Tanu Jindal *Editor*

New Frontiers in Environmental Toxicology

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ISBN 978-3-030-72172-5 ISBN 978-3-030-72173-2 (eBook)
https://doi.org/10.1007/978-3-030-72173-2

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About the Book

New Frontiers in Environmental Toxicology is an important book with emphasis on radiation toxicology, polar ecotoxicology, plastic toxicology, microbial toxicology, nanotoxicology, and pesticide toxicology.

The book aims to enlighten the reader on toxic pollutants in the environment and their harmful effect on human health and nature.

It focuses on the electromagnetic field of mobile phone radiation, polar Antarctica contamination, cyanobacteria extracts, phthalate ester in plastic, nanotoxicology exposure, and prophylactic approaches for OP poisoning effects on human health and ecosystem.

The book contains important aspects of environmental toxicology, features, characterization, applications, environmental routes for dispersion, nanotoxicity, ecotoxicity, and genotoxicity of nanomaterials have been discussed.

The present need is to discover novel sources of drugs, particularly natural resources which do not to have adverse effects on human beings and society. A search for these organisms for medicinal purposes has revealed important chemical prototypes in finding new agents, stimulating the use of refined physical techniques and new syntheses of molecules with the pharmaceutical application for human welfare.

We have discussed the toxicity of POPs in Antarctica, which is due to anthropogenic activities such as tourism and scientific activity. The presence of these persistent pollutants in Antarctica is an alarming situation and needs to be studied further to maintain its pristine environment. The presence of these persistent pollutants may be attributed to anthropogenic sources, orographic effects, migratory birds, and biomagnification.

Organophosphate pesticides are potent nerve agents. Improper use and poor monitoring lead to intentional and unintentional exposures. Most suicide cases are due to intentional consumption of OP. The book has covered the usage, consumption, and pathophysiology of OP exposure as well as recent advances in the prophylactic approach to combat human poisoning. It has also discussed the usage of oximes and their limitation, which is a major drawback in the treatment of OP poisoned cases.

Phthalates are esters of phthalic acid, compounds mainly used as plasticizers. Phthalates are widely used chemicals that are of significant research interest as their exposure causes various consequences for human health. The content discusses the toxicity of phthalates and its potential risks to human health.

We have also covered the important topic of electromagnetic field mobile phone radiation toxicity, where we have discussed the biological and health effects of electromagnetic fields (EMFs); it is usually the case that biology and health science are of the first concern, and engineering and physics often come second.

The book has sufficient up-to-date research and new critical perspectives of interest.

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About the Editor

Tanu Jindal completed her PhD in ecotoxicology from the Department of Zoology, University of Delhi, in 1999. She is group additional pro vice chancellor (R&D) and director of Amity Institute for Environmental Toxicology, Safety and Management (AIETSM) and Amity Institute of Environmental Sciences (AIES) at Amity University, Noida. She is responsible for PhD, MSc, and BSc environmental science courses to promote environmental research and studies with syllabus covering important and current area of environmental science. She has filed six patents on lysimetric-device, apparatus to estimate the loss of xenobiotics by volatilization and mineralization, natural pesticide, photochemical method to dispose of dilute pesticide waste and cost effective water testing kit. Prof. Jindal has completed projects such as Yamuna, Hindon, Ghagghar River and groundwater contamination through pesticides, MoEF&CC; Contamination of Soil and Groundwater through Leaching of Drains in Delhi, MoES; Groundwater Contamination of chlorpyrifos in Soils at Different pH with CWRDM, DST; Environmental Monitoring Studies at Antarctica, NCAOR, Goa; and Impact of Electro Magnetic Radiation, DST. Her recent initiatives are projects such as Development of Lysimeter, DST; Air Pollution Monitoring through CAAQMS-UPPCB; and Development of Emission Factors, CPCB. Establishment of the Pesticide Referral Laboratory and MRL fixation of pulses and spices were her research endeavors at ICAR. Her areas of expertise are ISO-17025, GLP-studies, radio and stable isotope tracer techniques, and GCMS and LCMS studies. She taught pesticides chemistry and toxicology at Delhi University for 7 years to MSc students. Prof. Jindal holds membership of eminent scientific societies. She has traveled extensively nationally and internationally presenting papers and has publications in refereed journals with high impact factor. She has published more than 38 papers in reputed journals and has been serving as an editorial board member of repute. Editor of The Year Award 2017 by MTRES, Excellence in Research and Teaching Award 2017 by National Environmental Science Academy, prestigious Scientist of the year Award – 2015, Environmentalist of the Year Award 2014 by NESA, New Investigator Award presentation at ACS, DST Young Scientist Award Project are to her credit. She has received travel awards from CSIR, DST, and INSA.

Electromagnetic Field Mobile Phone Radiation Toxicity

Neha Singh and Tanu Jindal

Abstract Over the last decade, the exponential growth of mobile communication has been accompanied by a parallel increase in density of electromagnetic fields (EMF). The continued expansion of mobile phone usage raises important questions as EMF, especially radio frequency (RF), have long been suspected of having biological effects. Because mobile phones and other wireless gadgets are held close to the body and are also used very frequently, these devices are potentially the most dangerous sources of EM radiation. This gave rise to an increasing concern for any unknown effects that may prove to be detrimental to human health.

Chronic exposure of humans to EMF emitted from various mobile phone sets and mobile towers has been understood to be a stress phenomenon that is linked with pathogenesis of various disease conditions including cancer. However, there is paucity of conclusive data that correlates the dosimetry of electromagnetic fields to biological effects produced by exposure.

Genotoxic effects are thought to be significant contributors to and/or initiators of carcinogenesis. Among the potentially genotoxic effects reported are that 2450 MHz radiation can cause DNA damage, chromosomal damage, cell death (apoptosis), reproduction ability, blood-brain barrier effect, hypersensitivity, etc. These effects are more fatal in children as their brain cells are more vulnerable.

Cell phones are becoming absolutely essential, but at the same time, radiation exposure needs to be monitored, and the public should be aware of its harmful impacts on health for its judicious use.

Keywords Blood-brain barrier · Cell death · DNA damage · Electromagnetic fields (EMF) · Hypersensitivity · Radio frequency (RF)

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_1

1 Introduction

There are 2.71 billion smartphone users in the world today out of which 66% of the users are addicted to their phones. As a result of enormous outburst in usage of cell phones across the globe, many experiments and researches have been conducted and are also in process to identify the plausible biological effects of these radiations especially on the brain as it absorbs maximum radiations in the body. The effect of mobile and cell tower radiations on biological entities is a subject of interest and study worldwide (Chauhan et al. 2018). In this chapter, we aim to review some research done which investigated the effect of radio frequency electromagnetic field (RF-EMF) radiations, emitted by mobile and cell towers on the brain showing evidences for causing brain tumour/cancer and blood-brain barrier and DNA damage of brain cells.

When we discuss the biological and health effects of electromagnetic fields (EMF), it's usually the case that biology and health science is of the first concern, and engineering and physics often comes second. It is necessary, however, to keep in mind that we are addressing interaction between electromagnetic field and the body. The physical interaction should be characterized and quantified first; only then the substantial phenomena occurring in tissue or cells are better understood. With all these quantitative descriptions, we could better define the biological and health effects that are subsequently observed. Thermographic methodology adopted directly measures elevations in temperature due to absorbed energy in the radio frequency-exposed object. If the temperature rise is abrupt enough to neglect heat conduction, the temperature rise is directly proportional to the SAR at any point (Taki et al. 2001).

2 Radiation Levels from Base Stations

In the natural world, we all are surrounded by electromagnetic fields emitted from various sources. However, high-intensity EMF radiation is emitted from mobile phones and cell towers and masts which may adversely affect our health (Singh and Jindal 2019). These radiations are not visible nor audible, but when one stays near such mobile base stations, they expose themselves to the artificial EMF radiated.

Artificial EMFs are generated from mobile and cell towers installed all around us by mobile operators for better reception. These towers are commonly known as base stations. A base station having a single or multiple antenna is a point of communication for mobile/cellular phones that receives and transmits the signals of a mobile network to the customers' phones. To obtain maximum coverage and signal strength, the antennae are fixed with certain power, gain and orientation on the base station. The mobile operators also take care that the there is no obstacles around the base

stations which may force it to emit higher power to cover the complete reception area (Singh et al. 2019). This often results in placing the base stations near residential areas, marketplaces, industrial areas and even in building roofs, where a large number of users are present. The presence of a base station near residential areas is known to have health effects on people residing in its vicinity. In some studies, it is observed that the people living in close proximity of a cellular mast (about 50–300 m) may show associations with several health disorders like depression, memory loss, fatigue, sleeping disorder, concentration difficulty, dizziness and likewise (Santini et al. 2002; Oberfeld et al. 2004).

3 Health Effects of Mobile Radiations

3.1 Thermal Effects

The effects of RF radiation depend upon its penetration potential in the human body and absorption power of different tissues. Absorption of RF-EMF radiation depends on the frequency of transmission, power density, distance from the radiating source and the organism size, shape and water content (Sivani and Sudarshanam, 2012). It is possible that different types of cells from different species might have a different response and sensitivity to RF-EMF exposure (Nylund and Leszczynski, 2006). The propagation of EMF through biological tissue differs from the propagation through free space and depends on the frequency and on electromagnetic properties of tissue. Earlier it was assumed that heating was the only effect that occurs due to RF radiations. Mobile phone is the only radiating material which always stays so close to a human body. Thermal effects are well understood effects of RF radiation when temperature increases in a living tissue by rotation of polar molecules induced by EMF. Due to rise in temperature, many changes have been characterized in many studies.

3.2 Blood-Brain Barrier Effects

There have been evidences suggesting that phosphorylation and increased expression of stress protein Hsp27 which regulates permeability of the blood-brain barrier is induced due to mobile phone radiations. This mechanism when occurs for a long period of time continuously may result in a health hazard due to the possible accumulation of brain tissue damage (Leszczynski et al. 2002). Studies have also shown that mobile phone radiations alter the blood-brain barrier, resulting in albumin discharge immediately and 14 days after 2 h of exposure (Eberhardt et al. 2008).

3.3 Electromagnetic Hypersensitivity

One area of research related to effects of electromagnetic field (EMFs) stresses on the difference between electromagnetic hypersensitive people and nonsensitive people as well as their physiological and psychological reactions when subjected to various stimuli (Augner et al. 2009). The latest findings show that electromagnetic hypersensitive people may report significantly reduced intracortical facilitation (Landgrebe et al. 2007). In other words, it could be said that some people may show higher sensitivity or reactivity when exposed to EMFs. However, not all studies back up these findings (Inomata-Terada et al. 2007).

3.4 Genotoxicity

The effects of RF-EMF on DNA damage have been reported in various studies in the last decade. A study showed that chronic exposure to microwave radiation at nonthermal levels causes statistically significant increase in DNA single-strand breaks in rat brain cells, (Paulraj and Behari 2006). DNA damage was reported in cells exposed to 24 h of low-intensity RF radiation which results in accumulated gene mutation (Phillips et al. 2009). Phillips et al. (2009) investigated the effects of acute exposure to RF-EMF radiation in DNA strand breaks in brain cells of rat.

3.5 Nervous System

Various studies have been conducted showing correlations between low levels of RF-EMF and damage of cell tissues as well as DNA which has been linked to brain tumour/cancer. Hardell et al. (2008) have reported carcinogenic effects of RF radiations emitted from mobile phones and likely to cause brain tumours with long-term usage as the brain being a near field organ. In another study Cardis et al. (2008) analysed the possible risks of RF exposures from mobile phones on the brain which is the region with greatest energy absorption. Children are more susceptible to the risk of DNA damage and subsequent cancers due to the effects of these RF-EMF radiations since their nervous system is still in the developmental stage and their rate of cellular activity and division is more rapid as compared to the adults (Sage and Carpenter 2009).

3.6 Immune System

Many of the effects of EMF radiation exposures is associated with the formation of free radicals which are known to be the factors that cause oxidative damage of various cellular structures and molecules (Aitken et al., 2005). These free radicals can result in the induction of reactive oxygen species (ROS) in human spermatozoa and may cause damage that can lead to immotility of sperms (Avendano et al., 2012). In a study, it was investigated if radio frequency (RF) exposure affects human immune system cells, and results showed significant changes in leucocyte behaviour (Aly et al., 2011). It has been well known that the human immune system plays an important role in fighting against infection and cancer.

3.7 Effect on Children

Children are the ones who are most affected as depth of the cell phone's radiation absorption into the brain is the largest for 5-year-old children, little less for 10-year-old children and least for an adult (Gandhi et al. 1996). In this paper we aim to review some research done which investigated the effect of radio frequency electromagnetic field (RF-EMF) radiations, emitted by mobile and cell towers on the brain showing evidences for having brain tumour/carcinogenic effect on the brain, mutation/DNA damage of brain cells and programmed cell death.

