (IIJIMA, 1991). "This last mentioned nanomaterial has been generating curiosity from the industries and some materials produced from carbon nanotubes are already on the market, among them biosensors (KRAUSS, 2009), biomolecular recognition devices and molecular carriers (KAM; JESSOP; WENDER , 2004), artificial water channels (LIU, 2009), cancer therapy and molecular photoacoustic imaging (LIU, 2006; WANG, 2011).

And by modifying the surface area of this nanomaterial, specific functions can be introduced, thus increasing the complexity and diversity of these nanomaterials. This type of material can be presented in two ways: a single wall (SWCNTs) or multiple walls (MWCNTs) (DONG & MA, 2015).

The interesting condition of this nanomaterial, despite its different applications and properties, is about its process of synthesis. Until then, it is necessary to use catalysts, and among the most used are Co, Fe, Ni and Mo and these catalysts become accumulated and become residual metal contaminants in these nanomaterials. Some carbon nanotubes are functionalized or coated with proteins, polymers or metals with the aim of improving their dispersion in solvents or obtaining more specific functions (ASCHBERGER *et al*., 2010).

The initial concern is about an exposure to this type of material that may be a potential health and environmental hazard (KIM *et al.,* 2011; DONG & MA, 2015). The justification would be precisely that the metals used in the synthesis as catalysts are aggregated and it is almost inevitable that these nanomaterials are not contaminated with residues of these catalysts, that is, when this type of material decomposes, nanometric particles of metal are formed. Therefore, the total removal of these metal catalysts and other types of residues from the carbon nanotubes that imply the application of this type of material, become a great challenge for the area of nanotechnology (GE *et al.,* 2011).

With the prospect of eliminating these types of residue, some purification methods were developed, including chemical, physical and chromatographic purification. But some purification processes may alter the properties of these nanomaterials. Therefore, metal catalyst residues often remain, even after complicated purification steps (GE *et al.,* 2011).

Carbon nanotubes can exist as compact tangles of nanotubes that can be considered as particles, or as breathable fibers with a high aspect ratio (length to width). The risk of these two forms of carbon nanotubes is different, for example; effects of nanotube particles would be confined to the lungs, possibly causing fibrosis and cancer, while the effects of nanotube fibers would affect the pleura (DONG & MA, 2015). The characteristics of the dimensions of carbon nanotubes, such as diameter and length, are directly related to the action on the target site of action of the nanotubes in the airways and in the pulmonary parenchyma. It is understood that the clearance of carbon nanotubes in the airways occurs by hair cells or by slow phagocytosis of macrophages. Consequently, accumulation occurs in the lungs, as the particles or fibers become deposited (LANONE *et al.,* 2013).

Some studies already carried out with a variety of different carbon nanotube samples, being single-wall or multiple-wall in research laboratories have demonstrated that they can provoke inflammatory responses (BHATTACHARYA *et al.,* 2013).

The implication of the behavior of carbon nanotubes when they meet biological systems creates the need for a careful toxicological evaluation for a better understanding of their biological impact, thus allowing greater exploitation in applications of different areas. This concern with the evaluation of the toxicity caused by carbon nanotubes opens a space for the introduction of nanotoxicity that evaluates the effects of these systems when they meet biological systems (DONG & MA, 2015).

In 2007, the Organization for Economic Co-operation and Development (OECD) created a program called: Sponsorship Program for the Testing of Manufactured Nanomaterials. This program was created with the aim of gaining insight into the safety of nanomaterials from the assessment of their effects on human health and the environment. A list of biopersistent nanomaterials, whose priority risk assessment among the top five nanomaterials, has been established by the OECD, they are: C60 fullerene, single-wall carbon nanotubes, multiple-wall carbon nanotubes, silver nanoparticles and iron nanoparticles (OECD, 2011).

The OECD reviews continuously the methodologies used and emphasizes that it is necessary to adapt these methodologies according to the characteristics of these manufactured nanomaterials (OECD, 2011).

