*Review Article*

Genotoxicity of Carbon Black Nanoparticles in Humans: A Review

ABSTRACT

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| Due to their special physicochemical properties carbon black nanoparticles (CBNPs) receive comprehensive industrial attention. The increasing application of nanoparticles creates critical safety problems regarding genetic material damage and human health complications from human contact. The pathways of DNA damage by CBNPs involve the generation of oxidative stress and inflammatory reactions and DNA direct binding that leads to genomic instability and elevated mutation rates. Contact with CBNPs causes reactive oxygen species to form thereby starting an oxidative stress chain reaction that leads to chromosomal damage. The results show that contact with CBNPs creates systemic body stress that raises vulnerability to cancer formation and multiple severe medical conditions. Proper regulatory safety measures and risk assessment protocols need to be applied through both technological improvements and safer material development to guarantee safety standards. Future investigations regarding CBNP exposure effects must study their enduring consequences along with setting maximum work safety boundaries because the International Agency for Research on Cancer has classified these compounds as potential cancer-causing substances. Understanding all dimensions of CBNPs provides essential bases for developing protective safeguards that maintain public health protection standards due to their industrial incorporation. |

*Keywords: Carbon Black Nanoparticles (CBNPs), Genotoxicity, Oxidative Stress and DNA Damage.*

1. INTRODUCTION

Carbon black nanoparticles (CBNPs) perform a wide range of functions across multiple industrial sectors including rubber products and coatings together with electronics applications yet these uses have sparked worries about DNA-damaging effects on human wellness. Nanoparticle exposure creates genotoxic effects which damage DNA thereby leading to mutations and cancer development so it stands among the critical health hazards. Oxidative stress together with inflammation caused by CBNPs leads to genetic harm through the direct contact between DNA molecules and reactive oxygen species (ROS) and through ROS-mediated oxidative damage. A review of CBNP genotoxicity in human subjects analyzes foraging evidence from laboratory tests and experimental tests and statistical population observation. The discussion provides details about DNA damage pathways and existing regulations and stresses the necessity for future research to both evaluate long-term risks and guarantee the proper safety standards of nanoparticles. The composition of CBNPs includes primarily elemental carbon as spherical aggregates that scientists have specifically designed to function as nanomaterials. The manufacturing method of CBNPs involves hydrocarbon thermal decomposition or incomplete hydrocarbon combustion leading to distinctive characteristics. CNTs possess distinctive features including strong UV absorption capabilities and excellent reinforcement properties along with high electrical conductivity because their dimensions measure between 10 and 500 nm. [1]

* 1. **Key Properties of CBNPs**

The various industrial sectors receive value from carbon black nanoparticles (CBNPs) through their distinct physicochemical properties. The nanostructured structure of CBNPs allows them to establish greater interaction with surroundings by generating increased surface area potential for chemical processes. Carbon black nanoparticles enhance the mechanical strength of composite materials because of their particular characteristic [2]. Thermal resistance properties make CBNPs withstand high temperatures whereas maintaining their structure thus making them ideal for rubber coatings rubber applications and thermal insulation applications [3]. These components feature natural electricity conductance that enables their use in conductive coating development and energy storage components as well as exceptional battery systems requiring superior charge performance [4]. The UV radiation protective feature of CBNPs makes them suitable for applications in cosmetics and coatings and plastics while their dark pigment properties enable their use in inks and paints and polymers [5]. Industrial applications of CBNPs lead to material issues while health-related environmental hazards demand proper regulatory mechanisms for their assessment.

* 1. **Widespread Industrial Applications**

Distinct physicochemical features found in carbon black nanoparticles enable them to function throughout many industrial sectors. The nanostructured nature of CBNPs allows them to achieve a high reactive surface area because of their typical surface characteristics which leads to improved mechanical reinforcement in composite materials [2]. Additional thermal stability makes CBNPs suitable for their use as thermal protection agents when applied to coating materials and to rubber production and insulation systems [3]. The conductive nature of CBNPs allows their application in electronic devices such as conductive coatings and energy storage systems as well as batteries because charge transfer capabilities are vital for these uses [4]. The absorbance of ultraviolet (UV) radiation exists in CBNPs which makes the particles applicable for UV protection within coatings and plastics and cosmetics and active black particles for inks paints and polymers because of their superior tinting ability and coloring reliability [5]. Many industries face significant concern regarding CBNP material applications because of its high versatility yet scientists require additional evaluation for environmental and health regulation.

* 1. **Human Exposure to Carbon Black Nanoparticles (CBNPs)**

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* 1. **Importance of Studying Genotoxic Effects of CBNPs**

DNA damage and mutational changes occur as a result of genetic system interactions with carbon black nanoparticles (CBNPs). CBNPs affect both occupational health conditions and consumer protection standards while offering sustainability to environmental systems. CBNP-induced genotoxicity shows manifestation through reactive oxygen species (ROS) formation which damages genetic material and increases both cancer development and mutation frequency [20]. Respiratory tissue shows the highest vulnerability to oncological growth when workers perform prolonged CBNP inhalation since this procedure produces both chronic lung inflammation and secondary DNA damage. The risk of lung cancer rises from prolonged inhalation exposure requiring essential exposure safety measures [21]. Multiple health risks make regulatory agencies review whether CBNPs should be categorized as carcinogenic pollutants. Additional research and stronger regulatory controls and risk assessment need immediate attention based on the International Agency for Research on Cancer Group 2B classification of CBNPs as possibly harmful to humans [11]. Knowledge about genotoxicity effects of CBNPs must increase as they gain wider industrial applications to establish safety protocols and protective measures.