3.8 Short-Term Heath Effects

There are also some short-term health effects that are known to be caused due to the use of mobile phones or keeping the phones close to the body. EMF radiations from cell towers and mobile phones may cause negative health impacts like headache, sleep disturbance, lack of concentration, dizziness, short-term memory loss, etc. A study conducted on medical students using mobile phones for more than 2 h showed signs of poor quality of sleep and even insomnia (Saxena et al. 2014). Another study conducted near a mobile base station showed delay in responses and effect on memory (Hutter et al. 2014).

4 Conclusions

So far, the lack of consistent and validated evidence makes it difficult to establish any cause-effect relationship between RF-EMF exposures and human health consequences. In vivo animal studies, one of the most significant effects of RF exposures

is disruption of the blood-brain barrier, but it is difficult to extrapolate the results to humans. Many studies claim that the effects displayed due to EMF exposures are more likely to be thermal and there are no convincing evidences of acute or chronic effects of EMF exposures on humans. This topic of research is ever since debatable and requires more consistent and reproducible evidences to prove ill effects of EMF exposures. Further, it still needs to be determined whether these biological effects could cause health hazards for which long-term studies and analysis are much needed. But the setting up of mobile towers all over in a haphazard manner has been reported to have some substantial effects on humans which could not be ignored. The government should be strict regarding the policies and the permissible limits of the EMF radiations from the cell towers to remove any chances of plausible effects.

References

A. A. Aly, S. B. Deris, & N. Zaki (2011). The effects on cells mobility due to exposure to EMF radiation. Advanced Computing: An International Journal

C. Augner, M. Florian, G. Pauser, G. Oberfeld, G.W. Hacker, (2009). GSM Base Stations: Short-Term Effects on Well-Being; Bioelectromagnetics

C. Avendano, A. Mata, C. A. Sanchez Sarmiento et al. (2012). Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. Fertil. Steril.

C. Sage, D. O. Carpenter, (2009). Public health implications of wireless technologies; Pathophysiology

Chauhan A, Garg S, Ranjan A, et al. (2018) Prevalence of microbial contamination of mobile cell phones in general population of Delhi, India. J Exp Clin Microbiol 2018; 1(1):12-15.

D. Leszczynski, S. Joenväärä, J. Reivinen, R.Kuokka, (2002). Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: Molecular mechanism for cancer- and blood-brain barrier-related effects; Differentiation

E. Cardis, I. Deltour, S. Mann, M. Moissonnier, M. Taki, N. Varsier, K. Wake, J. Wiart, (2008). Distribution of RF energy emitted by mobile phones in anatomical structures of the brain; Physics in Medicine and Biology

G. Oberfeld, et al. (2004). The microwave syndrome-further aspects of a Spanish study. Biological Effects of EMFs, Kos Greece.

H.P. Hutter, H. Moshammer, P. Wallner, M. Kundi (2014). Subjective symptoms, sleeping problems, and cognitive performance in subjects living near mobile phone base stations; Occupational Environment and Medicine

J.L. Eberhardt, B.R. Persson, A.E. Brun, L.G. Salford, L.O. Malmgren, (2008). Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones; Electromagnetic Biology Med

J.L. Phillips, N.P. Singh, H. Lai, (2009). Electromagnetic fields and DNA damage. Pathophysiology

L. Hardell, M. Carlberg, K. H. Mild, (2008). Methodological aspects of epidemiological studies on the use of mobile phones and their association with brain tumors; Open Environment Sciences

M. Landgrebe, S. Hauser, B. Langguth, U. Frick, G. Hajak, P. Eichhammer. (2007). Altered excitability in subjectively electrosensitive patients: Results of a pilot study; J Psychosom Res

M. Taki,; S. Watanabe, K. Wake, (2001). Characteristics, Dosimetry & Measurement of EMF.

N. Singh, T. Jindal, (2019). Impact of Mobile Phone Radiations on Germination Rate and Early Growth of Vigna Radiata (Mung Bean); Journal of Advanced Research in Dynamical and Control Systems

N. Singh, A. Ranjan, T. Jindal, (2019). Radio Frequency Emf Exposure Due To Mobile Tower Base Stations: Measurements And Analysis In Delhi- National Capital Region, India; Pollution Research

O.P. Gandhi ; G. Lazzi; C.M. Furse (1996). Electromagnetic absorption in the human head and neck for mobile telephones at 835 and 1900 MHz; IEEE Transactions on Microwave Theory and Techniques

R. Nylund, & D. Leszczynski, (2006). Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome-and proteome-dependent. Proteomics

R. J. Aitken, L. E. Bennetts, & D. Sawyer (2005). Impact of radiofrequency electromagnetic radiation on DNA integrity in the male germline. International Journal of Andrology

R. Paulraj, J. Behari, (2006). Single strand DNA breaks in rat brain cells exposed to microwave radiation; Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

R. Santini, P. Santini, J.M. Danze, P. Le Ruz, & M. Seigne, (2002). Study of the health of people living in the vicinity of mobile phone base stations: Incidence according to distance and sex, Pathology Biology

S. Inomata-Terada, S. Okabe, N. Arai, R. Hanajima, Y. Terao, T. Frubayashi, Y. Ugawa. (2007). Effects of high frequency electromagnetic field (EMF) emitted by mobile phones on the human motor cortex; Bioelectromagnetics

S. Sivani, & D. Sudarsanam (2012). Impacts of radio-frequency electromagnetic field (RF-EMF) from cell phone towers and wireless devices on biosystem and ecosystem-a review. Biology and Medicine

Y. Saxena, A. Shrivastava, P. Singh (2014). Mobile usage and sleep patterns among medical students, Indian Journal of Physiology and Pharmacology

Polar Ecotoxicology: Sources and Toxic Effects of Pollutants

Laxmikant Bhardwaj and Tanu Jindal

Abstract Anthropogenic activities in Antarctica have increased over the last two to three decades. Over the last 50 years, the development of tourism and research has affected the ecosystems of Antarctica through oil spills, fuel combustion, sewage discharge, and waste incineration. Antarctica is often thought a pristine land untouched by human disturbance and a continent of driest, windiest, coldest, and emptiest land which is largely covered by the Antarctic ice sheet. Persistent organic pollutants (POPs) are toxic compounds that resist photolytic, chemical, and biological degradation. POPs persist in the environment for a long time and are classified as a chlorinated pesticide, polychlorinated biphenyls (PCBs), and dioxins. They travel a long distance through a long-range transport mechanism.

At present, the levels of most persistent pollutants in Antarctic inhabitants are lesser than those in related species from other continents. Several factors such as population growth, global warming, and industrial developments in different countries of the Southern Hemisphere increase the impact of POPs in Antarctica. It is necessary to monitor POPs' contamination which is done by human activities to control further contamination. The presence of these persistent pollutants in Antarctica is an alarming situation and needs to be studied further to maintain the pristine environment of Antarctica. The presence of these persistent pollutants may be attributed to anthropogenic sources, orographic effects, migratory birds, and biomagnification.

Keywords Antarctica · Toxicity · Anthropogenic activity · Persistent organic pollutants (POPs)

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_2

1 Introduction

Antarctica is the southernmost as well as the remotest continent on Earth and geographically isolated from other continents. It is the fifth-largest of the seven continents. It is situated over the South Pole at latitude 66° 30' south. Barrientos-Díaz et al. (2008) reported that ~2% of the area of Antarctica is available for colonization by Antarctic inhabitants. In East Antarctica, 26 million km^3 of the ice sheet is present, and it is 83% of total ice in Antarctica (Hodgson et al. 2001). Cary et al. (2010) have reported that frequent freeze-thaw cycles, different pH levels, low temperatures, low organic nutrients, periods of prolonged dark in winter, ultraviolet radiation, water availability, and strong winds are found in Antarctica.

Since Antarctica is geographically isolated from other continents and distanced from human activities, it is a perfect place for collecting the baseline data for understanding the global environment. It also contains information about earth since its existence. It delivers the cleanest atmospheric environment available for the study of chemicals deposited in snow and ice sheets. Now Antarctica is facing the challenges of climate change, global warming, and biodiversity loss largely due to anthropogenic factors. Scientifically, it is a key component of the Earth's system to understand both present and past atmospheric weather and climate patterns, oceanic circulation patterns, and complex interactions between a wide range of ecosystems. Nearly 90% of the world's ice is locked up in the sheets of Antarctica, and about 70% of the freshwater is in the form of ice in Antarctica. The temperature over large parts of Antarctica is below the melting point and as a result of the precipitation almost always occurs in the form of snow.

POPs are man-made semi-volatile organic compounds. These pollutants may occur in different forms like natural as well as anthropogenic. They have fatal properties and are used worldwide. They are migrating from one continent to another by the process called "grasshopper effect." It is a process in which pollutants evaporate from a warmer region then enter into the atmosphere and condensed in a colder region (Hund 2014). These persistent pollutants are transported through air, water, and migratory species (Barrie et al. 1992; Fuoco et al. 2009; Bhardwaj et al. 2018). These pollutants can accumulate towards the pole and pass from one species to the next through the food chain (Wania and Mackay 1996).

PCBs, DDT, and dioxins are the most well-known POPs, and they are produced intentionally or unintentionally. Some PCBs which are used as heat exchange fluids in electrical transformers and large capacitors, additives to paints, lubricants, and DDT, which is still used to control mosquitoes that carry malaria in some parts of the world, are intentionally produced POPs. But dioxin is unintentionally produced POPs and is produced from the combustion of medical waste and municipal waste and some industrial processes. DDT is one of the most controversial POPs and was banned from 1972 in many countries, but it is still in use in some developing countries. It was also used in World War II to protect soldiers from malaria. In recent years many researchers are showing an interest in developing a low-cost method for the determination of POPs in trace amounts because of their bioaccumulation,

transformation, and toxicity. Less efforts have been made in the detection and analysis of POPs during recent decades; due to the continued development and refinement of specific techniques, a wide array of undetected contaminants of emerging environmental concern need to be identified and quantified in various Antarctic environmental components.

2 Sources of POPs in Antarctica

The sources of direct contamination in Antarctica are high-temperature incineration, food waste, wastewater treatment plant, and all waste disposal methods with sewage effluent often being a studied source of Antarctic POPs. Research stations themselves are also contamination sources with a wide range of synthetic organic compounds present in the building material, fittings, and external finishes and act as local point sources (Larsson et al. 1992; Hale et al. 2008). Jacob (2013) has reported that the POPs' concentration increases in the Antarctic environment due to the huge disposal of polycyclic aromatic hydrocarbons (PAHs) and brominated flame retardants (BFR) compounds which are arising from the combustion of fossil fuels. Dioxins and dibenzofurans are released into the atmosphere by natural processes such as volcanic eruption.

POPs are currently used in agriculture, industrial processes, and disease control but also contaminate all states of the world. Due to the fat-soluble nature, POPs easily accumulate in human tissue, leading to a continuously increasing disease risk throughout the lifespan, especially in the overweight population (Kim et al. 2010).

Fuoco et al. (1996) have reported that sediment, soil, and snow accumulate the valuable information of preceding environmental and climatic proceedings, known as recorders of POPs.

3 Toxic Effect of POPs in Antarctica

POPs are environmentally stable and highly toxic compounds that affect human health and the environment around the world. POPs are generated at one place but affect wildlife and people far from the place where they are used and released. POPs are endocrine disrupters and can damage the body systems like the endocrine system, reproductive system, immune system, and nervous system (Stockholm Convention Secretariat 2011). Long-term exposure to these pollutants can cause allergy and hypersensitivity, while short-term exposure of high concentration may cause illness and death. Through direct exposure and industrial accidents, these pollutants have a harmful effect on humans and may cause changes in development and behavior. People are mainly exposed to POPs through contaminated food and water and direct contact with chemicals. Crinnion (2011) stated that the major source of

human exposure to PCBs is through the dietary intake of contaminated foods and through inhalation of airborne pollutants.

POPs are carcinogenic in nature and can cause breast, leukemia, and pancreatic cancer. These chemicals cause several complications in female such as disbalance of mensuration cycle, preterm delivery, miscarriages, and shortened lactation period, while in the child they cause behavior problems, impaired memory, learning disabilities, and low birth weight. They are linked to several illnesses and disorders of different body systems such as the reproductive, immune, and nervous systems. The presence of these persistent pollutants in Antarctica and their accumulation into the Antarctic inhabitants may cause several problems such as genotoxicity, mutagenicity, and interference in the development of the young. Several researchers stated that POPs are linked to hypertension dyslipidemia and insulin resistance obesity (Lee et al. 2011; Penell et al. 2014; Lind et al. 2004). The toxic effect of these chemicals has been studied by Tanabe et al. (1983) in the Antarctic atmosphere from Japanese Antarctic research stations. Chiuchiolo et al. (2004) have reported DDTs in penguins, skuas, and krills.