Thus, the objective of this literature review is the association of the nanotoxicity presented by carbon nanotubes in biological systems against pulmonary diseases, among them pneumonias, emphysema, bronchitis, cystic fibrosis caused in people exposed to this type of nanomaterial. It is important to emphasize that this orientation was not developed to address specific characteristics of the nanomaterials and the respiratory system, but rather to review the association of carbon nanotubes with breathing problems already mentioned.

MATERIALS AND METHODS

This study constitutes a bibliographical review, developed with scientific production indexed in the following electronic databases: Science Direct, Pub Med, Scielo, Web of Science. The time cut covered the period between 2010 and 2017 and the main focus of this study were scientific papers focused on the toxicity of carbon nanotubes in the lungs. The descriptors used for the bibliographic review were: carbon nanotubes, toxicity and lung diseases. The summary and results of these articles were used for the development of this review.

After the survey, a final number of 22 articles was obtained, and 11 articles were studied and tabulated as to the tests performed to verify the toxicity of the carbon nanotubes in the lungs and the rest of the articles were used to understand the terms discussed in this article and construction of a review also addressed.

RESULTS AND DISCUSSIONS

In the search carried out in all mentioned databases, we found 22 articles related to the descriptors carbon nanotubes, toxicity and lung diseases, and only 11 articles performed some type of experiment, where they verified the existence or not of toxicity of the carbon nanotubes in the lungs. The period of publication was between 2010 and 2017, and the year 2017 concentrated the largest number, respectively five articles. The other years presented 1 or 2 articles. After reading and registration the selected articles, the 11 articles that compose an experiment to verify the toxicity of the carbon nanotubes in the lungs were evaluated, in terms of the type of carbon nanotubes (single wall or multiple walls), degree of purity and problematic presented in the lungs.

In most of the articles studied here, some kind of damage to the lungs was reported when contact on carbon nanotubes, among them, AISO *et al.* (2010); KASAI *et al.* (2016); Duke *et al.* (2017) & NIKOTA *et al.* (2017) reported fibrosis as the main clinical finding, these authors only studied carbon nanotubes with multiple walls. On the other hand, KATWA *et al.* (2012); GERNAND and ELIZABETH (2014); LUYTS *et al.* (2014) & MORIMOTO *et al.* (2017) have demonstrated that carbon nanotubes with single or multiple walls are capable of causing cellular inflammation when they come into contact with lung cell types, mainly provoking a neutrophil infiltration in this organ.

Among the other clinical findings also cited by some authors, are acute toxicity, lung tumors, absorption in lung epithelial cells and epithelial hyperplasia.

Another aspect that was evaluated is whether these authors took into account the degree of purity of these carbon nanotubes when they performed the toxicity tests, since it is already described in the literature that this toxicity can be given due to the presence of metallic residues that remain in this nanostructure, even after performing different types of purifications. One justification would be that very strict purification processes could alter the structure of this type of nanomaterial, by this way changing its properties. About 5 articles did not report the degree of purity presented by the studied carbon nanotubes, representing a number a little below half of the articles studied. This shows that the clinical findings found in these 5 studies may be associated with metallic residues that remained in the studied nanostructure and not specifically the type of nanostructure. The purity values cited by the authors were not always necessarily obtained by them during their studies, some used the degree of purity reported by the manufacturers of the carbon nanotubes and the values reported by all were between a range of 90% and 99,8% purity.

Only a single study that was carried out by HORIBATA *et al.* in 2017 did not show negative results, they did not verify the degree of purity of the carbon nanotubes and stated that only an intra-tracheal instillation of carbon nanotubes with multiple walls were not mutagenic.

Pulskamp *et al.* found in 2007 that commercially available carbon nanotubes (CNTs) do not cause acute toxicity but induce the production of reactive oxygen species (ROS) in human A549 lung cells and rat macrophages. However, the authors attributed high levels of ROS to metal residues found in carbon nanotubes.

