



Evaluación de genotoxicidad de nanopartículas de carbón usando células V79

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Julian David Rodríguez Tapia
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Tutor(es):
PhD. Grethel León Mejía

Cotutor
MsC. Alvaro Miranda Guevara

RESUMEN

La exposición crónica al material particulado derivado de la actividad minera se asocia con graves consecuencias clínicas. Los estudios han vinculado esta exposición con neumoconiosis, bronquitis crónica, enfisema, fibrosis y cáncer pulmonar en mineros de carbón, además de alteraciones en parámetros biológicos en las poblaciones aledañas. Sin embargo, establecer una causalidad directa es complejo debido a múltiples variables características de los modelos de exposición crónica. En orden de explorar los efectos de la exposición a nanopartículas de carbón proveniente de una de las minas cercanas a La Loma-Cesar, el presente trabajo buscó caracterizar el contenido de hidrocarburos aromáticos policíclicos (HAP) de las nanopartículas (NP) usando Cromatografía de Gases acoplada a Espectrometría de Massas (GC/MS) y se evaluaron los efectos de la exposición *in vitro* a diferentes concentraciones de NP en células de fibroblastos de pulmón de hámster chino (V79) usando el ensayo cometa alcalino y modificado con enzimas EndoIII y FPG. Dentro de los resultados obtenidos se destaca la presencia de HAPs en las NP tales como fluoranteno, naftaleno, antraceno, 7H-benzo[c]fluoreno, fenantreno, pireno, benceno[a]antraceno, criseno y algunos derivados alquilados. En el análisis de genotoxicidad de las NP sobre células V79 se encontró un efecto dosis-respuesta ante las concentraciones usadas. Estos datos respaldan la

hipótesis de que las nanopartículas de carbón derivadas de la actividad minera tienen un impacto en la integridad genética y pueden llevar a muerte celular programada. Estos resultados pueden direccionar futuras investigaciones y estudios adicionales para profundizar en la comprensión de los efectos, los cuales sean punto de apoyo para explorar estrategias de mitigación o de reducción de los riesgos asociados con la exposición a nanopartículas de carbón.

Palabras clave: Nanopartículas de carbón, genotoxicidad, estrés oxidativo, hidrocarburos aromáticos policíclicos, células V79.

ABSTRACT

Chronic exposure to particulate matter derived from mining activity is associated with serious clinical consequences. Studies have linked this exposure to pneumoconiosis, chronic bronchitis, emphysema, fibrosis, and lung cancer in coal miners, in addition to alterations in biological parameters in surrounding populations. However, establishing direct causality is complex due to multiple variables characteristic of chronic exposure models. In order to explore the effects of exposure to carbon nanoparticles from one of the mines near La Loma-Cesar, the present work sought to characterize the polycyclic aromatic hydrocarbon (PAH) content of the nanoparticles (NP) using Gas Chromatography coupled to Mass Spectrometry (GC/MS) and the effects of in vitro exposure to different concentrations of NP in Chinese hamster lung fibroblast cells (V79) were evaluated using the alkaline comet assay and modified with EndoH and FPG enzymes. Among the results obtained, the presence of PAHs in the NPs stands out, such as fluoranthene, naphthalene, anthracene, 7H-benzo[c]fluorene, phenanthrene, pyrene, benzene[a]anthracene, chrysene and some alkylated derivatives. In the genotoxicity analysis of the NPs on V79 cells, a dose-response effect was found with the concentrations used. These data support the hypothesis that carbon nanoparticles derived from mining activity have an impact on genetic integrity and can lead to programmed cell death. These results can direct future research and additional studies to deepen the understanding of the effects, which can serve as a basis for exploring mitigation or reduction strategies for the risks associated with exposure to carbon nanoparticles.

KeyWords: Carbon nanoparticles, genetic damage, oxidative stress, polycyclic aromatic hydrocarbons, mining activity, V79.