4 Conclusion and Path Forward

POPs' concentration has been found to be highly different in different regions of Antarctica, and it depends on the fate of these pollutants and climate change. For decreasing the risk of these toxic chemicals, we need a more effective monitoring system at the research station which checks the regular emission of these pollutants. Developing countries can prevent the emission of these toxic pollutants by improving the regulations and policies related to these pollutants. This is the primary step for monitoring the emission of POPs and limiting the propagation and migration of these persistent pollutants in the polar regions. Clean energy would be an option for decreasing the risk of these toxic pollutants. The usage of fossil fuels in transport, power plant, etc. is the key emission source of these pollutants. They are emitting PCBs and PAHs which are more persistent in the environment. Developed countries should help the developing countries for the setup of clean energy such as solar power plant and nuclear power plant. This step would be helpful for controlling the emission of these pollutants and also controlling the transportation of these persistent pollutants.

Tourism and scientific expedition also affects the pristine environment of Antarctica. The usage of transport facilities such as flights, helicopters, and ships is also a partial contributor to these pollutants in the Antarctic environment. The usage of personal care products, food supplies for the Antarctic researchers, and helpers are very much prone to carrying the POPs. They could be a contributor to these pollutants in the Antarctic environment. If they are not monitored properly, in the future they would be leaving the chemical footprints in Antarctica.

A group of research focusing on the above factors is urgently required in Antarctica to identify the gap areas which highlight the recent expansions in POPs'

research. The biomagnification processes and distribution patterns of the persistent pollutants in the Antarctic inhabitants and modeling of these pollutant transports are the thrust areas to explain the risk associated with persistent pollutants in the Antarctic ecosystem.

References

Barrie, Len A., Dennis Gregor, B. Hargrave, R. Lake, D. Muir, Russell Shearer, B. Tracey, and T. Bidleman. "Arctic contaminants: sources, occurrence and pathways." *Science of the total environment* 122, no. 1–2 (1992): 1–74.

Barrientos-Díaz, Leticia, Manuel Gidekel, and Ana Gutiérrez-Moraga. "Characterization of rhizospheric bacteria isolated from Deschampsia antarctica Desv." *World Journal of Microbiology and Biotechnology* 24, no. 10 (2008): 2289.

Bhardwaj, L., Chauhan, A., Ranjan, A., & Jindal, T. (2018). Persistent Organic Pollutants in Biotic and Abiotic Components of Antarctic Pristine Environment. Earth Systems and Environment, 1–20.

Cary, S. Craig, Ian R. McDonald, John E. Barrett, and Don A. Cowan. "On the rocks: the microbiology of Antarctic Dry Valley soils." *Nature Reviews Microbiology* 8, no. 2 (2010): 129.

Chiuchiolo, Amy L., Rebecca M. Dickhut, Michele A. Cochran, and Hugh W. Ducklow. "Persistent organic pollutants at the base of the Antarctic marine food web." *Environmental science & technology* 38, no. 13 (2004): 3551–3557.

Crinnion, Walter J. "Polychlorinated biphenyls: persistent pollutants with immunological, neurological, and endocrinological consequences." *Alternative Medicine Review* 16, no. 1 (2011).

Fuoco, Roger, M. Perla Colombini, Alessio Ceccarini, and Carlo Abete. "Polychlorobiphenyls in Antarctica." *Microchemical journal* 54, no. 4 (1996): 384–390.

Fuoco, Roger, Stefania Giannarelli, Y. Wei, Alessio Ceccarini, C. Abete, Sandro Francesconi, and Marco Termine. "Persistent organic pollutants (POPs) at Ross sea (Antarctica)." *Microchemical Journal* 92, no. 1 (2009): 44–48.

Hale, Robert C., Stacy L. Kim, Ellen Harvey, Mark J. La Guardia, T. Matt Mainor, Elizabeth O. Bush, and Elizabeth M. Jacobs. "Antarctic research bases: local sources of polybrominated diphenyl ether (PBDE) flame retardants." *Environmental science & technology* 42, no. 5 (2008): 1452–1457.

Hodgson, D. A., P. E. Noon, Wim Vyverman, C. L. Bryant, D. B. Gore, P. Appleby, M. Gilmour et al. "Were the Larsemann Hills ice-free through the last glacial maximum?." *Antarctic Science* 13, no. 4 (2001): 440–454.

Hund, Andrew J., ed. *Antarctica and the Arctic Circle: A Geographic Encyclopedia of the Earth's Polar Regions [2 volumes]*. ABC-CLIO, 2014.

Jacob, Jolly. "A Review of the accumulation and distribution of persistent organic pollutants in the environment." *International Journal of Bioscience, Biochemistry and Bioinformatics* 3, no. 6 (2013): 657.

Kim, Min-Ji, Philippe Marchand, Corneliu Henegar, Jean-Philippe Antignac, Rohia Alili, Christine Poitou, Jean-Luc Bouillot et al. "Fate and complex pathogenic effects of dioxins and polychlorinated biphenyls in obese subjects before and after drastic weight loss." *Environmental health perspectives* 119, no. 3 (2010): 377–383.

Larsson, Per, Cecilia Järnmark, and Anders Södergren. "PCBs and chlorinated pesticides in the atmosphere and aquatic organisms of Ross Island, Antarctica." *Marine Pollution Bulletin* 25, no. 9–12 (1992): 281–287.

Lee, Duk-Hee, Michael W. Steffes, Andreas Sjödin, Richard S. Jones, Larry L. Needham, and David R. Jacobs Jr. "Low dose organochlorine pesticides and polychlorinated biphenyls predict

obesity, dyslipidemia, and insulin resistance among people free of diabetes." *PloS one* 6, no. 1 (2011): e15977.

Lind, P. Monica, Jan Örberg, Ulla-Britt Edlund, Linnea Sjöblom, and Lars Lind. "The dioxin-like pollutant PCB 126 (3, 3′, 4, 4′, 5-pentachlorobiphenyl) affects risk factors for cardiovascular disease in female rats." *Toxicology Letters* 150, no. 3 (2004): 293–299.

Penell, Johanna, Lars Lind, Samira Salihovic, Bert van Bavel, and P. Monica Lind. "Persistent organic pollutants are related to the change in circulating lipid levels during a 5 year follow-up." *Environmental research* 134 (2014): 190–197.

Stockholm Convention Secretariat, 2011, UNEP, Sept 2015.

Tanabe, Shinsuke, Hideo Hidaka, and Ryo Tatsukawa. "PCBs and chlorinated hydrocarbon pesticides in Antarctic atmosphere and hydrosphere." *Chemosphere* 12, no. 2 (1983): 277–288.

Wania, Frank, and Donald Mackay. "Peer reviewed: tracking the distribution of persistent organic pollutants." *Environmental science & technology* 30, no. 9 (1996): 390A–396A.

Occurrence and Toxicity of Phthalates in Different Microenvironments

Arjun Suresh and Tanu Jindal

Abstract Phthalates are a broader group of chemicals with a variety of industrial uses, from the plastic manufacturer to food products, medical equipment, toys of children, cosmetics, etc. Phthalates are overused by several industries disregarding the growing concern on the toxic nature of numerous phthalates (DEHP, BBP, DnBP, DiBP) due to their low cost, attractive features and lack of other suitable options. Due to their repetitive usage in several products, they are released from various industrial sites and contaminate the environment. Humans are generally exposed to phthalates through several pathways including ingestion, inhalation and dermal causing developmental and reproductive toxicity. Therefore, concern has been grown over its safety and its potential toxic impacts on human health. This chapter discusses the toxic effects of phthalates in numerous microenvironments including air, water and soil and human health.

Keywords Phthalate · Toxicity · DEHP · Human health

Abbreviations

BzBP	Benzylbutyl phthalate
DMP	Dimethyl phthalate
DnBP	Di-n-butyl phthalate
DEP	Diethyl phthalate
DEHP	Di-2-ethylhexyl phthalate
DnOP	Di-isononyl phthalate
BBP	Benzyl butyl phthalate

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_3

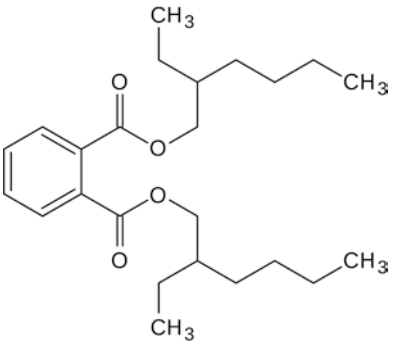
1 Introduction

Phthalates are massively used as additives and plasticizers in different industries; as the phthalates are not synthetically adhered to PVC, they can easily release and migrate into environments including air, water, food products and other substances. Humans can get exposed to phthalate either by direct contact or indirectly via its migration into other consumer products released into the environment by inhalation, ingestion and dermal pathways (Heudorf et al. 2007). The annual generation of phthalates is approximately 11 billion pounds due to their usage in broader domains including electronics items, pharmaceuticals, food packages, cosmetics, beauty products, etc. (Kim et al. 2019). Humans are frequently exposed to phthalates, and they have been detected in human saliva, blood, urine and breast milk (Högberg et al. 2008). Phthalates exert numerous biological impacts as they are endocrine disruptors (Mankidy et al. 2013). Phthalates have been reported to tarnish regular development of children and enhance the danger of various allergic ailments (Braun et al. 2013). Humans exposed to phthalates are likely to have risk of breast cancer (Hsieh et al. 2012). According to some available reports, phthalates are also having transgenerational impacts in that the impacts induced by phthalate can be transferred to consecutive generations (Zhou et al. 2017). The background information presented in this chapter provides an introductory review of the occurrence of phthalates and their toxic effects on various microenvironments.

2 Toxicity of Phthalates

In recent years, assumption over the severe effects of phthalates on organisms has been acknowledged by the scientists despite the toxicity of phthalates being already conceded since the 1950s. Research conducted on animals has exposed the basic side effects such as reproductive and developmental toxicity. The physicochemical characteristics of phthalates vary with the molecular structure that has a vapour phase with least vapour pressure. Phthalates are commonly fat-soluble substances which can impact their migration and dissolution. The phthalate exposure in organisms can occur with foodstuffs or any intracutaneous liquid or can be via the surrounding environment. Phthalate intake can take place through food products encompassing medicinal and nutritional substances. DEHP (di-2-ethylhexyl phthalate) is one of the highly used phthalates to enhance the elasticity of several plastic manufacturing industries. The chemical formula of DEHP is $C_{24}H_{38}O_4$. It has a molecular weight of approx. 390.56 $gmol^{-1}$ and contains eight carbon esters combined to a carboxylic benzene ring. DEHP is extremely toxic, with LC_{50} of 0.50 ppm that results in foetus fatality and quintessential toxicity symptoms including endocrine toxicity, cardio toxicity, neurotoxicity, renal toxicity, hepatotoxicity and ovarian and testicular toxicity in several animals (Rowdhwal and Chen 2018). Koch et al. (2006) reported DEHP as endocrine modulator in human beings (Fig. 1).

Fig. 1 Structure of DEHP



2.1 Phthalate Toxicity in Animals

In animals concern has been raised regarding deleterious impacts of phthalates; they can also induce toxic effect in the reproductive system of animals. DBP and DiBP are reported to disrupt the spermatogenesis process in zebrafish (Chen et al. 2020). DEHP has been reported as the most toxic phthalate on preliminary testicular cells of dogs. The spotlight on the hazard of phthalates on reproductive organs relies mostly on human medicines, though food making and associated animals are generally disregarded. As the phthalate toxicity is global and they are ubiquitously present in the environment as a biggest human health concern where exposure through foodstuffs, water, atmosphere and dermal contact on a regular basis is everywhere, focus should be given to farm and other associated animals as well (Cil and Akçay 2020)

2.2 Phthalate Toxicity in Humans

Exposures of phthalates in human beings are pervasive due to the lack of restrictions in the usage of phthalates in body care products (Pak et al. 2011). Phthalates has endocrine-disrupting features, and its exposure in higher concentrations results in lethal diseases such as cancer, deformations, kidney and liver damage. Phthalate can also dangerously affect other processes in humans such as metabolic, thyroid signalling and immunity. Through subsequent exposure to phthalate during foetus development, phthalates may be transmitted from mother to foetus through blood and into born babies through the breast milk, and that exposure of phthalates can severely impact the endocrine system that is crucial for several biological processes including reproductive, developmental, etc. Additionally, the presence of phthalates has also been reported in breast milk, seminal fluid and placenta.

2.3 Phthalate Toxicity in Food

Foodstuffs are the biggest source of exposure to phthalates in the human body. The European Food Safety Authority (EFSA) over a conjecture of researchers on food products, additives and other foodstuffs has set the tolerable daily dosage (TDI) for several dominant phthalates and studied that exposure of phthalates in the human body via various foodstuffs is within the range approximately between 0.03 and 0.2 mg/kg of body weight. Customarily phthalates utilized for foodstuffs including tubes, food packaging and disposables have been reported as a biggest source of phthalate contamination (EFSA 2005).