Tsukahara & Haniu, in 2011, used highly purified multi-walled carbon nanotubes and demonstrated that even without the generation of reactive oxygen species (ROS) that are attributed to metal impurities, there was membrane damage, increased cytokine release, and induction to cell necrosis.

Love *et al.* (2012) have stated that carbon nanotubes are toxic and can affect different cell types, but it is necessary to understand that factors such as metal impurities, particulate state, structural differences and the surface properties of CNTs are linked to the cytotoxicity of these nanomaterials. And that to advance in this study requires a more detailed characterization of the carbon nanotubes before the toxicity tests and carry out a standardization of the tests used.

Ge *et al.,* (2012) suggests that it is essential to systematically understand how endogenous and exogenous factors can influence the toxicity of carbon nanotubes. They further claim that the presence of metallic and carbonaceous impurities and the mechanism of toxicity are the crux of carbon nanotubes, but unfortunately, they are still not entirely clear.

Thus, we can understand that nanotechnology has played an important role in the technological area and directly affects the development of science, but due to this growth in a short time, some precautions regarding the risks caused by some nanomaterials go unnoticed and there is still no conclusive results if these nanomaterials may or may not pose risks to health and the environment in the future.

The following table describes the main studies on the toxicity of carbon nanotubes against lung diseases between 2010 and 2017:

Table 1 - Description and results of studies carried out in the years 2010 - 2017 on nanotoxicity of single or multiple wall carbon nanotubes.

Autor	Journal	CNT	Purity	Results
AISO *et al.*, 2010	Industrial Health	MWCNT	Uninformed	Inflammation, hyperplasia of type II cells, microgranulomas and fibrosis.
KATWA *et al.*, 2012	J. Small	MWCNTs	Uninformed	Inflammatory pulmonary responses.
LUYTS *et al.*, 2014	Particle and Fibre Toxicology	MWCNTs	Uninformed	Pronounced inflammatory response in the lung.
DUKE *et al.*, 2017	Particle and Fibre Toxicology	MWCNT	Uninformed	Fibrosis.

HORIBATA *et al.*, 2017	Genes and Environment	MWCNTs	Uninformed	The findings indicated that a single intratracheal instillation of MWCNTs was not mutagenic for both bone marrow and rat lung.
ASCHBERGER *et al.*, 2010	Critical Reviews in Toxicology	MWCNT	90% purity	The results indicated that the major risks to humans result from chronic occupational inhalation, especially during activities involving high CNT release and uncontrolled exposure.
ALI-BOUCETA *et al.*, 2011	Jornal small	MWNTs	94% purity	Absorption in lung epithelial cells
GERMAND and ELIZABETH, 2014	Risk Analysis	CNT	95% purity	Pulmonary inflammation
KASAI *et al.*, 2016	Particle and Fibre Toxicology	MWCNT	Around 99,6 and 99,8 % purity	Epithelial hyperplasia, granulomatous alteration, fibrosis, bronchioloalveolar carcinoma and adenomas.
MORIMOTO *et al.*, 2017	Advance Industrial Science and Technology	MWCNT	99,6% purity	Acute pulmonary toxicity and pulmonary neutrophil infiltration.
NIKOTA *et al.* 2017	Particle and Fibre Toxicology	MWCNT	93% purity	Fibrotic disease and inflammatory response.

CONCLUSION

It is noticed that only seven of the 11 studies addressed in this review presented the purity value of the carbon nanotubes used in their studies, and only two studies performed characterization tests for analysis of metals present in nanomaterials. For future perspectives, a purification method is required to ensure the exclusion of any kind of metallic waste while at the same time not altering any characteristics of the nanomaterial while maintaining its properties intact. Finally, this study has shown that before performing any type of test to check the toxicity of carbon nanotubes in the lungs or other organs that may be affected, it is necessary to perform characterization and identification tests on the impurities of this nanomaterial. Only after that, there can be safely associated that the toxicity presented in in vitro and in vivo tests is really from carbon nanotubes and not from carbon nanotubes metal waste.