REFERENCIAS

1. Balat M. Coal in the Global Energy Scene. *Energy Sources, Part B: Economics, Planning, and Policy.* 2009 Dec;28(5):50–62.
2. Lien L. Advances in coal mining technology. In: *The Coal Handbook: Towards Cleaner Production.* Elsevier; 2013. p. 193–225.
3. Sahu SP, Yadav M, Rani N, Das AJ. Assessment of occupational health exposure to particulate matter around opencast coal mines, India: a case study. *Arabian Journal of Geosciences.* 2018 Jul;12;11(14):373.
4. Arregocés HA, Rojano R, Restrepo G. Meteorological factors contributing to organic and elemental carbon concentrations in PM10 near an open-pit coal mine. *Environmental Science and Pollution Research.* 2022 Apr;6;29(19):28854–65.
5. Yang Y, Ligouis B, Pies C, Grathwohl P, Hofmann T. Occurrence of coal and coal-derived particle-bound polycyclic aromatic hydrocarbons (PAHs) in a river floodplain soil. *Environmental Pollution.* 2008 Jan;151(1):121–9.
6. Sun L, Liao X, Yan X, Zhu G, Ma D. Evaluation of heavy metal and polycyclic aromatic hydrocarbons accumulation in plants from typical industrial sites: potential candidate in phytoremediation for co-contamination. *Environmental Science and Pollution Research.* 2014 Nov 20;21(21):12494–504.
7. Kwon HS, Ryu MH, Carlsten C. Ultrafine particles: unique physicochemical properties relevant to health and disease. *Exp Mol Med.* 2020 Mar 17;52(3):318–28.
8. Environmental Protection Agency. Air Quality Criteria for Particulate Matter (Final Report, 2004). Vol. EPA 600. 2004.
9. Larionov A, Volobaev V, Zverev A, Vdovina E, Bach S, Schetnikova E, et al. Chemical Composition and Toxicity of PM10 and PM0.1 Samples near Open-Pit Mines and Coal Power Stations. *Life.* 2022 Jul 13;12(7):1047.
10. Pietrojasti A. Health implications of engineered nanomaterials. *Nanoscale.* 2012;4(4):1231.
11. Brugge D, Durant JL, Rioux C. Near-highway pollutants in motor vehicle exhaust: A review of epidemiologic evidence of cardiac and pulmonary health risks. *Environmental Health.* 2007 Dec 9;6(1):23.
12. Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, et al. Translocation of Inhaled Ultrafine Particles to the Brain. *Inhal Toxicol.* 2004 Jan;16(6–7):437–45.
13. Terzano C, Di Stefano F, Conti V, Graziani E, Petroianni A. Air pollution ultrafine particles: toxicity beyond the lung. *Eur Rev Med Pharmacol Sci.* 2010 Oct;14(10):809–21.
14. Renwick LC, Donaldson K, Clouter A. Impairment of Alveolar Macrophage Phagocytosis by Ultrafine Particles. *Toxicol Appl Pharmacol.* 2001 Apr;172(2):119–27.
15. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. *Environ Health Perspect.* 2005 Jul;113(7):823–39.

16. Blamey Benavides X, Mosquera E, Díaz F. Estudio exploratorio II: Identificación de nanopartículas en procesos industriales de soldadura y de minería. *Ciencia & trabajo.* 2016;18(55):28–36.
17. Huertas JI, Huertas ME, Solís DA. Characterization of airborne particles in an open pit mining region. *Science of The Total Environment.* 2012 Apr;423:39–46.
18. Ren M, Zheng L, Hu J, Chen X, Zhang Y, Dong X, et al. Characterization of polycyclic aromatic hydrocarbons in soil in a coal mining area, East China: Spatial distribution, sources, and carcinogenic risk assessment. *Front Earth Sci (Lausanne).* 2022 Oct 31;10.
19. Masto RE, Singh MK, Rout TK, Kumar A, Kumar S, George J, et al. Health risks from PAHs and potentially toxic elements in street dust of a coal mining area in India. *Environ Geochem Health.* 2019 Oct 4;41(5):1923–37.
20. Wu D, Wang Z, Chen J, Kong S, Fu X, Deng H, et al. Polycyclic aromatic hydrocarbons (PAHs) in atmospheric PM_{2.5} and PM₁₀ at a coal-based industrial city: Implication for PAH control at industrial agglomeration regions, China. *Atmos Res.* 2014 Nov;149:217–29.
21. Trechera P, Moreno T, Córdoba P, Moreno N, Zhuang X, Li B, et al. Comprehensive evaluation of potential coal mine dust emissions in an open-pit coal mine in Northwest China. *Int J Coal Geol.* 2021 Feb;235:103677.
22. Espitia-Pérez L, da Silva J, Espitia-Pérez P, Brango H, Salcedo-Arteaga S, Hoyos-Giraldo LS, et al. Cytogenetic instability in populations with residential proximity to open-pit coal mine in Northern Colombia in relation to PM₁₀ and PM_{2.5} levels. *Ecotoxicol Environ Saf.* 2018 Feb;148:453–66.
23. Bray C, Battye W, Uttamang P, Pillai P, Aneja V. Characterization of Particulate Matter (PM_{2.5} and PM₁₀) Relating to a Coal Power Plant in the Boroughs of Springdale and Cheswick, PA. *Atmosphere (Basel).* 2017 Sep 23;8(10):186.
24. Timonen H, TK, AM, RF, VY, BM, OP, HR, AE & SS. Sources and composition of particulate matter in boreal arctic environment next to an active mining area. *Boreal Env Res.* 2018;23:105–25.
25. Abdel-Shafy HI, Mansour MSM. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum.* 2016 Mar;25(1):107–23.
26. Achten C, Hofmann T. Native polycyclic aromatic hydrocarbons (PAH) in coals – A hardly recognized source of environmental contamination. *Science of The Total Environment.* 2009 Apr;407(8):2461–73.
27. Liu J, Liu G, Zhang J, Yin H, Wang R. Occurrence and risk assessment of polycyclic aromatic hydrocarbons in soil from the Tiefa coal mine district, Liaoning, China. *Journal of Environmental Monitoring.* 2012;14(10):2634.
28. Topinka J, Rossner P, Milcova A, Schmuczerova J, Svecova V, Sram RJ. DNA adducts and oxidative DNA damage induced by organic extracts from PM_{2.5} in an acellular assay. *Toxicol Lett.* 2011 May;202(3):186–92.
29. Ohno M, Sakumi K, Fukumura R, Furuichi M, Iwasaki Y, Hokama M, et al. 8-oxoguanine causes spontaneous de novo germline mutations in mice. *Sci Rep.* 2014 Apr 15;4(1):4689.