**2. MECHANISMS OF GENOTOXICITY**

The potential for carbon black nanoparticles to harm DNA cells poses significant genotoxic dangers because these nanoparticles are operational and lead to molecular damage that create mutations and chromosomal changes that produce cancer. The mechanisms of genotoxicity for CBNPs are investigated by researchers through studies of oxidative stress processes together with direct DNA damage mechanisms as well as inflammatory-related effects and DNA repair interferences.

**2.1. Oxidative Stress and Reactive Oxygen Species (ROS) Generation**

The high surface activity and reactivity of the carbon black nanoparticles (CBNPs) results in oxidative stress as the main mechanism of genotoxicity since these properties generate reactive oxygen species (ROS) including superoxide anions (O₂⁻), hydroxyl radicals (•OH) and hydrogen peroxide (H₂O₂) [20]. Oxidative base lesions along with 8-hydroxy-2'-deoxyguanosine (8-OHdG) and both single-strand breaks (SSBs) and double-strand breaks (DSBs) develop when ROS interact with DNA which creates genomic instability and raises mutation rates [22]. Oxidative stress effects from CBNP exposure generate multiple detrimental effects by causing lipid peroxidation together with protein oxidation which destroys cell equilibrium while additionally producing secondary chemical compounds and DNA damage with blocked repair functionalities. [2] Lung epithelium and immune cells generate more ROS when exposed to CBNP while the cells develop DNA fragmentation, chromosomal abnormalities and micronuclei which serve as genotoxic stress and cancer indicators [21]. The dominant cause of CBNP genotoxic effects stems from oxidative stress according to existing research which demands more investigation about antioxidant defense and protective methods against oxidative damage for risk populations.

**2.2. Direct Interaction and Structural Damage to DNA**

Both oxidative damage and DNA physical interactions with carbon black nanoparticles CBNPs result in genomic instability alongside DNA physical damage. Experimental data shows that CBNPs enter through the nuclear barrier to bind DNA strands which causes breaks and mutations that increase cancer formation [15]. Electron microscopy shows that CBNP aggregates exist within the nucleus thus demonstrating their ability to disrupt chromatin structure and gene expression [17]. Due to their structural characteristics CBNPs exhibit the capacity to integrate between DNA base pairs and grasp DNA repair proteins thus impeding the DNA repair mechanisms [23]. The modified structure of CBNPs raises their potential to cause mutations that increase the risk of cancer development. Additional research is needed regarding CBNP-genetic material interactions together with protective mechanisms against DNA damage caused by nanoparticles.

**2.3. Inflammation Mediated Genotoxicity**

DNA-damaging genotoxicity in CBNPs results mainly from chronic inflammation by carbon black nanoparticles (CBNPs) which functions both directly and indirectly to harm DNA. The activation of immune response due to CBNPs leads to cytokine production including IL-6 and TNF-α and IL-1β which create secondary DNA damage through oxidative stress mechanisms alongside cellular balance disruption [13]. Genotoxicity rises as a result of inflammatory activities because reactive oxygen species (ROS) together with reactive nitrogen species (RNS) produce oxidative stress which causes DNA strand breaks and base changes and chromosome changes [12]. Highly reactive intermediates form when myeloperoxidase together with other oxidative enzymes release from activated macrophages and infiltrating neutrophils at CBNPs sites and these intermediates directly damage DNA as well as proteins and cellular membranes [14]. Tissues undergoing long-term unresolved inflammatory responses develop fibrosis which converts cells and damages DNA structure before causing cancer formation [11]. Research must advance to understand the connection between inflammation and CBNPs-induced genotoxicity as well as find strategies to lower DNA damage from inflammatory processes experienced by those exposed to CBNPs.

**2.4. Interference with DNA Repair Mechanisms**

Genotoxic effects from Carbon black nanoparticles (CBNPs) develop because these particles harm DNA directly and hinder DNA repair systems in cells which enables persistent genetic damage and elevated mutations that result in genomic instability. Science demonstrates that CBNPs stop DNA repair enzymes from carrying out their essential functions during base excision repair (BER) and nucleotide excision repair (NER) cellular processes [20]. Scientific research shows that contact with CBNPs leads to p53 inhibition thus blocking a vital tumor-suppressing protein which serves as a DNA damage signal and controls cell cycles and triggers programmed cell death. A disrupted p53 signaling pathway causes defective cell replication regulation when DNA damage occurs thus leading to cancer development and higher mutation rates [22]. The chemical compounds present in CBNPs impose harmful effects on epigenetic regulation while simultaneously blocking interactions between repair machinery through physical contact. Several studies show DNA methylation and histone modification differences in cells with CBNP contact that could lead to repair gene suppression and oncogenic network support and thus increase genetic instability [16]. The research demands investigation into how DNA repair inhibition by CBNPs affects prolonged effects and identifies protective measures against their genetic toxicities. The genetic damages caused by carbon black nanoparticles (CBNPs) develop according to their physical and chemical characteristics such as dimensions along with surface area and active chemical makeup. Biological interactions between CBNPs are affected by physical properties which determine their mechanism of oxidative damage and cell penetration along with DNA degradation ability.