2.4 Phthalate Toxicity in Water

The aquatic environment is a compelling source of phthalates containing the wastewater effluent discharge, landfill leachate and precipitation. There has been no record found over the presence of phthalates at national levels to identify their hazardous concentration levels. The environmental quality standard for surface water and standards for potable drinking water limits the values for phthalates. Plastic containers constructed using PVC-consisting phthalates utilized as plasticizers have been used around the world for packaging of drinking water. The concern over the usage of plastic bottles globally has been consistently growing over the recent years (Imran et al. 2020).

2.5 Phthalate Toxicity in Cosmetics

Phthalates have been broadly used in cosmetic industries including skincare products (moisturizers, softeners, shampoos, etc), nail paints and sealants. Numerous phthalates have been reported in cosmetic materials such as DEHP, DEP, DBP and DBB. DEHP has been reported in 67 cosmetics, and 309 patents were held involving 120 nail paints and enamels and 27 manicuring development. These substances may severely impact the human health via direct dermal contact such as skin, hair, nails and mucous membranes.

2.6 Phthalate Toxicity in Soil

The major sources of phthalates in soil are agriculture irrigation, pesticides and industrial effluents. There are several routes through which phthalates can enter into the soil. The retention and migration of phthalates in soil depend on different kinds

of soil including weather parameters (temperature, humidity); anthropogenic influences and land usage impact the soil by disintegration and leaching of phthalates into the soil system. The presence of phthalate concentrations significantly impacts the soil microorganisms which are critical for nutrient recycling, pest control and managing the soil texture. The lysimetric studies reported the presence of DEHP in the soil as well as leachate pointing out that it could transmit deeper into soil profile and can cause the risk of groundwater contamination (Suresh et al. 2019).

2.7 Phthalates Toxicity in Indoor Air and Dust

Humans used to stay indoors relatively longer, and indoor sources of contamination are generally linked with prohibited ventilation system at home and low degradation rate of contaminants which ultimately results in higher concentrations of pollutants. Indoor air has been reported as the most potential source of environmental risk to humans. Several endocrine-disrupting substances including plastic products, households and detergents are found as critical indoor pollutants (less studied). Several studies reported phthalates as dominant air pollutants, in that DEP and DnBP have been reported at higher concentrations in air as well as dust (Rudel et al. 2003) (Table 1).

Table 1 Concentrations of phthalates in the environment and their toxic effects on human health

SN	Sources	Phthalate compounds	Observed concentration	Toxic effects	References
1	River water	DEP, DMP, BBP, DEHP	313–4640 ng/l	Impacts aquatic organisms (increased mortality, decreased body weight and distortion of sex ratio in embryos)	Selvaraj et al. (2015)
2	Indoor air	DEP, DBP, DEHP	Less than 10 ppb	Upper airway irritation and asthma	Chou and Wright (2006)
3	Soil	DMP, DEP, DnBP, DnOP, DEHP and BBP	0.032–6.29 mg/μg	Carcinogenic risk in humans through dietary pathways	L Niu et al. (2014)
4	Foodstuff (grain, bread and cereal products)	DEHP	300 ug/kg	Reproductive toxicity in adults, insulin resistance and type II diabetes, obesity, allergy, asthma, cancer	Serrano et al. (2014)

3 Conclusion

The presence of phthalates in the environment is hazardous for human health and the ecosystem. Over the past decades, researches have indicated that phthalates can leach into the ecosystem from varying sources and affect human health and the environment. Measures of phthalate toxicity vary widely according to phthalate type and its molecular structure. Leaching of phthalate can lead to contamination of food, air, soil, water, etc. which ultimately disturbs human health and environment. Various studies available on the human health impact of phthalate are showing evidence of a wide range of adverse effects in the urinary tract, semen, pregnancy, reproductive tract, kidneys, lungs, foetus and heart. Further studies have to be carried to understand the detailed pathway of phthalate degradation to develop regulatory measures.

References

European Food Safety Authority (EFSA). (2005). Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *EFSA Journal, 3*(10), 282.

Heudorf, U., Mersch-Sundermann, V. and Angerer, J., 2007. Phthalates: toxicology and exposure. *International journal of hygiene and environmental health*, *210*(5), pp.623–634.

Imran, M. A., Tong, Y., Hu, Q., Liu, M., & Chen, H. (2020). Effects of persulfate activation with pyrite and zero-valent iron for phthalate acid ester degradation. *Water, 12*(2), 354.

Kim, H., Nam, K., Oh, S., Son, S., Jeon, D., Gye, M.C. and Shin, I., 2019. Toxicological assessment of phthalates and their alternatives using human keratinocytes. *Environmental research*, *175*, pp.316–322.

Högberg, J., Hanberg, A., Berglund, M., Skerfving, S., Remberger, M., Calafat, A.M., Filipsson, A.F., Jansson, B., Johansson, N., Appelgren, M. and Håkansson, H., 2008. Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. *Environmental health perspectives*, *116*(3), pp.334–339.

Mankidy, R., Wiseman, S., Ma, H. and Giesy, J.P., 2013. Biological impact of phthalates. *Toxicology letters*, *217*(1), pp.50–58.

Koch, H.M., Preuss, R. and Angerer, J.D., 2006. Di (2-ethylhexyl) phthalate (DEHP): human metabolism and internal exposure–an update and latest results 1. *International journal of andrology*, *29*(1), pp.155–165.

Braun, JM, et al., 2013. Phthalate exposure and children's health; Curr. Opin. Pediatr. 25, 247–254

Hsieh, T.H., Tsai, C.F., Hsu, C.Y., Kuo, P.L., Hsi, E., Suen, J.L., Hung, C.H., Lee, J.N., Chai, C.Y., Wang, S.C. and Tsai, E.M., 2012. n-Butyl benzyl phthalate promotes breast cancer progression by inducing expression of lymphoid enhancer factor 1. *PloS one*, *7*(8), p.e42750.

Zhou, C., Gao, L. and Flaws, J.A., 2017. Exposure to an environmentally relevant phthalate mixture causes transgenerational effects on female reproduction in mice. *Endocrinology*, *158*(6), pp.1739–1754.

Rowdhwal, S.S.S. and Chen, J., 2018. Toxic effects of di-2-ethylhexyl phthalate: an overview. *BioMed research international*, *2018*.

Pak, V.M., McCauley, L.A. and Pinto-Martin, J., 2011. Phthalate exposures and human health concerns: A review and implications for practice. *AAOHN journal*, *59*(5), pp.228–235.

Rodgers, K.M., Rudel, R.A. and Just, A.C., 2014. Phthalates in food packaging, consumer products, and indoor environments. In *Toxicants in food packaging and household plastics* (pp.31–59). Springer, London.

Suresh, A. and Jindal, T., 2020. Phthalate Toxicity.

Latini, G., 2005. Monitoring phthalate exposure in humans. *Clinica Chimica Acta*, *361*(1–2), pp.20–29.

Ventrice, P., Ventrice, D., Russo, E. and De Sarro, G., 2013. Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity. *Environmental toxicology and pharmacology*, *36*(1), pp.88–96.

Schettler, T.E.D., 2006. Human exposure to phthalates via consumer products. *International journal of andrology*, *29*(1), pp.134–139.

Lyche, J.L., Gutleb, A.C., Bergman, Å., Eriksen, G.S., Murk, A.J., Ropstad, E., Saunders, M. and Skaare, J.U., 2009. Reproductive and developmental toxicity of phthalates. *Journal of Toxicology and Environmental Health, Part B*, *12*(4), pp.225–249.

Liu, X., Shi, J., Bo, T., Zhang, H., Wu, W., Chen, Q. and Zhan, X., 2014. Occurrence of phthalic acid esters in source waters: a nationwide survey in China during the period of 2009–2012. *Environmental pollution*, *184*, pp.262–270.

Al-Saleh, I., Shinwari, N. and Alsabbaheen, A., 2011. Phthalates residues in plastic bottled waters. *The Journal of toxicological sciences*, *36*(4), pp.469–478.

Koo, H.J. and Lee, B.M., 2004. Estimated exposure to phthalates in cosmetics and risk assessment. *Journal of Toxicology and Environmental Health, Part A*, *67*(23–24), pp.1901–1914.

He, L., Gielen, G., Bolan, N.S., Zhang, X., Qin, H., Huang, H. and Wang, H., 2015. Contamination and remediation of phthalic acid esters in agricultural soils in China: a review. *Agronomy for Sustainable Development*, *35*(2), pp.519–534.

Rudel, R. A., Camann, D. E., Spengler, J. D., Korn, L. R., & Brody, J. G. (2003). Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environmental Science & Technology, 37*(20), 4543–4553.

Chou, K. and Wright, R.O., 2006. Phthalates in food and medical devices. *Journal of Medical Toxicology*, *2*(3), pp.126–135.

Niu, L., Xu, Y., Xu, C., Yun, L. and Liu, W., 2014. Status of phthalate esters contamination in agricultural soils across China and associated health risks. *Environmental Pollution*, *195*, pp.16–23.

Selvaraj, K.K., Sundaramoorthy, G., Ravichandran, P.K., Girijan, G.K., Sampath, S. and Ramaswamy, B.R., 2015. Phthalate esters in water and sediments of the Kaveri River, India: environmental levels and ecotoxicological evaluations. *Environmental geochemistry and health*, *37*(1), pp.83–96.

Serrano, S.E., Braun, J., Trasande, L., Dills, R. and Sathyanarayana, S., 2014. Phthalates and diet: a review of the food monitoring and epidemiology data. *Environmental Health*, *13*(1), p.43.

Mariana, M., Feiteiro, J., Verde, I. and Cairrao, E., 2016. The effects of phthalates in the cardiovascular and reproductive systems: a review. *Environment international*, *94*, pp.758–776.

Pan, T.L., Wang, P.W., Aljuffali, I.A., Hung, Y.Y., Lin, C.F. and Fang, J.Y., 2014. Dermal toxicity elicited by phthalates: evaluation of skin absorption, immunohistology, and functional proteomics. *Food and chemical toxicology*, *65*, pp.105–114.

Cil, K.T.P.A.B. and Akçay, A.F.E., 2020. Companion animals get close to the toxic aspects of antropogenic world: cytotoxicity of phthalates and bisphenol A on dog testicular primary cells.

Suresh, A., Jindal, T. and Khan, S.A., 2019. An Integrated Approach for Risk Assessment of Groundwater Contamination Using Lysimetric Studies. *Pollution Research*, *38*(2), pp.491–494.

Chen, H., Chen, K., Qiu, X., Xu, H., Mao, G., Zhao, T., Feng, W., Okeke, E.S., Wu, X. and Yang, L., 2020. The reproductive toxicity and potential mechanisms of combined exposure to dibutyl phthalate and diisobutyl phthalate in male zebrafish (Danio rerio). Chemosphere, p.127238.

Suresh, A. and Jindal, T., 2019. Lysimetric Studies to Estimate the Solute and Contaminant Transport in Hydrogeological Profile. *Ecology, Environment and Conservation*, *25*, pp.S102–S105.

Cytotoxic and Antibiotic Properties of Cyanobacterial Extracts

Abhishek Chauhan, Anuj Ranjan, Rupesh Kumar Basniwal, and Tanu Jindal

Abstract The current world has many challenging situations that have to be dealt with very carefully. Among these states of affairs, the most prevailing is to discover novel sources of drugs, particularly, natural resources, which are supposed not to have adverse effects on human beings and society. During the last few decades, therefore, micro-algae are chosen among the researchers throughout the world. These photosynthetic microorganisms can yield proteins, carbohydrates, and lipids as a result of photosynthesis, thus referred to as important biological resources having a wide range of biotechnological applications in the modern world due to their ability to grow rapidly even in harsh environmental conditions. A search of these organisms for medicinal purposes has revealed important chemical prototypes for the finding of new agents, stimulating the use of refined physical techniques and new syntheses of molecules with the pharmaceutical application for human welfare. Several strains such as *Anabaena*, *Lyngbya*, *Calothrix*, *Spirulina*, *Nostoc*, *Hapalosiphon*, *Phormidium*, and *Oscillatoria* have been identified so far which can produce a wide variety of secondary metabolites having therapeutic potentials. These organisms are even being altered genetically using biotechnological interventions for the production of various active compounds having antibacterial activity such as bacteriocin, ambigol A, parsiguine, hapalindole, and hormothamnin A; antifungal activity such as fischerellin A, phytoalexin, tolytoxin, laxaphycins, ambiguines, and calophycin; and cytotoxic activity such as scytophycins, tantazoles, tolytoxin, acutiphycins, toyocamycin, and tubercidin.