30. Parry EM. Detection and characterization of mechanisms of action of aneugenic chemicals. *Mutagenesis*. 2002 Nov 1;17(6):509–21.
31. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. *IARC Monogr Eval Carcinog Risks Hum*. 2010;92:1–853.
32. Pham AN, Xing G, Miller CJ, Waite TD. Fenton-like copper redox chemistry revisited: Hydrogen peroxide and superoxide mediation of copper-catalyzed oxidant production. *J Catal*. 2013 May;301:54–64.
33. Go LHT, Cohen RA. Coal Workers' Pneumoconiosis and Other Mining-Related Lung Disease. *Clin Chest Med*. 2020 Dec;41(4):687–96.
34. Romero M, Varona M, Ibáñez-Pinilla M, Briceño L. Prevalence of pneumoconiosis and spirometric findings in underground mining workers in Cundinamarca, Colombia. *Revista de la Facultad de Medicina*. 2019 Oct 1;67(4):393–8.
35. Laney AS, Weissman DN. Respiratory Diseases Caused by Coal Mine Dust. *J Occup Environ Med*. 2014 Oct;56(Supplement 10):S18–22.
36. Buttling LG, McKnight MX, Kolivras KN, Ranganathan S, Gohlke JM. Maternal proximity to Central Appalachia surface mining and birth outcomes. *Environmental Epidemiology*. 2021 Feb;5(1):e128.
37. Ruktanonchai CW, McKnight MX, Buttling L, Kolivras K, Krometis LA, Gohlke J. Identifying exposure pathways mediating adverse birth outcomes near active surface mines in Central Appalachia. *Environmental Epidemiology*. 2022 Jun;6(3):e208.
38. Hendryx M, Entwhistle J. Association between residence near surface coal mining and blood inflammation. *Extr Ind Soc*. 2015 Apr;2(2):246–51.
39. León-Mejía G, Quintana M, Debastiani R, Dias J, Espitia-Pérez L, Hartmann A, et al. Genetic damage in coal miners evaluated by buccal micronucleus cytome assay. *Ecotoxicol Environ Saf*. 2014 Sep;107:133–9.
40. León-Mejía G, Espitia-Pérez L, Hoyos-Giraldo LS, Da Silva J, Hartmann A, Henriques JAP, et al. Assessment of DNA damage in coal open-cast mining workers using the cytokinesis-blocked micronucleus test and the comet assay. *Science of The Total Environment*. 2011 Jan;409(4):686–91.
41. Ávila Júnior S, Possamai FP, Budni P, Backes P, Parisotto EB, Rizelio VM, et al. Occupational airborne contamination in south Brazil: 1. Oxidative stress detected in the blood of coal miners. *Ecotoxicology*. 2009 Nov 18;18(8):1150–7.
42. Plinio Enrique Bustamante Ortega; Rafael Eduardo García Molano; Oswald Maya Sánchez; Juan Felipe Rodríguez López; Tatiana Aguilar Londoño. Minería de carbón en colombia transformando el futuro de la industria. Ministerio de Minas y Energía. 2022;
43. Drummond Ltd. <https://www.drummondltd.com/nuestras-operaciones/minas/>. Nuestras operaciones: Minas.
44. Guerrero-Castilla A, Olivero-Verbel J, Marrugo-Negrete J. Heavy metals in wild house mice from coal-mining areas of Colombia and expression of genes related to oxidative stress, DNA damage and exposure to metals. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2014 Mar;762:24–9.