The genotoxicity of carbon black nanoparticles (CBNPs) depends on their size dimensions and surface area and chemical properties which control how cells take them inside and what happens to DNA. Small-sized CBNPs (<100 nm) enhance cellular permeation thus making them more reactive helping them interact with DNA and cause strand breaks and chromosome changes [15,17]. The extended system retention of these larger-sized particles lasts longer periods of time leading to elevated risks of mutagenesis and carcinogenesis [24]. Ultrafine CBNPs (<50 nm) also permeate barriers like the alveolar-capillary barrier to reach organs like the liver and brain, and smaller nanoparticles (<20 nm) permeate skin to deliver systemic absorption as well as dermal cell DNA damage [12,14].

A critical factor in genotoxicity exists between the relationship between surface area and particle size because smaller nanoparticles generate higher surface-to-volume ratios consequently promoting reactive oxygen species formation which causes DNA damage [20]. The high surface area of CBNPs has the ability to capture essential DNA repair enzymes while blocking their DNA damage repair mechanism [22]. The DNA damage caused by these CBNPs with small size ranges leads to enhanced oxidative DNA damage which manifests through elevated 8-OHdG levels and micronuclei formation when compared to bigger nanoparticles [2]. Additionally, CBNP aggregation influences bioavailability, and dispersed nanoparticles are more genotoxic and cytotoxic than agglomerates [7,3].

The surface chemistry of CBNPs is also implicated in their genotoxicity since charge, coating, and functional groups influence DNA interaction and reactivity. The positively charged CBNPs bind more with the negatively charged DNA, enhancing genotoxicity, and oxidized CBNPs with oxygen functionalities (-OH, -COOH) yield higher ROS levels resulting in greater breaks in DNA [21,19]. Genotoxicity is regulated by lowering or enhancing the surface modifications; polymer and protein coatings enhance biocompatibility with lower ROS and DNA damage but metal coatings (e.g., Fe, Cu, Ni) enhance oxidative stress and mutagenicity [4,8]. The evaluation of genotoxic potential relies heavily on nanoparticle properties making it necessary to develop safer CBNP designs while controlling modifications to minimize health risks upon exposure.

**3. IN VITRO STUDIES**

In vitro tests play a key role in determining the genotoxicity of carbon black nanoparticles (CBNPs) in human cell models such as lung epithelial cells, fibroblasts, and immune cells. It determines the mechanisms of DNA damage such as oxidative stress, direct interaction with DNA, and inflammation. An overview of notable in vitro experiments of genotoxicity of CBNPs is given below.

**3.1. Genotoxic effects in Human lung Epithelial cells**

Alveolar epithelial cells are the main targets of CBNP inhalation and are critical in genotoxicity studies. Researchers performed a substantial study that evaluated carbon black nanoparticles (CBNPs) genotoxicity while using the A549 human lung epithelial cells as the standard model system in respiratory toxicity research. Complete DNA strand breakage occurred from CBNP treatment according to their analysis through increased tail moment detection on comet assays and formation of micronuclei which showed whole genetic damage. Excessive reactive oxygen species (ROS) led to oxidative DNA modification according to reported findings and this mechanism destabilized genetic material. The oxidative stress halted the cell cycle progression, at the G1/S checkpoint, indicating that cells had tried to stop proliferation to enable repair of the DNA. Further, the study verified that CBNPs activated transcription factors AP-1 (Activator Protein-1) and NF-κB (Nuclear Factor Kappa-B), which are critical regulators of inflammatory and stress-response pathways. Activation of the pathways indicates an aggressive inflammatory nature to CBNP-induced genotoxicity, which can facilitate DNA damage by secondary mechanisms of cytokine-mediated oxidative stress. These findings establish the bivalent nature of CBNPs to induce direct induction of DNA damage and inflammatory processes to induce genomic instability, and warn of long-term lung toxicity and potential carcinogenic risk of occupational and environmental exposure to these nanoparticles. [25]

Researchers reviewed carbon black nanoparticle (CBNP) genotoxicity effects in mammalian cells which revealed toxicity increases by nanoparticle size. Smaller CBNP particles measuring less than 100 nm displayed more genetic toxicity because they entered cells efficiently and produced more ROS. Ultrafine nanoparticles showed membrane penetration capabilities and they built up in cell cytoplasm and nucleus where they interacted with DNA structures which led to chromosomal anomalies and DNA breaks and micronucleus events. The production of ROS was more pronounced in tiny particles which resulted in oxidative stress that damaged DNA while simultaneously preventing its repair. Results from the study established that secondary genotoxic events occur when CBNPs initiate inflammatory processes which generate oxidative stress which in turn results in non-exposed cell damage by indirect mechanisms. A recent study reveals that wildfires release CBNPs in different measurement sizes which correlates with variations in genetic damage risks thus necessitating additional research about exposure effects in diverse settings [26].

Scientists conducted detailed investigations of carbon black nanoparticles (CBNPs) genotoxic effects on 16HBE human bronchial epithelial cells to understand their effects on cell division management and genetic material integrity. These researchers observed that CBNP exposure caused substantial damage to Polo-like kinase 1 (Plk1) signaling pathways that regulate both mitotic progression and chromosome segregation as well as DNA damage response. The research revealed that CBNPs contaminated Plk1 activity in abnormal ways that created chromosomal misalignment while causing aneuploidy and mitotic arrest together with all typical genomic instability indicators. The disruption of Plk1 signaling pathways led to reactive oxygen species (ROS) overproduction that started DNA strand breaks and caused oxidative stress to trigger apoptotic cell death. Caspase-dependent apoptotic paths began once these actions occurred which caused greater cell death while also damaging the epithelial barrier structures. These observations demonstrate that CBNP exposure can start a carcinogenic process because Plk1 regulates chromosomal stability while triggering continual genetic changes and lung epithelial cell apoptosis. Studies stress the significance of understanding how nanoparticle exposure affects cell cycle regulatory functions to discover long-term lung health threats from CBNPs [27].