Keywords Cyanobacteria · Cytotoxic activity · Bioactive compounds · Biotechnological interventions

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_4

1 Introduction

Cyanobacteria also known as blue-green algae (BGA) are photosynthetic microorganisms that have grown on earth for more than 3 billion years with some genera showing only minor morphological changes since that time. It has been estimated that about 2000 strains of freshwater and marine BGA are distributed all over the world. They show notable ecological diversity. Because of extensive eutrophication of lakes, ponds, and some parts of oceans, BGA often form blooms, which lead to water hygienic problems (Chorus and Bartram 1999; Duy et al. 2000). They may cause unpleasant tastes and odors through the excretion of volatile compounds (Jones and Korth 1995). Several genera of cyanobacteria form toxic water blooms and different cyanobacterial toxins have been characterized (Hunter 1995; Goyal et al. 2015; Kaushik and Chauhan 2009). Microalgae are known for several biological activities and are used for the production of various value-added products worldwide. Pharmaceutical drug discoveries (New Drug Discovery Research) for the past 40 years depended deeply on the procedure of empirical transmission of a large number of pure antimicrobial compounds to provide new leads. BGA is a group of extraordinary diverse Gram-negative prokaryotes that originated 3.5 billion years ago and are among the oldest phototrophic organisms. Their development without organic substrate can be a practical advantage over the microorganisms. Cyanobacteria do not require carbon or energy sources in their growth medium. Thus, they require only a basic inorganic medium, which has several logical advantages when performing the mass culture and purification of active compounds. Secondary metabolites refer to those compounds that are not used by the organisms for their primary metabolisms. Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetic importance. Secondary metabolites include several types of compounds that may act as hormones, antibiotics, allelochemicals, toxins, and biotoxins that are found in surface supplies of fresh water (Carmichael 1992; Patterson et al. 1994; Moore 1996; Smith and Doan 1999). The ability of such compounds to kill bacteria and fungi has been well documented (Bonjouklian et al. 1988). The properties of secondary metabolites in nature are not completely understood (Metting and Pyne 1986; Inderjit and Dakshini 1994).

According to light-harvesting pigments of cyanobacteria includes phycobiliproteins or just phycobilins which are phycoerythrin (PE in some), phycoerythrocyanin (PEC in some), phycocyanin (PC in all), allophycocyanin (APC in all), chlorophyll a (Chl a), and carotenoids (especially β-carotene, zeaxanthin, echinenone, myxoxanthophyll, and oscilloxanthin). PE may contain only phycoerythrobilin as chromophore (C-PE) or a mixture of phycoerythrobilin and phycourobilin. All known species of cyanobacteria have some PC and APC. The growth rate and production of metabolites in cyanobacteria are influenced by cultural conditions, optimization of media, time of harvest, and physical parameters such as light and temperature, and then they can lead to greatly enhanced product yield. The generation time for cyanobacteria is relatively slow, and typical doubling time ranges from 0.3 to 1.4/day under optimum conditions (Kaushik 1987). The optimized production of

significant compounds under restricted cultural conditions is believable. The less accessibility and high cost of new generation antibiotics necessitate looking for the substances from alternative medicines with claimed antimicrobial activity. Today, most of the diseases caused by pathogens can be cured with the help of available antibiotics, but the discovery of any new antibiotic generally follow up with a course of resistance mechanism building up against it among the target organisms. This phenomenon known as "antibiotic resistance" is developing among microbial species at an appreciable rate, is a formidable complication of prudent and overuse of available antibiotics, and is imposing a serious health threat to human welfare.

Generally, all cyanobacterial vegetative cells contain carboxysomes, pseudocrystalline aggregates of the key enzymes of CO2 fixation via the reductive pentose phosphate pathway, and glycogen is a general carbohydrate reserve material of cyanobacteria. Other cellular inclusions include poly-β-hydroxybutyrate (PHB) granules, cyanophycean granules, polyphosphate granules, carboxysomes or polyhedral bodies, and gas vesicles (Stanier 1988).

It has been accepted in general that light-harvesting pigments include chlorophyll a, phycobiliproteins, phycocyanin, phycoerythrin in some, and carotenoids (especially β-carotene and zeaxanthin). Because of chlorophyll a, the cells absorb red light of 680 nm. The photosynthetic apparatus is present in the form of thylakoids, which are flattened membranous sacs located within the cell. The surface of thylakoids is studded with phycobilisomes, which contain phycobilin pigment. However, chlorophyll b and chlorophyll c are absent (Holt et al. 1994; Pelczar et al. 1993).

The cyanobacteria bear the characteristics to secrete vitamins, amino acid, fatty acid, carbohydrates, and metabolites like primary and secondary amines, histamines, histidine, tannins, terpenoids, bromophenol, and polysaccharides. Few of these compounds are proven to be biologically active (Metting and Pyne 1986). The recent examples are cyanovirin-N secreted by *Nostoc* ellipososoporum and anti-HIV glycolipids secreted by isochyrosis and bromophenol, which are secreted by *Calothrix* sp. (Jaspars and Lawton 1998).

They have become targets for screening programs in search of novel compounds of potential medicinal value. The study of cyanobacteria as a source of new chemical entities is an exciting field that needs to be investigated further.

2 Cyanobacteria as a Source of Antimicrobial Compounds

The first partly identified antimicrobial compound isolated from algae were obtained from unicellular green algae particularly, *Chlorella*, which contained a substance termed as "*chlorellin*" that exhibited inhibitory activity against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* (Pratt et al. 1944). Chlorellin is composed of peroxides of unsaturated fatty acids (Spoehr et al. 1949).

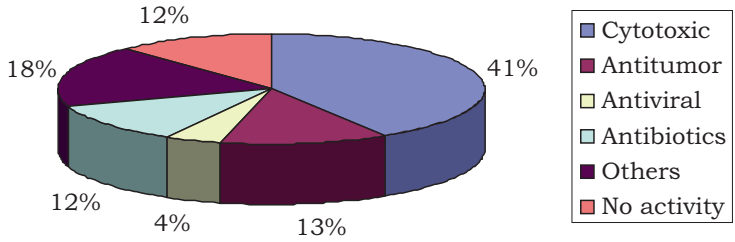

Fig. 1 Cytotoxic and Antibiotic activity of Cyanobacteria

The cultivable microorganisms for the screening of novel pharmaceuticals are of paramount importance. Historically, actinomycetes have been the most prolific producers of metabolites with significant antimicrobial activities. They also have been, not surprisingly, the most closely examined group of organisms, and at present, the yield is known compounds at a rate over 95% of all active leads discovered in primary screening (Bradner 1980). However, the search for cyanobacteria with antimicrobial activity has gained importance in recent years due to growing worldwide concern about an alarming increase in the rate of infection by antibiotic-resistant pathogen and newly emerged diseases (Hayashi et al. 1994). They can be cultured in the laboratory with relative ease to provide a consistent source of biologically active secondary metabolites (Patterson et al. 1993). Screening of cyanobacteria for antimicrobial compounds having pharmaceutical value has received ever-increasing attention. The molecules isolated from several cyanobacteria have a broad spectrum of biological activities including toxins, antibiotics, fungicides, and algicides (Browitzka 1995). Random screening of cyanobacteria will continue to play an important role in the drug discovery process for the foreseeable future (Fig. 1).

3 Bioactive Compounds Isolated from Cyanobacteria

Cyanobacterial lipopeptides include different compounds like cytotoxic (41%), antitumor (13%), antiviral (4%), and antibiotics(12%), and 18% of activity includes antimalarial, antimycotics, multidrug resistance reversers, antifeedant, herbicides, and immunosuppressive agent (Burja et al. 2001). The cyanobacteria besides producing toxins are known to have a wide array of active substances with antibacterial, antiviral, fungicidal, enzyme inhibiting, immunosuppressive, cytotoxic, and algaecidal activity isolated from cyanobacterial biomass or in some cases from the medium of laboratory cultures (Namikoshi and Rinehart 1996; Banker and Carmeli 1998)**.**

Cytotoxic Activity Fish and Codd (1994) studied the cytotoxic activity of *Phormidium* sp. (a thermotolerant cynobacterium). Falch et al. (1992) isolated polyhalogenated aromatic compound from *Fischerella ambigua* having cytotoxic

Table 1 Cytotoxic antibiotic discovered during the screening of cyanobacteria

Micro-algae	Antibiotic	References
Oscillatoria	Acutiphycins	Barchi Jr. et al. (1984)
Nostoc	Indolocarbazoles	Knubel et al. (1990)
Scytonema	Mirabilene isonitriles	Carmeli et al. (1990a)
Cylindrospermum, *Nostoc*	Paracyclophanes	Moore et al. (1990)
Scytonema	Scytophycins	Ishibashi et al. (1986) and Carmeli et al. (1990b)
Scytonema	Tantazoles	Carmeli et al. (1990c)
Scytonema	Tolytoxin	Carmeli et al. (1990b)
Tolypothrix	Toyocamycin	Stewart et al. (1988)
Tolypothrix	Toyocamycin-5′-alpha-D-glucopyranose	Stewart et al. (1988)
Tolypothrix	Tubercidin-5′-alpha-D-glucopyranose	Stewart et al. (1988)
Plectonema	Tubercidin	Stewart et al. (1988)

activity. Several compounds such as scytophycins, tantazoles, tolytoxin, acutiphycins, toyocamycin, tubercidin, etc. have been isolated so far which are having cytotoxic activity (Table 1).

4 Cyanobacterial Toxins: Cyanotoxins

Cyanobacteria are known to produce various secondary metabolites with diverse biological and biochemical properties. Cyanotoxins are cyanobacterial secondary metabolites; they are a diverse group of compounds both from a chemical and toxicological point of view. Toxicities include neurotoxicity, hepatotoxicity, cytotoxicity, and dermatotoxicity. Cyanotoxins have several main groups peptides, heterocyclic compounds (alkaloids), or lipidic compounds. The microcystins are the most common algae toxin found and are associated with *Microcystis*, *Anabaena*, *Oscillatoria*, *Nostoc*, *Hapalosiphon*, and *Anabaenopsis* species. The first known reported incidence of cyanobacteria toxin poisoning was from an Australian lake in 1878. Toxicity of cyanobacteria have been reported approximately 1000 years ago when General Zhuge Ling reported mortality in troops that drank water from a river in southern China that was green (Table 2).

Table 2 Toxin from cyanobacteria (according to Chorus and Bartram 1999)

S. No.	Cyanobacterial spp.	Toxin	Mechanisms of toxicity	References
(1). 5	*Lyngbya*, *Oscillatoria*, *Schizothrix*	Lyngbyatoxin-a (cytotoxic, dermatotoxic, gastroenteritis)	Dermonecrotic, protein kinase C activator, and potent tumor promoters	Cardellina et al. (1979) and Fujiki et al. (1981, 1984)
(2). 6	*Anabaena, Anabaenopsis*, *Aphanocapsa, Aphanizomenon*, *Arthrospira*, *Cyanobium*, *Cylindrospermopsis*, *Fischerella*, *Hapalosiphon*, *Limnothrix*, *Lyngbya*, *Microcystis*, *Nostoc, Oscillatoria (Planktothrix)*, *Phormidium*, *Planktothrix*, *Rivularia*, *Synechocystis*, *and Synechococcus*	Microcystins (hepatotoxic)	Inhibitors of protein phosphatases 1, 2A, and 3, tumor promoter, genotoxicity	Honkanen et al. (1990), MacKintosh et al. (1990) and Gulledge et al. (2002)
(3). 7	*Nodularia*	Nodularins (hepatotoxic)	Inhibitors of protein phosphatases 1, 2A, and 3, tumor promoter	Yoshizawa et al. (1990) and Gulledge et al. (2002)
(4). 8	*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Planktothrix*, *Raphidiopsis*, *Scytonema*	Saxitoxins (neurotoxic)	Blocking neuronal communication by binding to the voltage-gated Na+ channels	Strichartz et al. (1986) and Su et al. (2004)
(5). 9	*Anabaena*, *Microcystis*, *Nostoc*, *Planktothrix*	β-N-methylamino-L-alanine (BMAA) (neurotoxic)	Motor system disorder, glutamate agonist, increasing the intracellular concentration of calcium in neurons, and inducing neuronal activity by hyperexcitation	Brownson et al. (2002) and Lobner et al. (2007)

(continued)

Table 2 (continued)

S. No.	Cyanobacterial spp.	Toxin	Mechanisms of toxicity	References
(6). 10	*Anabaena*, *Anacystis*, *Microcystis*, *Oscillatoria*, *Spirulina*, *and almost all cyanobacteria*	Lipopolysaccharides (LPS) (dermatotoxic)	Impairment of immune and detoxification system, irritation, and allergic effects	Mankiewicz et al. (2003) and Wiegand and Pflugmacher (2005)
(7). 11	*Lyngbya*	Kalkitoxin (neurotoxic)	Blocking voltage-gated sodium channels	Wu et al. (2000), LePage et al. (2005)
(8). 12	*Lyngbya*	Jamaicamides neurotoxic (cytotoxic)	Blocking voltage-gated sodium channels	Edwards et al. (2004)
(9). 13	*Anabaena*, *Oscillatoria (Planktothrix)*, *Phormidium*, *Raphidiopsis*	Homoanatoxin (neurotoxic)	Blockade of the neuromuscular transmission	Aas et al. (1996)
(10). 14	*Microcystis*, *Planktothrix*	Cyanopeptolin (neurotoxic activity)	Transcriptional alterations of genes belonging to DNA	Faltermann et al. (2014)
(11). 15	*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Oscillatoria (Planktothrix)*, *Rhaphidiopsis*, *Umezakia*	Cylindrospermopsin (hepatotoxic, nephrotoxic, and cytotoxic)	Irreversible inhibition of protein and glutathione synthesis, implicating cytochrome P-450, overexpression of DNA damage repair proteins	Humpage et al. (2000), Froscio et al. (2003) and Neumann et al. (2007)
(12). 16	*Lyngbya*, *Schizothrix*, *Trichodesmium*, *Oscillatoria*	Aplysiatoxins (dermatotoxic)	Potent tumor promoters and protein kinase C activators	Fujiki et al. (1982)
(13). 17	*Lyngbya*	Antillatoxin (neurotoxic)	Blocking neuronal communication by binding to the voltage-gated Na+ channels	Berman et al. (1999) and Li et al. (2001)
(14). 18	*Anabaena*, *Aphanizomenon*, *Cylindrospermum*, *Microcystis*, *Planktothrix*, *Raphidiopsis*	Anatoxin (a neurotoxic)	Depolarizing neuromuscular blocking	Devlin et al. (1977) and Carmichael (1998)
(15). 19	*Anabaena*	Anatoxin-a(s) (neurotoxic)	Inhibition of ach-esterase activity, hyperexcitability of nerve	Matsunaga et al. (1989)

5 Antibiotic Properties of Cyanobacteria

Antifungal Activity Flavonoid-type compounds, fischerellin A, phytoalexin, tolytoxin, laxaphycins, ambiguines, calophycin, and sctophytin have been isolated from *Oscillatoria*, *Scytonema pseudohofmanii*, *Anabaena laxa*, and many more cyanobacterial species which are supposed to have anti-candidal and antifungal activity (Frankmolle et al. 1992).