45. León-Mejía G, Rueda RA, Pérez Pérez J, Miranda-Guevara A, Moreno OF, Quintana-Sosa M, et al. Analysis of the cytotoxic and genotoxic effects in a population chronically exposed to coal mining residues. *Environmental Science and Pollution Research*. 2023 Mar;4;30(18):54095–105.
46. León-Mejía G, Vargas JE, Quintana-Sosa M, Rueda RA, Pérez JP, Miranda-Guevara A, et al. Exposure to coal mining can lead to imbalanced levels of inorganic elements and DNA damage in individuals living near open-pit mining sites. *Environ Res*. 2023 Jun;227:115773.
47. Miranda-Guevara A, Muñoz-Acevedo A, Fiorillo-Moreno O, Acosta-Hoyos A, Pacheco-Londoño L, Quintana-Sosa M, et al. The dangerous link between coal dust exposure and DNA damage: unraveling the role of some of the chemical agents and oxidative stress. *Environ Geochem Health*. 2023 Oct 4;45(10):7081–97.
48. Flint OP. *In Vitro Toxicity Testing: Purpose, Validation and Strategy*. Alternatives to Laboratory Animals. 1990 Nov 13;18(1_part_1):11–8.
49. León-Mejía G, Silva LFO, Civeira MS, Oliveira MLS, Machado M, Villela IV, et al. Cytotoxicity and genotoxicity induced by coal and coal fly ash particles samples in V79 cells. *Environmental Science and Pollution Research*. 2016 Dec 16;23(23):24019–31.
50. Matzenbacher CA, Garcia ALH, dos Santos MS, Nicolau CC, Premoli S, Corrêa DS, et al. DNA damage induced by coal dust, fly and bottom ash from coal combustion evaluated using the micronucleus test and comet assay in vitro. *J Hazard Mater*. 2017 Feb;324:781–8.
51. Caria H, Chaveca T, Laires A, Rueff J. Genotoxicity of quercetin in the micronucleus assay in mouse bone marrow erythrocytes, human lymphocytes, V79 cell line and identification of kinetochore-containing (CREST staining) micronuclei in human lymphocytes. *Mutation Research/Genetic Toxicology*. 1995 Jun;343(2–3):85–94.
52. SARIGÖL KILIÇ Z, ÇAL T, ÜNDEĞER BUCURGAT Ü. Evaluation of the Methylation and Acetylation Profiles of Dinitroaniline Herbicides and Resveratrol on the V79 Cell Line. *Turk J Pharm Sci*. 2020 Dec 1;17(6):631–7.
53. Olive PL, Banáth JP. The comet assay: a method to measure DNA damage in individual cells. *Nat Protoc*. 2006 Jun 27;1(1):23–9.
54. Cannan WJ, Pederson DS. Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin. *J Cell Physiol*. 2016 Jan 28;231(1):3–14.
55. Vamvakas S, Vock EH, Lutz WK. On the Role of DNA Double-Strand Breaks in Toxicity and Carcinogenesis. *Crit Rev Toxicol*. 1997 Jan 25;27(2):155–74.
56. Garm C, Moreno-Villanueva M, Bürkle A, Larsen LA, Bohr VA, Christensen K, et al. Genetic and environmental influence on DNA strand break repair: A twin study. *Environ Mol Mutagen*. 2013 Jul 25;54(6):414–20.
57. Maynard S, Schurman SH, Harboe C, de Souza-Pinto NC, Bohr VA. Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis*. 2008 Sep 12;30(1):2–10.
58. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. *The FASEB Journal*. 2003 Jul;17(10):1195–214.