**3.2. Genotoxic Effects in Human Fibroblasts and Dermal Cells**

Carbon black nanoparticles pose significant work-related dangers through skin contacts so researchers study genotoxic effects on skin cellular structures using fibroblast models. The research team performed systematic evaluation to study how carbon black nanoparticles (CBNPs) affect human promyelocytic fibroblast-like cells through oxidative stress and immune disruption mechanisms and DNA damage analysis. Testing showed that nanoparticles from carbon black exposure produced swift increases in reactive oxygen species (ROS) within cells which led to oxidative damage of DNA molecules. When immune signal transduction pathways failed to operate properly after CBNP exposure the cells produced higher levels of pro-inflammatory cytokines in particular IL-6, TNF-α, and IL-1β. The pro-inflammatory condition of cells created additional DNA damage throughout neighboring cells which implies that such effects might act as bystanders. After CBNP exposure mitochondria did not function correctly thus both raising oxidation levels and damaging cellular energy processes which leads to persistent genomic instability. Research findings indicate CBNP has a possible permanent risk for affected cells and workers exposed to it in professional environments since it drives both tissue breakdown and carcinogenesis potential through fibroblast malfunction. Data from the study emphasizes the requirement of strict laws that protect against CBNP health dangers and more investigation needs to study cellular processes involved in long-term exposure [28].

Research investigators studied the genotoxicity effects of carbon black nanoparticles (CBNPs) on fibroblast cells through their analysis of chronic DNA damage and inflammatory response. The researchers established that CBNP treatment generated oxidative conditions that led to DNA breakage and chromosomal abnormalities and micronucleus formation which constitute major elements in genotoxic damage and genomic destabilization. The scientists reported DNA damage persisted beyond the removal period of CBNPs thus showing that these nanoparticles lead to lasting genetic alterations instead of brief impacts. CBNP introduction to cells led to severe inflammation by triggering increased production of cytokines IL-6 and TNF-α and IL-1β. The authors confirmed their hypothesis that sustained inflammation functions as the primary mechanism which leads to genotoxic effects caused by nanoparticles through proving its enhancement of both oxidative stress and different secondary DNA damage products. Prolonged exposure of fibroblasts to CBNPs altered cell cycle control mechanisms thus increasing the probability of cell transformation that leads to potential carcinogenesis. The observational data supports the belief that increased CBNP exposure durations raise the cancer risk whereas occupational situations wherein employees breathe nanoparticles several times result in the highest cancer potential. Additional observations of people exposed to CBNPs and stricter safety measures need immediate implementation because they protect against potential adverse health effects [29].

**3.3. Genotoxic Effects in Human Immune Cells (THP-1 Monocytes, Macrophages, and Lymphocytes)**

The inflammatory process triggered by CBNP exposure depends significantly on immune cell responses which in turn lead to DNA damage effects. Scientists conducted a systematic study of carbon black nanoparticle genotoxicity effects on THP-1 human monocytes. Oxidative stress together with inflammation and DNA damage emerged as a result of CBNP exposure according to these findings. Exposure to CBNPs caused significant increases of reactive oxygen species (ROS) which led to DNA damage detectable through comet assays as well as γ-H2AX foci formations. The scientific literature proves that DNA damage resulting from oxidative stress leads to both mutagenesis and genomic instability. The exposure triggered major activation of immune cells through extensive activation of primary inflammatory cytokines IL-6 and TNF-α. Activation of secondary genotoxicity occurred through extended chronic inflammation which caused DNA damage in cells which remained unexposed to the inflammation mediators and oxidative stress. The systemic toxicity risk of CBNPs emerges from immune activation that leads to oxidative and inflammatory stress spread across the entire body. Research evidence shows that workers exposed to CBNPs for extended periods develop permanent immune system overactivation and sustained genetic damage that raises the risk of DNA mutations and cancer development. The analysis specifies that secondary genotoxicity must receive emphasis in risk assessments due to requirements for stringent exposure guidelines and protective measures against nanoparticle-induced immune system-mediated DNA damage [30].

The impact of carbon black nanoparticles (CBNPs) on systemic genotoxicity received investigation outside the lungs through a study dedication. The study examined how CBNPs affected DNA in liver cells through pulmonary exposure despite not accumulating at the liver during inhalation. Research results demonstrated how CBNPs transfer through the lungs to bloodstream prior to reaching the liver where they provoke oxidative stress along with inflammation that results in DNA strand breakage. A technique measuring 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels validated that the exposure to CBNPs triggers ROS-mediated genetic damage in liver cells. The comet assay investigation revealed elevated DNA fragment levels which demonstrated straight DNA injury. The study demonstrated that systemic inflammation from CBNP contact triggered the main origin of secondary genotoxicity. Research findings showed elevated levels of inflammatory cytokines IL-6, TNF-α, IL-1β in circulating blood which potentially led to DNA damage in liver cells because of inflammation-directed oxidative stress. Systemic inflammatory response further confirms the fact that the exposure of CBNPs is not restricted to lung toxicity alone but is also capable of causing far-reaching biological implications to distant organs. The study adopted A549 human lung epithelial cells to understand carbon black nanoparticles (CBNPs) genotoxicity effects under respiratory toxicity research testing conditions. DNA strand breakages occurred fully as per their study results when exposing cells to CBNP since tail moment increased on comet assays revealed DNA damage and micronuclei formation displayed entire genetic damage. Research reports show that excessive ROS triggers oxidative DNA modifications causing genetic material destabilization. Despite current findings scientists need to investigate CBNPs' biodistribution along with their long-term effects on non-pulmonary tissues alongside more rigorous norms to prevent systemic nanoparticle toxicity [31].