Antibacterial Activity Kaushik and Chauhan (2008a, b) had reported the antibacterial activity of several species of cyanobacteria such as Anabaena, *Lyngbya*, *Calothrix*, *Spirulina*, *Nostoc*, *Hapalosiphon*, *Phormidium*, *Oscillatoria*, etc. against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, etc.) and Gram-negative bacteria (*Escherichia coli*, *Peudomonas aeruginosa*, *Salmonella typhi*, etc.). These organisms even are being altered genetically using biotechnological interventions for the production of various active compounds having antibacterial activity such as bacteriocin, ambigol A, parsiguine, hapalindole, and hormothamnin A. Garima et al. (2013) have evaluated *Phormidium* for its significant antibacterial and anticandidal activity. Various species of Anabaena were evaluated for their antimicrobial activity, and active extracts were further screened for the presence of various chemical constituents through HPTLC and UV-HPLC (Kaushik et al. 2009a; Chauhan et al. 2010). Kaushik et al. (2008a, b) and Kaushik et al. (2009b) also examined *Nostoc commune* and *Lyngbya majuscula* for potent antimicrobial activity against clinically significant microorganisms, and further methanolic extract was analyzed through UV-HPLC and HPTLC analysis, respectively (Kaushik and Chauhan 2008a, b; Kaushik et al. 2008a, b; Kaushik et al. 2009a, b, c, d).

Antimycobacterial Activity Rao et al. (2007) reported the antimycobacterial activity of different spp. of many cyanobacteria, viz., *Hapalosiphon* sp., *Anabaena* sp., *Lyngbya* sp., *Westeillopsis prolifica*, *Spirulina* sp., *Anabaena variabilis*, *Anabaena cylindrica*, *Oscillatoria* sp., and *Scytonema* sp. against various species of Mycobacteria (*Mycobacterium tuberculosis ATCC 27294*, *M. tuberculosis MDR*, *M. avium*, *M. intracellulare*, *and M. aurum).*

6 Path Forward

Cyanobacteria offer a great opportunity as these are considered to be one of the potential organisms useful to mankind in many ways. Worldwide attention is drawn towards cyanobacteria for their possible use in several therapeutic and other products. Keeping in view the applied application of micro-algae and their product, it has been concluded that now this area is exploring rapidly. The major challenge in front of the current world is to fight effectively against the new emerging diseases

specifically microbial infections and to discover new drugs for mankind and society. At the same time, there is an urgent need to think from basic to applied research to commercialize several value-added products.

References

Aas, P., Eriksen, S., Kolderup, J., Lundy, P., Haugen, J.E., Skulberg, O.M., etal. (1996). Enhancement of acetylcholine release by homoanatoxin-a from Oscillatoria Formosa. Environ. Toxicol. Pharmacol. 2, 223–232.doi: https://doi.org/10.1016/S1382-6689(96)00059-2

Banker, R. and Carmeli, S. 1998. Tenuecyclamides A-D, cyclic hexapeptides from cyanobacterium *Nostoc* spongiaeforme var. tenue. J.Nat. Prod. 61, 1248-1251.

Barchi, J.J. Jr., Moore, R.E. and Patterson, G M.L. 1984. Acutiphycinn and 20, 21- didehydroacutiphycin, new antineoplastic agents from the cyanophyte Oscillatoria acutissima. J. Am. Chem. Soc. 106, 8193-8197.

Berman, F.W., Gerwick, W.H., and Murray, T.F. (1999). Antillatoxin and kalkitoxin, ichthyotoxins from the tropical cyanobacterium Lyngbya majuscula, induce distinct temporal patterns of NMDA receptor mediated neurotoxicity. Toxicon 37, 1645–1648.doi:https://doi.org/10.1016/S0041-0101(99)00108-7

Bonjouklian, R., Moore, R.E., Mynderse, J.S., Patterson, G.M.L. and Smitka, T.A.1988. New hapalindoles. European patent application EP 0260964 A2, 1-26.

Bradner, W.T., 1980. Fermentation approaches to new drug development. In: Crooke, S.T. & Prestayko, A.W. Cancer and Chemotherapy. Academic Press, New York. Pp. 313-25.

Browitzka, M.A., 1995. Microalgae as a source of pharmaceuticals and other biologically active compounds. J. Appl. Phycol. 7, 3-15.

Brownson, D.M., Mabry, T.J., and Leslie, S.W. (2002). The cycad neurotoxic amino acid, beta-N-methylamino-L-alanine (BMAA), elevates intracellular calcium levels in dissociated rat brain cells. J. Ethnopharmacol. 82, 159–167.doi: https://doi.org/10.1016/S0378-8741(02)00170-8

Burja, A.M., Banaigs, E.B., Abou-Mansour, B.J.G. and Wright, P.C. 2001. Marine cyanobacteria - a prolific source of natural products. Tetrahedron. 57, 9347–9377.

Cardellina, J.H., Marner, F.-J., and Moore, R.E. (1979).Seaweed dermatitis: structure of lyngbyatoxin A. Science 204, 193–195.doi:https://doi.org/10.1126/science.107586.

Carmeli, S., Moore, R.E., Patterson, G.M.L., Corbett, T.H., and Valeriote, F.A. 1990c Tantazoles, unusual cytotoxic alkaloids from the blue-green alga Scytonema mirabile. J. Am. Chem. Soc. 112, 8195-8197.

Carmeli, S., Moore, R.E.and Patterson, G.M.L. 1990a Isonitriles from the blue-green alga Scytonema mirabile. J. Org.. Chem. 4431-8.

Carmeli, S., Moore, R.E.and Patterson, G.M.L. 1990b Tolytoxin and New Scytophycins from three species of Scytonema. J. Nat. Prod. 53, No-6, 1533-1542.

Carmichael, W.W. 1992. Cyanobacterial secondary metabolites-the cyanotoxin. J. Appl. Bacteriol. 72, 445-459.

Carmichael, W.W. (1998).“Toxins of freshwater algae,” in Handbook of Natural Toxins, Marine Toxins and Venoms, Vol. 3,ed. A.T. Tu (New York, NY: Marcel Dekker),121–147.

Chauhan, A., Chauhan, G., Gupta, P.C., Goyal, P., and Kaushik, P. 2010. In vitro antibacterial evaluation of Anabaena sp. against several clinically significant microflora and HPTLC analysis of its active crude extracts. Indian Journal of Pharmacology, 42(2), 104-106.

Chorus, I. and Bartram, J. 1999. Toxic cyanobacteria in the water. E & F.N. Spon, London.

Devlin, J.P., Edwards, O.E., Gorham, P.R., Hunter, N.R., Pike, R.K., and Starvic, B. (1977). Anatoxin-a, a toxic alkaloid from Anabaena flos-aquae NRC-44h. Can. J. Chem. 55, 1367–1371. doi:https://doi.org/10.1139/v77-189

Duy, T.N., Lam, P.K., Shaw, G.R. and Connell, D.W. 2000. Toxicological and risk assessment of freshwater cyanobacterial (blue-green algae) toxin in water. Rev. Environ. Contam. Toxicol. 163, 113–185.

Edwards, D.J., Marquez, B.L., Nogle, L.M., McPhail, K., Goeger, D.E., Roberts, M.A., etal. (2004). Structure and biosynthesis of the jamaicamides, new mixed polyketide-peptide neurotoxins from the marine cyanobacterium Lyngbya majuscula. Chem. Biol. 11, 817–833. doi:https://doi.org/10.1016/j.chembiol.2004.03.030

Falch, B.S., Konig, G.M., Wright, A.D. 1992. Antibacterial and cytotoxic compounds from blue-green algae Fischerella ambigua. Planta Medica, 58, 654-655.

Faltermann, S., Zucchi, S., Kohler, E., Blom, J.F., Pernthaler, J., and Fent, K. (2014). Molecular effects of the cyanobacterial toxin cyanopeptolin (CP1020) occurring in algal blooms: global transcriptome analysis in zebrafish embryos. Aqua. Toxicol. 149, 33–39.doi:https://doi.org/10.1016/j.aquatox.2014.01.018

Fish, S.A. and Codd, G.A. 1994. Bioactive compound production by thermophilic and thermotolerant cyanobacteria (blue-green algae). World J. Microbiol. Biotech. 10, 338-341.

Frankmolle, W.P., Larsen, L.K., Caplan, F.R., Patterson, G.M.L., Knubel, G, Levine, I.A., and Moore, R.E., 1992. Antifungal cyclic peptides from the terrestrial blue green alga Anabaena laxa. Isolation and biological properties. J antibiotics. 45, 1451-1457.

Froscio, S.M., Humpage, A.R., Burcham, P.C., and Falconer, I.R. (2003). Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. Environ. Toxicol. 18, 243–251.doi: https://doi.org/10.1002/tox.10121

Fujiki, H., Mori, M., Nakayasu, M., Terada, M., Sugimura, T., and Moore, R.E. 1981).Indole alkaloids: dihydroteleocidin B, teleocidin, and lyngbyatoxin Aas Members of a new class of tumor promoters. Proc. Natl. Acad.Sci.U.S.A. 78, 3872–3876.doi:https://doi.org/10.1073/pnas.78.6.3872.

Fujiki, H., Suganuma, M., Hakii, H., Bartolini, G., Moore, R.E., Takayama, S., et al. (1984).A two-stage mouse skin carcinogenesis study of lyngbyatoxin A. J. Cancer Res. Clin.Oncol. 108, 174–176.doi:https://doi.org/10.1007/BF00390993

Fujiki, H., Suganuma, M., Nakayasu, M., Hoshino, H., Moore, R.E., and Sugimura, T. (1982). The third class of new tumor promoters, polyacetates (debromoaplysiatoxin and aplysiatoxin), can differentiate biological actions relevant to tumor promoters. Gan 73, 497–499.

Garima, Chauhan, A., Goyal, P., and Kaushik, P. 2013. Antibacterial and anticandidal screening of extracellular and intracellular extracts of Phormedium, a cyanobacterium. Int. J. Chem. And Life Sciences, Vol. 2(3), 1107-1111.

Goyal, P., Chauhan, A. and Verma, A (2015) Targeting cyanobacteria as a novel source of biofuel. Int. J. Curr. Microbiol. App.Sci. 4(2): 981-993. (IF:2.015) ISSN:2319-7692.

Gulledge, B.M., Aggen, J.B., Huang, H.-B., Nairn, A.C., and Chamberlin, A.R. (2002). Themicrocystins and nodularins: cyclic polypeptide inhibitors of PP 1 and PP2A. Curr. Med. Chem. 9, 1991–2003.doi:https://doi.org/10.2174/0929867023368845

Hayashi, O., Katoh, T. and Okuwaki, Y. 1994. Enhancement of antibody production in mice by dietary Spirulina platensis. J. Nutr. Sci. Vitaminol (Tokyo).

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. 1994. Bergey's Manual of Determinative Bacteriology (9th edition). Williams & Wilkins, 428, East Perston Street, Baltimore, Maryland 21202, USA. pp.VII+787

Honkanen, R.E., Zwiller, J., Moore, R.E., Daily, S.L., Khatra, B.S., Dukelow, M., et al. (1990). Characterization of microcystin-LR, a potent inhibitor of type 1 and type 2 A protein phosphatises. J. Biol. Chem. 265, 19401–19404.