59. Collins AR. The Comet Assay for DNA Damage and Repair: Principles, Applications, and Limitations. *Mol Biotechnol.* 2004;26(3):249–61.
60. Huang B, Liu G, Wang P, Zhao X, Xu H. Effect of Nitric Acid Modification on Characteristics and Adsorption Properties of Lignite. *Processes.* 2019 Mar 22;7(3):167.
61. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res.* 1988 Mar;175(1):184–91.
62. Lu Y, Liu Y, Yang C. Evaluating In Vitro DNA Damage Using Comet Assay. *Journal of Visualized Experiments.* 2017 Oct 11;(128).
63. Møller P. Genotoxicity of environmental agents assessed by the alkaline comet assay. *Basic Clin Pharmacol Toxicol.* 2005;96 Suppl 1:1–42.
64. Linstrom PJ& MWG. Linstrom, P.J. & Mallard, W.G. (2022). NIST chemistry WebBook, NIST Standard Reference Database Number 69. In: National Institute of Standards and Technology. Gaithersburg MD; 2022.
65. Gualtieri M, Mantecca P, Corvaja V, Longhin E, Perrone MG, Bolzacchini E, et al. Winter fine particulate matter from Milan induces morphological and functional alterations in human pulmonary epithelial cells (A549). *Toxicol Lett.* 2009 Jul;188(1):52–62.
66. Huang X, Shi X, Zhou J, Li S, Zhang L, Zhao H, et al. The activation of antioxidant and apoptosis pathways involved in damage of human proximal tubule epithelial cells by PM2.5 exposure. *Environ Sci Eur.* 2020 Dec 16;32(1):2.
67. Deng X, Zhang F, Rui W, Long F, Wang L, Feng Z, et al. PM2.5-induced oxidative stress triggers autophagy in human lung epithelial A549 cells. *Toxicology in Vitro.* 2013 Sep;27(6):1762–70.
68. Allen M, Millett P, Dawes E, Rushton N. Lactate dehydrogenase activity as a rapid and sensitive test for the quantification of cell numbers in vitro. *Clin Mater.* 1994 Jan;16(4):189–94.
69. Miranda Guevara AJFMOAHAQSMPLLRJTFJPMFMLWLMG. Análisis in vitro de citotoxicidad y genotoxicidad causado por nanopartículas de carbón. In: Calidad de aire, cambio climático y salud pública . Hill Consulting; 2023.
70. Wang G, Zheng X, Duan H, Dai Y, Niu Y, Gao J, et al. High-content analysis of particulate matters-induced oxidative stress and organelle dysfunction in vitro. *Toxicology in Vitro.* 2019 Sep;59:263–74.
71. Geiser M, Rothen-Rutishauser B, Kapp N, Schürch S, Kreyling W, Schulz H, et al. Ultrafine Particles Cross Cellular Membranes by Nonphagocytic Mechanisms in Lungs and in Cultured Cells. *Environ Health Perspect.* 2005 Nov;113(11):1555–60.
72. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology.* 2011 May;283(2–3):65–87.
73. Luo H, Lu Y, Shi X, Mao Y, Dalal NS. Chromium (IV)-mediated fenton-like reaction causes DNA damage: implication to genotoxicity of chromate. *Ann Clin Lab Sci.* 1996;26(2):185–91.



74. León-Mejía G, Sosa MQ, Rohr P, Kvitko K, Henriques JAP, da Silva J. Occupational Exposure to Coal, Genotoxicity, and Cancer Risk. In: Environmental Health Risk - Hazardous Factors to Living Species. InTech; 2016.
75. Bjelland S. Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2003 Oct 29;531(1–2):37–80.
76. Desler C, Johannessen C, Rasmussen LJ. Repair of DNA damage induced by anthanthrene, a polycyclic aromatic hydrocarbon (PAH) without bay or fjord regions. *Chem Biol Interact*. 2009 Feb;177(3):212–7.
77. Chen C, editor. Selected Topics in DNA Repair. InTech; 2011.
78. Gurbani D, Bharti SK, Kumar A, Pandey AK, Ana GREE, Verma A, et al. Polycyclic aromatic hydrocarbons and their quinones modulate the metabolic profile and induce DNA damage in human alveolar and bronchiolar cells. *Int J Hyg Environ Health*. 2013 Aug;216(5):553–65.
79. Jacob J, Raab G, Soballa V, Schmalix WA, Grimmer G, Greim H, et al. Cytochrome P450-mediated activation of phenanthrene in genetically engineered V79 Chinese hamster cells. *Environ Toxicol Pharmacol*. 1996 Feb;1(1):1–11.
80. Beach AC, Harmon J. Additive effects and potential inhibitory mechanism of some common aromatic pollutants on in vitro mitochondrial respiration. *J Biochem Toxicol*. 1992 Sep 8;7(3):155–61.
81. Harmon HJ, Sanborn MR. Effect of naphthalene on respiration in heart mitochondria and intact cultured cells. *Environ Res*. 1982 Oct;29(1):160–73.
82. Schirmer K, Dixon DG, Greenberg BM, Bols NC. Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill. *Toxicology*. 1998 May;127(1–3):129–41.
83. Sun K, Song Y, Zong W, Tang J, Liu R. Anthracene-induced DNA damage and oxidative stress: a combined study at molecular and cellular levels. *Environmental Science and Pollution Research*. 2020 Nov 19;27(33):41458–74.