An investigation studied DNA damage pathways triggered by carbon black nanoparticles (CBNPs) within macrophages and lung epithelial cells that elaborate about genotoxicity through reactive stress and dangerous peroxynitrite. When exposed to CBNPs human cells demonstrated excessive nitrative stress and oxidative stress together with DNA strand damage and chromosomal mutation. The research revealed that carbon black nanoparticles generate peroxynitrite (ONOO⁻) through the combination of superoxide (O₂⁻) and nitric oxide (NO). DNA damage occurs through peroxynitrite action mainly affecting guanine bases which produces 8-nitroguanine as a mutagen that enhances genomic instability and promotes cancer formation. The study validated 8-nitroguanine DNA adducts in CBNP-treated cells through immunostaining analyses and mass spectrometry findings which proved nitrative stress in genotoxicity. The exposure to CBNP stimulates immune cells to produce inducible nitric oxide synthase which leads to extensive NO generation and persistent nitrative stress. Necrosis along with apoptosis developed following repeated CBNP exposure which could potentially result in long-term chronic lung DNA damage and persistent inflammation of the lungs. This research demonstrates that DNA damage caused by nitrative stress becomes the primary risk factor for pulmonary diseases such as cancer and lung fibrosis among workers exposed to CBNP. The study demonstrates the requirement to study nitrative stress in relation to nanoparticle-induced genotoxicity while advocating for rigorous safety measures to block CBNP exposures [32].

**3.4. Types of genotoxic endpoints measured (e.g., DNA strand breaks, chromosomal aberrations, micronucleus formation)**

Evaluation of the genotoxic hazard of carbon black nanoparticles (CBNPs) encompasses analysis of important indicators of DNA damage, chromosomal instability, and mutagenic activity. The assessment includes DNA strand breaks as well as chromosomal aberrations and micronucleus formation with examination of oxidative DNA damage followed by mutation assessment. The assessment of CBNP-induced genotoxicity requires these factors to determine health risks during occupational and environmental conditions.

**3.4.1. DNA strand Breaks (Single and Double-strand Breaks)**

Genotoxic stress that arises from both oxidative or nitrative stress and carbon black nanoparticles (CBNPs) can be measured through DNA strand break analysis. Two commonly used laboratory methods for detecting single-strand breakages (SSBs) and double-strand breakages (DSBs) are the comet assay and γ-H2AX staining. Science shows that carbon black nanoparticle exposure builds reactive nitrogen species (RNS) comprising peroxynitrite (ONOO⁻ which originates from superoxide (O₂⁻) and nitric oxide (NO). The DNA-modifying effects of peroxynitrite result in damaged DNA strands together with mutations and genome instability [33]. When exposed to CBNP the cells increase their iNOS expression leading to sustained DNA damage through nitrative stress [34]. CBNP-activated macrophages produce inflammation that increases pro-inflammatory cytokine-caused genotoxicity which leads to elevated oxidative stress and DNA damage in surrounding non-exposed cells [34]. Long-term health consequences of inhaling CBNP emerge from observations when workers are at risk in exposed environments. Further safety measures are necessary to reduce nanoparticle-induced lung toxicity and nitrative stress as an important cause of nanoparticle-induced genotoxicity.

**3.4.2. Chromosomal Aberrations (CAs)**

The term chromosomal aberrations describe chromosomal alterations that result mainly from DNA damage and replication faults as well as repair system failures. Research data show that when 16HBE cells experienced exposure to carbon black nanoparticles their Polo-like kinase 1 signaling network essential for mitosis and chromosome segregation became disrupted. The Plk1 signal became abnormal due to oxidative stress from CBNP exposure leading to mispacked chromosomes together with excessive formation of centrosomes and defective spindle structure formation. The cell framework developed these anomalies that caused aneuploidy as well as micronuclei and chromosomal instability (CIN) which represent fundamental tumor-developing characteristics. The toxic properties of CBNPs may result in mitotic errors that increase genome instability and cancer hazards particularly within exposure-prone locations. More research together with strict safety standards must be conducted to determine the lasting effects of CBNP-caused chromosomal alterations [27].

**3.4.3. Micronucleus Formation (MN Assay)**

The nuclear structures called micronuclei represent damaged or improperly separated chromosomes and chromosomal instability which form from unthriving chromosome fragments when cell division occurs during mitosis. Scientists used systematic review to study size-dependent genotoxic effects of carbon black nanoparticles (CBNPs) on mammalian cells both in the initial phase and secondary stage. Small-sized CBNPs with dimensions under 100 nm demonstrated higher genotoxic effects than their bigger counterparts. The toxic effect increased because cells took up more particles and their surface area increased while they produced greater levels of oxidative stress. The review demonstrated that CBNPs exposure produces concentrated patterns of micronucleus (MN) formation which is commonly used to measure chromosomal damage and DNA instability. Cellular entry happened more efficiently for smaller nanoparticles while they demonstrated higher binding capacity to DNA and mitotic components so they caused better opportunities for chromosomal mis arrangement and destruction. The production of ROS by CBNPs caused DNA damage through single or double strand breaks and disrupted accurate cell division progression. The review demonstrates inflammatory responses from CBNP-treated cells lead to DNA damage in beside cells without treatment which confirms the overall risk potential at the exposure site. Research results demonstrate that the susceptibility to genotoxic harm from CBNPs rises as the particle dimensions decrease since small sizes enable DNA damage both directly and through inflammation-triggered oxidative stress generation. There is an urgent requirement to investigate exposure safety thresholds and regulatory protocols because nanoparticles are extensively utilized in industrial settings [26].