REFERENCES

AISO, S. YAMAZAKI1, K. UMEDA, Y. I ASAKURA, M. KASAI, T. TAKAYA, M. TOYA, T. KODA, S. NAGANO, K. ARITO, H. FUKUSHIMA, S. Pulmonary Toxicity of Intratracheally Instilled Multiwall Carbon Nanotubes in Male Fischer 344 Rats. **Industrial Health**, n. 48, p. 783-794, 2010.

ALI-BOUCETTA, H. AL-JAMAL, K. T. MÜLLER, K. H. LI, S. PORTER, A. E. EDDAOUDI, A. MAURIZIO, P. PRATO, BIANCO, A. KOSTARELOS, K. Cellular Uptake and Cytotoxic Impact of Chemically Functionalized and Polymer-Coated Carbon Nanotube. **Jornal small**, n. 22, p. 3230-3238, 2011.

ASCHBERGER, K. JOHNSTON, H, J. STONE, V. AITKEN, R. J. HANKIN, S. M. Peters, S. A. K. TRAN, C. L. CHRISTENSEN, F. M. Review of carbon nanotubes toxicity and exposure-Appraisal of human health risk assessment based on open literature. **Critical Reviews in Toxicology**, v. 40, n. 9, p. 759-790, 2010.

BHATTACHARYA, K. ANDÓN, F. T. EL-SAYED, R. FADEEL, B. Mechanisms of carbon nanotube-induced toxicity: Focus on pulmonary inflammation. **Advanced Drug Delivery Reviews**, v. 65, n. 15, p. 2087-2097, 2013.

DONG, J. & MA, Q. Advances in mechanisms and signaling pathways of carbon nanotube toxicity. **Nanotoxicology**, v. 9, n. 5, p. 658-676, 2015.

DUKE, K. S. TAYLOR-JUST, A. J. IHRIE, M. D. SHIPKOWSKI1, K. D. THOMPSON, A. E. DANDLEY E. C. PARSONS, G. N. BONNE, J. C. STAT1- dependent and - independent pulmonary allergic and fibrogenic responses in mice after exposure to tangled versus rod-like multi-walled carbon nanotubes. **Particle and Fibre Toxicology**, v. 14, n. 26, 2017.

GE, C. LI, Y. YIN, J. LIU, Y. WANG, L. ZHAO, Y. CHEN, C. The contributions of metal impurities and tube structure to the toxicity of carbon nanotube materials. **NPG Asia Materials**, v.4, p.32, 2012.
GERNAND, J. M. CASMAN, E. A. A Meta-Analysis of Carbon Nanotube Pulmonary Toxicity Studies--How Physical Dimensions and Impurities Affect the Toxicity of Carbon Nanotubes. **Risk Analysis**, v. 34, n. 3, 2014.

HORIBATA, K. UKAI, A. OGATA, A. NAKAE, D. ANDO, H. KUBO, Y. NAGASAWA, A. YUZAWA, K. HONMA, M. Absence of *in vivo* mutagenicity of multiwalled carbon nanotubes in single intratracheal instillation study using F344 gpt delta rats. **Genes and Environment**, v. 39, n. 4, 2017.

IIJIMA, S. Helical microtubules of graphitic carbon. **Nature**, v. 354, p. 56-58, 1991.

KAM, N. W. S., JESSOP, T. C., WENDER, P. A. & Dai, H. Nanotube molecular transporters: internalization of carbon nanotube-protein conjugates into mammalian cells. **J. Am. Chem. Soc.** v. 126, p. 6850-6851 (2004).

KASAI, T. UMEDA, Y. OHNISHI, M. MINE, T. KONDO, H. TAKEUCHI, T. MATSUMOTO, M. FUKUSHIMA, S. Lung carcinogenicity of inhaled multiwalled carbon nanotube in rats. **Particle and Fibre Toxicology**, v. 13, n. 53, 2016.