Humpage, A.R., Fenech, M., Thomas, P., and Falconer, I.R. (2000). Micronucleus induction and chromosome loss in transformed human white cells indicate clastogenic and aneugenic action of the cyanobacterial toxin, cylindrospermopsin. Mutat. Res. Genet.Toxicol.Environ.Mutagen. 472, 155– 161. doi:https://doi.org/10.1016/S1383-5718(00)00144-3

Hunter, P.R. 1995. Cyanobacterial toxins and their potential risk to drinking water supplies. Microbiol. Eur. 3, 8-10.

Ishibashi, M., Moore, R.E., Patterson, G.M.L., Xu C. and Clardy, J., 1986. Scytophycins, cytotoxic and antimycotic agents from the Cyanophyte Scytonema Pseudohofmanni. J. Org Chem. 51, 5300-5306.

Inderjit and Dakshini, K.M.M. 1994. Algal allelopathy. The Botanical Review. 60, 182-196.

Jaspars, M. and Lawton, L.A. 1998. Cyanobacteria-a novel source of pharmaceuticals. Current Opinion Drug Discovery and Development 1(1), 77–84.

Jones, G.J. and Korth, W. 1995. In situ production of volatile odor compounds by river and reservoir phytoplankton population in Australia. Water Sci. Technol. 31, 145-151.

Kaushik, B.D., 1987. Laboratory methods for Blue-Green Alage. Pub. Assoc. Publishing Co. New Delhi. pp.1–171.

Kaushik, P and Chauhan A. Cyanobacteria: Antibacterial Activity New India Publishing Agency, 101, Vikas Surya Plaza, C.U. Block, L.S.C. Mkt., Pitampura, New Delhi 110088, ISBN 10: 8190723774, ISBN13: 9788190723770 xiii, 198 p, figs. and tables. Year: 2009

Kaushik, P. and Chauhan, A (2008a) Antibacterial potential of aqueous and organic extracts of N. commune a cyanobacterium. VEGETOS (An International Journal of Plant Sciences). Vol. 21, N0.1.77-80. (NAAS:6.2, IF: 0.04) ISSN: 0046-899.

Kaushik, P. and Chauhan, A. 2008b. In vitro antibacterial activity of laboratory grown culture of Spirulina platensis. Ind. Journal of Microbiology. Vol. 48. No. 1.

Kaushik, P. Chauhan, A., Chauhan, G. and Goyal P (2008a) Evaluation of Nostoc commune for potential antibacterial activity and UV-HPLC analysis of methanol extract. The Internet Journal of Microbiology. Volume 5 Number 1. ISSN: 1937-8289.

Kaushik, P., Chauhan, A., Garima, and Goyal P (2009d) Antibacterial Potential and UV-HPLC Analysis of Laboratory-Grown Culture of Anabaena variabilis. Internet Journal of Food Safety, Vol. 11, 2009, p. 11–18 ISSN 1930-0670

Kaushik, P., Garima, Chauhan, A., and Goyal P (2009c) Screening of Lyngbya majuscula for potential antimicrobial activity and HPTLC analysis of active methanolic extract. Journal of Pure and Applied Microbiology, Vol. 3, No. 1, 169–174.

Kaushik, P., Garmia, Chauhan, A. and Goyal, P. 2009b. Screening of Lyngbya majuscula for potential antibacterial activity and HPTLC analysis of active methanolic extract. Journal of Pure & Applied Microbiology, 3(1), 169–174.

Kaushik, P., Chauhan, A., Chauhan, G. and Goyal, P. 2008b. Evaluation of Nostoc commune for potential antibacterial activity and UV-HPLC analysis of methanol extract. The Internet Journal of Microbiology. Volume 5, Number 1.

Kaushik, P., Chauhan, A., Chauhan, G. and Goyal, P. 2009a. Antibacterial potential and UV-HPLC analysis of laboratory-grown culture of Anabaena variabilis. Internet Journal of Food Safety, 11, 11-18.

Knubel, G., Larsen, L.K., Moore, R.E., Levine, I.A., and Patterson, G.M.L. 1990. Cytotoxic, antiviral indolocarbazoles from a blue green alga belonging to the Nostocaceae. J. Antibiotics, 43, 1236. 1239.

LePage, K.T., Goeger, D., Yokokawa, F., Asano, T., Shioiri, T., Gerwick, W.H., etal. (2005).The neurotoxic lipopeptide kalkitoxin interacts with voltage-sensitive sodium channels in cerebellar granule neurons. Toxicol. Lett. 158, 133–139.doi: https://doi.org/10.1016/j.toxlet.2005.03.007

Li, W.I., Berman, F.W., Okino, T., Yokokawa, F., Shioiri, T., Gerwick, W.H., etal. (2001). Antillatoxin is a marine cyanobacterial toxin that potently activates voltage-gated sodium channels. Proc. Natl. Acad.Sci.U.S.A. 98, 7599–7604.doi: https://doi.org/10.1073/pnas.121085898

Lobner, D., Piana, P.M.T., Salous, A.K., and Peoples, R.W. (2007). β-N-methylamino-L-alanine enhances neurotoxicity through multiple mechanisms. Neurobiol. Dis. 25, 360–366.doi:https://doi.org/10.1016/j.nbd.2006.10.002

MacKintosh, C., Beattie, K.A., Klumpp, S., Cohen, P., and Codd, G.A. (1990).Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett. 264,

Mankiewicz, J., Malgorzata, T.M., Walter, Z., and Maciej, Z.M. (2003). Natural toxins from cyanobacteria. Acta Biol. Cracovien.Ser.Bot. 45, 9–20.

Matsunaga, S., Moore, R.E., Niemczura, W.P., and Carmichael, W.W. (1989). Anatoxin-a(s), a potent anticholinesterase from Anabaena flos-aquae. J. Am. Chem. Soc. 111, 8021–8023. doi:https://doi.org/10.1021/ja00202a057.

Metting, B. and Pyne, J.W. 1986. Biologically active compounds from microalgae. Enzyme and microbiological technology. 8, 386-394. Microcystis aeruginosa. Phytotherapy Res. 11, 93–96.

Moore, B. S., Chen, J.L., Patterson, G. M.L. and Moore, R.E. 1990. Paracyclophanes from blue-green algae. J. Am. Chem. Soc. 112, 4061–4063.

Moore, R.E. 1996. Cyclic peptides and depsipeptides from cyanobacteria: a review. J. ind. Microbiol. 16, 134–143.

Namikoshi, M. and Rinehart, K.L., 1996. Bioactive compounds produced by cyanobacteria. J. Ind. Microbio. 17, 373-384.

Neumann, C., Bain, P., and Shaw, G. (2007). Studies of the comparative in vitro toxicology of the cyanobacterial metabolite deoxycylindrospermopsin. J. Toxicol. Environ. Health 70, 1679–1686.doi:https://doi.org/10.1080/15287390701434869

Patterson, G.M.L., Baker, K.K., Baldwin, C.L., Bolis, C.M., Caplan, F.R., Larsen, L.K., Levine, I.A., Moore, R.E., Nelson, C.S., Teschappat, K.D., and Tuang, G.D. 1993. Antiviral activity of cultured blue-green algae (Cyanophyta). J. Phycol. 29, 125–130.

Patterson, G.M.L., Larsen, L.K., Moore, R.E., 1994. Bioactive natural product from blue-green algae. J. Appl. Phycol. 6, 151–157.

Pelczar, M.J., Chan, E.C.S and Krieg, N.R. 1993. Microbiology. Tata McGraw-Hill Publishing Company Ltd. New Delhi. pp-VIII, 1-918.

Pratt, R., Daniels, T.C., Eiler, J.J., Gunnison, J.B., Kumler, W.D., Oneto, J.F., Strait, L.A., Spoehr, H.A., Hardin, G, J. Milner, H.W., Smith, J.H.C., and Strain, H.H. 1944. Chlorellin, An antibacterial substance from Chlorella. Science. 99, No-2574, 351–352.

Rao, M., Malhotra, S., Fatma, T. and Rattan, A. 2007. Antimycobacterial Activity from Cyanobacterial Extracts and Phytochemical Screening of Methanol Extract of Hapalosiphon. Pharmaceutical Biology. Vol. 45 No. 2. pp. 88-93.

Smith, G.D. and Doan, N.T. 1999. Cyanobacterial metabolites with bioactivity against phytosynthesis in cyanobacteria, algae and higher plants. J. Appl. Phyco. 11, 337-344.

Spoehr, H.A., Smith, J.H.C., Strain, H.H., Milner, H.W. and Hardin, G.J. 1949. Carnegie Inst. Wash. Publ. 58-61.

Stanier, G. 1988. Fine structure of cyanobacteria. Methods in enzymology, 167, 157-172.

Stewart, J.B., Bornemann, V., Chen, J.L., Moore, R.E., Caplan, F.R., Karuso, H, Larsen, L.K. and Patterson, G.M.L. 1988. Cytotoxic, fungicidal nucleosides from blue-green algae belonging to the Scytonemataceae. J. Antibiotics, 41, 1048-1056.

Strichartz, G., Rando, T., Hall, S., Gitschier, J., Hall, L., Magnani, B., et al. (1986). On the mechanism by which saxitoxin binds to and blocks sodium channels. Ann. N.Y. Acad. Sci. 479, 96–112. doi:https://doi.org/10.1111/j.1749-6632.1986.tb15564.x

Su, Z., Sheets, M., Ishida, H., Li, F., and Barry, W.H. (2004). Saxitoxin blocks L-Type/Ca. J. Pharmacol. Exp. Therapeut. 308, 324–329.doi: https://doi.org/10.1124/jpet.103.056564

Wiegand, C., and Pflugmacher, S. (2005). Ecotoxicological effects of selected cyanobacterial secondary metabolites: a short review. Toxicol. Appl. Pharmacol. 203, 201–218.doi:https://doi.org/10.1016/j.taap.2004.11.002

Wu, M., Okino, T., Nogle, L.M., Marquez, B.L., Williamson, R.T., Sitachitta, N., etal. (2000). Structure, synthesis, and biological properties of kalkitoxin, a novel neurotoxin from the marine cyanobacterium Lyngbya majuscula. J. Am. Chem. Soc. 122, 12041–12042.doi:https://doi.org/10.1021/ja005526y

Yoshizawa, S., Matsushima, R., Watanabe, M.F., Harada, K.-I., Ichihara, A., Carmichael, W.W., etal. (1990). Inhibition of protein phosphatases by microcystis and nodularin associated with hepatotoxicity. J. Cancer Res. Clin. Oncol. 116, 609–614.doi:https://doi.org/10.1007/BF01637082

Nanotoxicology: Exposure, Mechanism, and Effects on Human Health

Alishba Tanya John, Shikha Wadhwa, and Ashish Mathur

Abstract The increasing negative effects of nanomaterials on humans and the environment have led to the increase in a new subtopic in the branch of nanotechnology, known as "nanotoxicology." The issue of toxicity of nanomaterials has been a relatively new topic of concern with regard to nanotechnology, and this is because of the lack of techniques and experimental conditions in order to evaluate and monitor nanotoxicity. With the increase in concerns related to nanotoxicity, many global organizations have taken up the initiative to spread awareness and to design various strategies to assess the toxic effect of nanomaterials on human health and the environment**.** Nanoparticles can be produced by a variety of physical, chemical, and biological methodologies. Researchers have also been interested in trying to find out many new and interesting ways to synthesize nanoparticles for varied particle applications. The current chapter describes many such aspects of nanotoxicology including exposure, mechanism, and effects on human health.

Keywords Nanomaterials · Human exposure · Nanotoxicity · Microculture tetrazolium assay · Nitrosative stress response

1 Introduction

Nanotechnology is the science of synthesizing, fabricating, and utilizing structures and devices having at least one dimension in the nano-regime (which is one billionth of a meter). The term "nano" originated from the Greek word "nanos," which means "dwarf," and it is for the same reason that whenever we talk about nanotechnology or nanoscience, very small particles come into our minds (Klefenz 2004).

Nanomaterials are classified into 1D, 2D, and 3D nanomaterials based on the concept of quantum confinement. Quantum confinement or quantum size effect refers to the phenomenon of nanomaterials where its size and structure is a result of the direct influence of small length scale on the material's bandgap structure

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_5

Table 1 Classification of nanomaterials based on quantum confinement

Nanostructure	Quantum confinement	Number of free dimensions	Examples
0D	3	0	Nanocrystals, clusters, quantum dots
1D	2	1	Nanowires, nanotubes, nanobelts, nanorods
2D	1	2	Nanofilms, nanomembranes, nanosponges
3D	0	3	Superlattices, bulk materials

(Takagahara and Takeda 1992). The length scale resembles the system of quantum confinement in the range of 1–25 nm, corresponding to the "geometrical constraint" electrons present in nanomaterials. These feel boundaries and respond accordingly to the particle size by adjusting their energies. Table 1 shows examples of different nanomaterials that are classified based on quantum confinement.