**4. IN VIVO STUDIES**

In vivo experiments provide comprehensive information regarding carbon black nanoparticles' (CBNPs) genotoxicity within whole organisms as a function of their systemic toxicity and health significance. Animal and human models have explored exposure channels such as inhalation, oral intake, and dermal application to assess their impact on DNA integrity, inflammation, and potential for cancer development.

**4.1. Animal Model studies on CBNP- Induced Genotoxicity**

**4.1.1. Pulmonary Exposure and Systemic DNA Damage**

A genotoxicity study of carbon black nanoparticles (CBNPs) in mice following pulmonary exposure showed their systemic genotoxicity and biodistribution. CBNPs demonstrated DNA-damaging effects in the lung tissue as well as in the liver tissues illustrating their ability to transfer through bloodstream circulation. The tests of Comet assays alongside γ-H2AX staining revealed massive DNA strand breaks and genetic instability throughout lung and liver organs while the expression of oxidative stress presented higher reactive oxygen species (ROS) and increased peroxidation levels. The elevated levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) as a result of CBNP exposure led to increased oxidative stress and subsequent DNA fragmentation alongside mitochondrial dysfunction in the liver. Data indicates that CBNPs may cause damage to the genetic material across the system which could lead to such long-term health problems as cancer alongside organ system dysfunction. The research demonstrates that ongoing industrial practices with CBNPs require exposure risk evaluation procedures for personnel who handle these materials. Additional research on human exposure exposure to carbon black nanoparticles is necessary because evidence shows these particles affect organs beyond lungs thus challenging typical respiratory protective measures while prompting regulatory authorities to implement stricter workplace safety guidelines [29].

Research shows that carbon black nanoparticles (CBNPs) enter the bloodstream after lung exposure and cause liver genotoxicity by affecting primary DNA structures in tissue. Results from Comet assays combined with γ-H2AX staining showed extensive DNA damage within hepatic cells which supports the observation that CBNPs move through bloodstream circulation rather than remaining within the respiratory tract. Liver tissue analysis through transmission electron microscopy (TEM) reveals how CBNPs avoid lung defense systems which results in genetic damage occurring outside the respiratory system. The analysis established that lung inflammation from CBNP exposure results in pro-inflammatory cytokines (IL-6 and TNF-α and IL-1β) becoming systemically active which then causes secondary genotoxic effects in the liver. Research findings indicate that inhaled CBNPs could create widespread risks because they affect liver function which is an important concern for exposure sites where these particles exist both in occupational settings and in environmental areas [31].

**4.1.2. Hepatic and Systemic Genotoxicity**

Researchers studied the toxic effects of ultrafine carbon black nanoparticles (CBNPs) on liver tissues of mice within a live experimental setting to show how the toxic substance spreads through the body beyond respiratory functions. The study confirmed liver tissue impairment caused by increased ROS markers alongside decreased antioxidants while detecting DNA strand breaks that produced chromosomal abnormalities and γ-H2AX foci structures as genotoxic indicators in liver cells. Extensive inflammatory reactions developed from CBNP exposure because these particles triggered increased secretion of inflammatory cytokines IL-6 and TNF-α and IL-1β and attracted immune cells causing DNA and mitochondrial damage through oxidative stress processes. The prolonged contact with CBNPs led to permanent tissue deformations of liver structures while simultaneously killing hepatocytes by apoptosis and necrosis and leading to fibrosis formation. The research indicates these CBNPs function as initiators of fibrosis and hepatocellular carcinoma per microscopic test data. Studies have shown the existence of major health risks to workers engaged in industrial processing of manufactured products and coated and electronic material production because CBNPs travel through tissue structures from the lungs to reach the liver. According to research data from this study the industry needs to advance exposure control strategies while simultaneously creating liver-damage surveillance systems to manage nanoparticle genotoxicity hazards that arise at work environments. [35]

A four-generational animal study examined maternal carbon black nanoparticle (CBNP) inhalation on male reproductive health through assessment of vital reproductive metrics alongside genetic effects discovery. The sperm count of male offspring decreased while the sperm motility deteriorated and testicular abnormalities developed which combined to compromise male fertility. The cellular oxidative stress in germ cells manifested through elevated ROS levels together with higher lipid peroxidation and reduced antioxidant enzyme functioning which led to DNA damage and chromosomal instability as well as meiotic malfunction. The studied germ cells presented chronic double-strand breaks diagnosed with γ-H2AX staining and comet assays and spermatocytes showed chromosomal deformation via micronucleus formation. DNA methylation along with histone modifications served as epigenetic indicators of transmissible reproductive health effects. Industrial usage of CBNPs creates significant concerns about long-term reproductive hazards and multigenerational risks thus requiring both better workplace protection methods and continuous nanoparticle epigenetic modification research. [36]