KATWA, P. WANG, X. URANKAR, R. N. PODILA, R. HILDERBRAND, S. C. FICK, R. B. RAO, A. M. KE, P. C. WINGARD, C. G. M. BROWN, J. M. A Carbon Nanotube Toxicity Paradigm Driven by Mast Cells and the IL-33/ST2 Axis. **J. Small**, v. 24, n. 8, p. 2904-2912, 2012.

KIM, W. KIM, H. J. LEE, S. H. CHANG, M. B. MAENG, W. J. Effect of extruded canola seed supplementation on blood metabolites and lactational performance in lactating dairy cows. **J. Anim. Sci. Technol**., v. 43, n. 6, p. 859-872, 2011.

KRAUSS, T. D. Biosensors: nanotubes light up cells. **Nat. Nanotechnol**. v. 4, n. 85-86, 2009.

LANONE, S. ANDUJAR, P. KERMANIZADEH, A. BOCZKOWSK, J. Determinants of carbon nanotube toxicity. **Advanced Drug Delivery Reviews**, v. 65, n. 15, p. 2063-2069, 2013.

LIU, B., LI, X., LI, B., XU, B. & ZHAO, Y. Carbon nanotube based artificial water channel protein: membrane perturbation and water transportation. Nano Lett. v. 9, p. 1386-1394, 2009.

LIU, Z., CAI, W., HE, L., NAKAYAMA, N., CHEN, K., SUN, X., CHEN, X. & DAI, H. In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. **Nat. Nanotechnol**, v. 2, p. 47-52, 2006.

LOVE, S. A. MAURER-JONES, M. A. THOMPSON, J. W. LIN, Y. HAYNES, C. L. Assessing Nanoparticle Toxicity. **Annual Review of Analytical Chemistry**. v.5, p.181-205, 2012.

LUYTS, K. SMULDERS, S. NAPIERSKA, D. KERCKHOVEN, S. V. POELS, K. SCHEERS, H. HEMMERYCKX, B. NEMERY, B. HOYLAERTS, M. F. HOET, P. H. M. Pulmonary and hemostatic toxicity of multi-walled carbon nanotubes and zinc oxide nanoparticles after pulmonary exposure in Bmal1 knockout mice. **Particle and Fibre Toxicology**, v. 11, n. 61, 2014.

MORIMOTO, Y. IZUMI, H. YOSHIURA, Y. FUJISAWA, Y. YATERA, K. FUJITA, K. MARU, J. ENDOH S. HOND, K. Basic study of intratracheal instillation study of nanomaterials for the estimation of the hazards of nanomaterials. **Industrial health**, v. 56, p. 30-39, 2018.

NIKOTA, J. BANVILLE, A. GOODWIN, L. R. WU, D. WILLIAMS, A. YAUK, C. L. WALLIN, H. VOGEL, U. HALAPPANAVAR, S. Stat-6 signaling pathway and not Interleukin-1 mediates multi--walled carbonnanotube-induced lung fibrosis in mice: insights from an adverse outcome pathway framework. **Particle and Fibre Toxicology**, v. 14, n. 37, 2017.

Organização para a Cooperação e Desenvolvimento Econômico - OECD, 2011. Disponível em: http://www.oecd.org. Acesso em 04 de Agosto de 2018.

PULSKAMP, K. DIABATE, S. KRUG, H. F. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. **Toxicol. Lett**. v. 168, p. 58-74, 2007.

TSUKAHARA, T. HANIU, H. Cellular cytotoxic response induced by highly purified multi-wall carbon nanotube in human lung cells. **Mol. Cell. Biochem**. v. 352, p. 57-63, 2011.

WANG, L., LIU, Y., LI, W., JIANG, X., JI, Y., WU, X., XU, L., QIU, Y., ZHAO, K. & WEI, T. Selective targeting of gold nanorods at the mitochondria of cancer cells: implications for cancer therapy. **Nano Lett**., v. 11, p. 772-780, 2011.