**4.1.3. Dermal and Oral Exposure Effects**

Micronucleus and comet assay experiment was also conducted to investigate the genotoxicity of carbon black nanoparticles (CBNPs) after dermal and oral exposure in rats, and extensive DNA fragmentation and oxidative damage was observed. Their work confirmed that CBNPs had penetrated the barrier of the skin, leading to DNA strand breaks and chromosomal aberrations in skin and blood cells, suggesting penetrability to systemic circulation. Lights demonstrate that CBNPs cause genotoxic effects in intestines and liver cells because CBNPs enter the human body through the gastrointestinal system and spread throughout the body. Research indicated oral exposure elevated 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels as well as reactive oxygen species (ROS) that paired with elevated pro-inflammatory cytokines (IL-6) and TNF-α contributing to DNA damage and mitochondrial dysfunction. Laboratory tests indicate that CBNPs may cause systemic health issues which extend past inhalation exposure. Therefore, industries need to adopt protective measures to reduce risks from skin contact and ingestion specifically when dealing with CBNPs used in cosmetics and coating materials as well as packaging products. [37]

**4.2. Human Epidemiological Studies on CBNP Genotoxicity**

Research about the DNA damage risks from long-term CBNP contact shows limited availability of human epidemic data while demonstrating elevated risks of DNA damage and cancer development. DNA repair gene expression underwent significant modifications after conducting gene expression profile tests on workers subjected to extended carbon black nanoparticle (CBNP) exposure. The investigation showed that extensive occupational contact with CBNPs resulted in DNA response pathways' regulatory dysfunction and major changes in genes which maintain BER and NER repair activity. The examination revealed reduced expression levels of XRCC1 OGG1 XPA proteins which maintain DNA repair capacity and discovered exposure at CBNP facilities creates genomic defect repair difficulties leading workers to susceptibility against mutations and cancer formation. Research results showed elevated inflammatory marker expression together with stress-related gene expression increase due to CBNPs exposure which results in chronic inflammatory processes leading to more DNA damage. Observational results stress Ebola virus Disease that CBNP exposure creates in professional environments requiring better administrative practices and exposure detection alongside defensive legislation to avoid genotoxic stress leading to cancer development. [38]

Scientists conducted extensive research on cancer risks among carbon black factory workers to evaluate chronic carbon black nanoparticles (CBNP) exposure effects on human health. Lung cancer risks from persistent CBNP exposure were identified by the researchers although they emphasized additional scientific investigation should address the distractors caused by workplace chemical exposures and tobacco smoking and environmental pollution. Research on employment groups throughout multiple worker populations showed elevated rates of lung cancer alongside respiratory diseases thus suggesting CBNP-driven toxic effects in the lungs. The report indicated that long-term airborne exposure to CBNP potentially causes lung cancer development through processes that include oxidative stress together with chronic inflammation and DNA damage. The authors stressed the requirement of continued long-term follow-up of the exposed workers, enhanced workplace protection measures, and further research aimed at delineating the independent role of CBNPs in lung cancer risk. [39]

**4.3. Different exposure route and their implications for human health**

Numerous scientific reports validate that individuals who encounter carbon black nanoparticles (CBNPs) suffer from systemic genetic damage which manifests as DNA strand breaks and chromosomal aberrations and oxidative DNA lesions in various body parts including lungs, liver and blood [29, 31]. The method used to transport nanoparticles into the body determines toxicity symptoms because pulmonary exposure results in lung inflammation which allows nanoparticles to spread throughout the bloodstream toward distant organs [29, 31]. Contact with the skin leads to penetration through the skin as well as local oxidative stress and systemic inflammatory responses that escalate genotoxic risk [37]. After intestinal absorption of ingested CBNPs harmful effects appear in extrapulmonary tissues leading to toxic damage and general DNA damage [35]. Prolonged occupational contact with CBNPs raises the danger of developing cancer alongside DNA repair pathway disruption and persistent inflammation that ultimately generates lung cancers and tumors elsewhere [38,39]. Research indicates CBNP exposure produces reproductive health problems because it generates oxidative DNA damage in germ cells and creates sperm quality reductions as well as heritable epigenetic modifications [36]. The evidence highlights the necessity to establish regulatory restrictions for CBNP exposure assessment since industrial and environmental work areas commonly present prolonged human contact with these pollutants.

**5. HUMAN EXPOSURE AND RISK ASSESSMENT**

Working alongside CBNPs present occupational and environmental health hazards which primarily affect manufacturing sector employees as well as construction personnel and printing industry personnel who regularly encounter airborne nanoparticle emissions. Prolonged workplace exposure to CBNPs most commonly affects workers through breathing the particles and skin exposure and eating contaminated materials which leads to bodywide damage as well as oxidizing agents and inflammatory reactions along with genetic cell toxicity [38,39]. Recent research indicates that industrial workers exposed to CBNPs face lung inflammation together with respiratory conditions while also increasing their risk of developing lung cancer. Furthermore, scientists suggest through their research that CBNPs have the potential to move through the body to distant organs such as the liver and reproductive system thereby causing worry about systemic effects [29,31]. The general public faces regular low-dose exposure to CBNPs mainly because these pollutants from vehicles and industries are transmitted to air, water and soil which ultimately accumulates in the environment [35].

Health risk assessment for CBNP exposure remains challenging because individuals differ in their exposure concentrations and sensitivities and because proper long-term research is lacking. Risk assessment becomes problematic for CBNP because no exposure limits have been set like for controlled hazards including silica or asbestos [37]. Particle size together with surface chemistry and aggregation variations contribute to exposure differences by giving responsive and deeply penetrating particles [26]. Little standardized human study findings along with monitoring data create barriers to precisely detecting cancer risks and reproductive toxicity and transgenerational effects [36]. The identification of precautionary safety standards together with strong regulations plus extensive examinations becomes necessary to understand CBNP health risks because of these circumstances.

**6. REGULATORY AND SAFETY CONSIDERATIONS**

The worldwide regulatory standards for carbon black nanoparticles (CBNPs) exist under different limits established by authorities such as the Occupational Safety and Health Administration (OSHA) and National Institute for Occupational Safety and Health (NIOSH) in the USA but also the European Chemicals Agency (ECHA) and International Agency for Research on Cancer (IARC). OSHA established the permissible exposure limit (PEL) of carbon black at 3.5 mg/m³ for an 8-hour time-weighted average (TWA) yet nanoscale particles are excluded from this standard [40]. According to NIOSH research the maximum acceptable exposure thresholds should be 0.1 mg/m³ for fine carbon black along with 0.001 mg/m³ for ultrafine/nanoscale carbon black while respiratory toxicity and cancer-causing effects exist as the primary risks [29]. The European Union's REACH regulation mandates risk assessment and notification in terms of nanoparticle-specific risk, but setting tighter limits of exposure to CBNPs poses problems [41]. The IARC has carbon black classified as a Group 2B carcinogen (possibly carcinogenic to humans), on the basis of long-term animal studies suggesting inhalation exposure to lung cancer risks [42].

Despite the existence of contemporary regulation, challenges still face the development of overall safety standards for carbon-based nanomaterials (CBNPs), due to scarce comprehensive long-term human research, uncertainty in the exposure levels, and inadequate protective practice in the workplace. The experts refer to the need for novel regulatory approaches, including tighter occupational exposure levels, mandatory nanoparticle-specific labeling, tighter PPE regulations, and harmonized tox tests [43]. Progressive policy requirement depends on improved nanotoxicology capabilities to study the extended effects of nanomaterials which include DNA damage and reproductive harm and generational risks. Furthermore, the use of tighter workplace monitoring procedures and exposure monitoring will be needed in reducing chronic health hazards of exposure to CBNPs.

**7. FUTURE DIRECTIONS**

The genotoxic properties of carbon black nanoparticles (CBNPs) exist but scientists lack complete understanding about their extended health consequences and poison pathways together with dose thresholds. The current research focuses on acute toxicity assessments of in vitro environments although human studies on chronic exposure exposure and regulatory threshold development need establishment [44]. Research must focus on understanding how CBNPs create DNA damage by analyzing oxidative stress and inflammation-mediated genetic damage due to their effects on cancer development and metabolic problems [45]. Researchers are worried about both heritable genetic alterations and reproductive hazards that CBNPs can cause [27].

Scientific investigation of the future needs to generate quantity-based data for nano-specific properties such as particle size and surface chemistry and aggregation state because these features influence biological responses and hazardous effects [46]. Workplace safety must be improved while controls need to be tightened to reduce human exposure to harmful substances through the use of safer materials. The development of modified nanoparticles designed to reduce oxidative potential represents an approach to mitigate genotoxic risks when the industry advances its use of these materials [47]. In order to decrease occupational exposure rates healthcare officials must implement advanced personal protective equipment (PPE) combined with air purification systems alongside continuous monitoring platforms [48]. Reduction of exposure depends on public health education along with necessary safety training requirements for industries at high risk. The assessment of nanomaterials which degrade safely or pose less toxicity than current CBNPs enables industries to maintain productivity together with sustainable alternatives [41]. Protection of health and promotion of technology can be achieved through toxicological investigations as well as regulatory innovations and safer technologies applied in conjunction with a comprehensive risk reduction strategy.

8. Conclusion

The genotoxic effects of human exposure to carbon black nanoparticles (CBNPs) over a long period creates DNA oxidative damage and chromosomal abnormalities as well as DNA repair system malfunction which results in cancer threats together with reproductive risks. Research findings show that CBNPs create DNA strand breaks and oxidative lesions in lung tissues and reproductive cells and liver tissues and blood cells thus proving their capacity to translocate systemically throughout the body [49,50,51]. Long-term exposure to CBNPs as identified by occupational research shows a connection between inflammatory DNA damage and disruption of DNA repair genes and elevated risk for lung cancer but additional research is needed to separate these risks from smoking and air pollution influence [43, 52]. Research shows that CBNP exposure generates transgenerational genetic risks which becomes evident from observations of oxidative DNA damage as well as epigenetic alterations in germ cells [53].

More research needs to investigate the sustained human health risks and toxicities from CBNPs since industry continues to increase their applications within manufacturing and construction as well as for coatings and consumer products. Present standards for CBNP exposure remain insufficient since specific safety rules for nanoparticles at workplaces need further development [54,55]. Preventing genotoxic hazards requires stronger regulations along with enhanced monitoring as well as tighter exposure limits and better alternative nanomaterials for safety. Better risk assessment models need epidemiological studies of large scale, extended exposure measurements and universal toxicology testing procedures for assessment improvement. By focusing on scientific development and workplace safety regulations and relevant standards the future risks from CBNP exposure will decrease substantially to protect human health effectively.

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