Nanoparticles can be produced by a variety of physical, chemical, and biological methodologies. Researchers have also been interested in trying to find out many new and interesting ways to synthesize nanoparticles for varied particle applications. Conventionally, synthesis of nanomaterials is classified into two broad categories: top-down and bottom-up approaches. Synthesis of nanomaterials using the top-down approach is through the breakdown of macro/bulk particles into smaller particles with the help of various physical techniques such as crushing, grinding, milling, etc. The drawbacks of the top-down approach are that it does not result in uniform particle shape and size and energy utilized in different physical methods is too high. While on the other hand, the bottom-up approach synthesizes nanomaterials from atom to molecule to clusters. The advantage of this approach is that nanoparticles synthesized have a uniform size distribution and shape (Hahn 1997). Figure 1 illustrates different techniques under the umbrella of top-down and bottom-up approaches.

Nanoparticles have extremely small size, reduced properties, and high surface-to-volume ratio that leads to both physical and chemical differences in their properties as compared to macro particles with the same chemical configuration, owing to which these particles have many applications in different fields such as medical imaging, nanocomposites, filters, drug delivery, and cancer treatment (Table 2) (Daniel and Astruc 2004).

2 Global Market of Nanomaterials

Nanotechnology and its outputs have become an inevitable part of our daily routines as many commercially available merchandises have been using nanomaterials in various products such as sunscreens, automobiles, and the new-age television systems. The increase in the production and usage of goods with nanomaterials proves

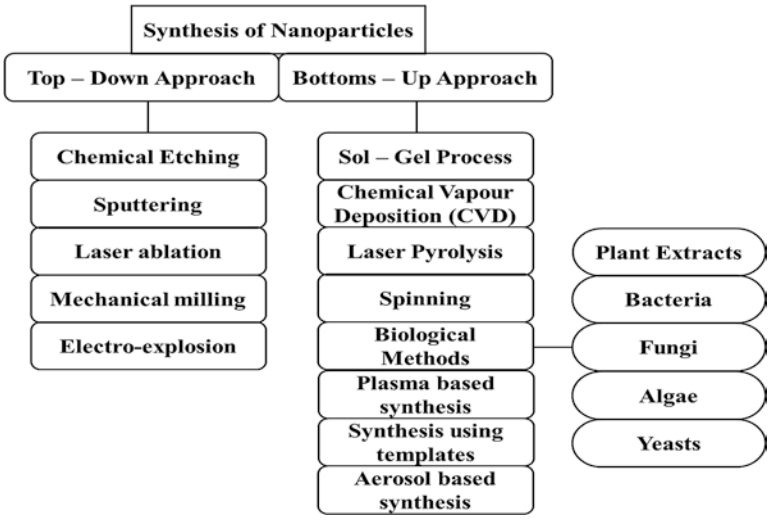

Fig. 1 Techniques for synthesis of nanoparticles

Table 2 Nanomaterials used in different applications

S. no.	Application	Nanoparticle used	References
1	Agriculture	Ag NPs, aluminosilicate NPs, CNTs, TiO_2 NPs	Singh et al. (2015)
2	Biosensor	Au NPs, various magnetic NPs, CNTs, graphene	Holzinger et al. (2014)
3	Cosmetics	ZnO NPs, TiO_2 NPs, fullerenes, lipid nanoparticles	Mousavi (2017)
4	Dentistry	CNTs, hydroxyapatite, iron oxide, ZrO_2 NPs, SiO_2 NPs, TiO_2 NPs, Ag NPs	Priyadarsini et al. (2017)
5	Drug delivery	Chitosan NPs, liposomes, fullerenes, SiO_2 NPs	Jong et al. (2008a)
6	Electronics	CNTs, Al_2O_3 NPs, TiO_2 NPs, CuO NPs, SiO_2 NPs, ZnO NPs	Bahiraei and Heshmatian (2018)
7	Environment	Chitosan NPs, SiO_2 NPs, TiO_2 NPs	Dong et al. (2014)
8	Food industry	Ag NPs, TiO_2 NPs, silver zeolites	Berekaa (2015)
9	Health care	SiO_2 NPs, CNTs, Au NPs, iron oxide NPs	Hwang et al. (2018)
10	Wastewater treatment	TiO_2 NPs, iron oxide NPs, Au NPs, Ag NPs, Fe NPs, carbon-based nanomaterials	Lu and Astruc (2018)

that it has the potential to influence many sectors of the global economy. The global market of nanotechnology-based products have been estimated to rise up to $90.5 billion by the year 2021 from $39.2 billion with a compound annual growth rate (CAGR) of 18.2% based on the reports that were recorded in 2016 by BCC Research (BccResearch 2017). However, many industries and market research experts believe that nanotechnology and nanomaterials will not receive much attention. One such industry is the Deloitte Touche Tohmatsu Limited, where the experts have declared

only a CAGR of 15.5% increase in the global market of nanomaterials in the period of 2012–2019 (Dickson 2015). The factors that have the potential to threaten the market of nanomaterials globally are the main concerns related to its toxicity at different levels to humans and the environment. Toxicity of nanomaterials is not only a concern themselves but also various hazardous chemicals used during their preparation, release of different toxic intermediate complexes, and the toxic wastes generated from the processes throughout its production and manufacturing (Inshakova and Inshakov 2017).

Nanomaterials utilized in commercially ready products can be categorized into three segments (Market, Vertical Farming 2016a; Nanomaterials Market 2011; Abraham 2011):

(i) Metal, metal oxides, carbon-based materials, dendrimers, nano-cellulose, and nano-clay
(ii) Nanoparticles, nanotubes, nanowires, and nanofibers
(iii) Graphene, fullerenes, carbon black, ceramics, and silica

Experts have reported that use of metal and metal oxides nanomaterials will dominate the market and occupy about 25% with a CAGR of 20.7% from 2016 to 2022 (Market, Vertical Farming 2016b), of which titanium dioxide and silicon dioxide will be the most employed in different consumer products. Silver nanoparticles owing to its excellent antimicrobial and antibacterial characteristics occupies 50% of the market and have been incorporated in a variety of products applied in the different industries such as health care, cosmetics, electronics, textiles, etc. The usage of silver nanoparticles has been estimated to increase with a CAGR of 13% from 2016 to 2024 (Silver Nanoparticles Market Size by Application 2017). Nano-clay is the next big nanomaterial to rule the market. According to the reports of Transparency Market Research, global economy of nano-clay is expected to rise at a CAGR of 24.9% until 2020 (Nanoclay market 2016). Carbon-based nanomaterials have also been gaining a lot of importance in terms of commercial applications and can be expected to cause the next industrial revolution; however, only a few producers and suppliers in different segments of various industries dominate the market. Out of all the carbon-based materials, carbon nanotubes have most of the market share. The total global turnover of carbon nanotubes in the year 2010 was approximately $668.3 million of which the individual share of multiwalled carbon nanotubes was $631.5 million and that of single-walled carbon nanotubes was $36.8 million, which has been estimated to grow at a CAGR of 10.5% (Production and Applications of Carbon nanotubes 2011). Figure 2 represents the market share of different nanomaterials used globally for various applications.

The increased usage of different forms of nanomaterials in various applications has led researchers to ponder upon the toxic effects these materials can have on human health and the environment. Since then nanotoxicology has become a class of study on its own under the class of nanotechnology. Shvedova et al. had introduced the term “nanotoxicology” almost a decade ago to enlighten users about its potential toxicity corresponding to its unique physical and chemical properties; however, the interaction of nanoparticles with cells and tissues of human body was

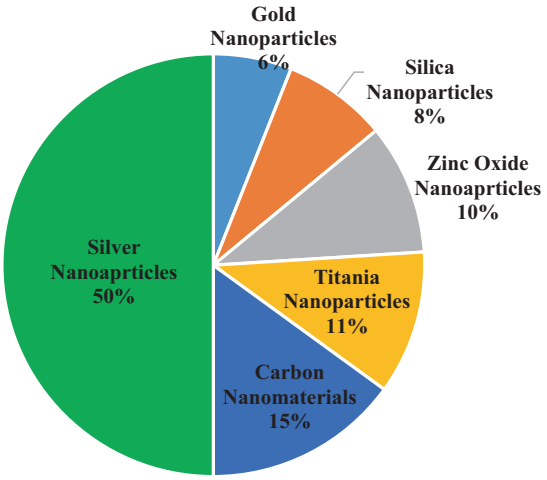

Fig. 2 Different nanomaterials occupying the global market for the year 2016 (Silver Nanoparticles Market Size by Application 2017)

very unpredictable and ambiguous back then (Shvedova et al. 2016). With the advancement in science and technology and the immense knowledge people have been gathering in terms of nanotechnology and biological molecular mechanisms, it has become very easy to understand the mechanism of nanotoxicity, and also various methods have been cultivated for identification and detection of the toxic effects nanomaterials have on human health and the environment.

3 Routes of Human Exposure to Nanomaterials

Exposure of human beings and the environment to nanomaterials takes place at different levels of its production, application, and elimination. At all these different stages, nanomaterial toxicity is a major concern as the level of exposure of toxic materials might vary depending on a number of factors such as the population being exposed, time and interval of exposure, and the nature of the substance that is being exposed (Civeira et al. 2016). As for instance, in an industrial scenario, workers are exposed to nanomaterials during the entire process of synthesis of the material as a consequence of which toxicity might be induced into their body. Once the material has been synthesized and has moved into the commercial market for mass production, workers can be exposed to toxic nanomaterial at different stages such as synthesis, packaging, transport, recovery, and storage (Leo et al. 2013). Elimination of nanomaterials to the environment poses a threat of exposure of nanomaterials not only to human health but also to the environment in the form of industrial waste, directly or indirectly to air, soil, or water. Hence, human beings and the environment may be adversely affected due to the contamination of air, water, or soil (Buzea et al. 2007). Therefore, with an attempt to protect and preserve human population, it is very important to understand different modes of exposure of environment to nanomaterials.

3.1 Skin

The skin is the most abundant and prime defense organ present in the human body, because of which exposure of toxic nanomaterials to humans is direct and is increasing at an alarm rate (Mackevica and Hansen 2016). Toxic effects from exposed nanomaterials can spread either directly within the skin cells or can be absorbed and transported into the bloodstream to different organs of the body, giving rise to numerous detrimental effects (Warheit and Maria Donner 2015). Toxicity from nanomaterials can also spread into the human blood on purposeful application of skin care products manufactured using nanoparticles such as sunscreens or other drug treatment cosmetics (Hagens et al. 2007).

Titanium dioxide nanoparticles are an essential class of metal oxide nanoparticles that are being utilized extensively in the cosmetic industry in various sunscreens and different skin care products; thus it is one of the most commonly exposed nanomaterials to human skin. It has been reported that titanium dioxide nanoparticles can penetrate into the human stratum corneum and into the epidermal and even dermal layers triggering toxic effects (Shakeel et al. 2016). Quantum dots are another category of nanomaterials that have numerous unique physical, chemical properties, and these have been reported to penetrate through the intact stratum corneum barrier and pass into the epidermal and dermal layers and get localized, hence causing toxic effects (Ryman-Rasmussen et al. 2006a). Quantum dots have also been reported to penetrate into the subcutaneous lymphatics and various regional lymph nodes thereby causing systemic effects (Gopee et al. 2007). Fullerene-based peptides have been reported of having the capability to transport into the intact dermal layer and cause mechanical stressors, because of which disruption of many biological functions occurs (Rouse et al. 2007). Researchers have also reported that single-walled and multiwalled carbon nanotubes with different surface coatings have harmful effects on epidermal keratinocytes and fibroblasts thus having the ability of altering human genes and even disrupting the process of protein expression (Haliullin et al. 2015).

3.2 Gastrointestinal Tract

The gastrointestinal tract as an organ system performs functions that are responsible for the consumption and digestion of food items, absorption of nutrients, and expulsion of unwanted waste from the body. Nanomaterials can be incorporated into the gastrointestinal tract from food, water, cosmetics, medicines, and drugs or from different drug delivery systems or even after the clearance of mucus from the respiratory tract; therefore the gastrointestinal tract is considered as an important target in terms of nanoparticle exposure (Som et al. 2011).

Copper nanoparticles found in the gastrointestinal have been reported to be much more toxic than bulk copper nanoparticles. They have also shown to cause adverse damage to the liver, the kidney, and the spleen (Chen et al. 2006). Recent reports have concluded that use of biodegradable nanoparticle for the delivery of oral drugs to the gastrointestinal tract reduced the toxic effects on nanomaterials (Bergin and Witzmann 2013).

3.3 Respiratory Tract

The respiratory system of the human body provides an easy access to particulate materials inside the body because of which diseases resulting from various airborne particles has become an interesting topic of study for many researchers (Pelclova et al. 2015). The extremely small size of nanoparticles allow its entry and deposition into the bloodstream and different parts of the respiratory system leading to numerous toxic effects. Difference in the grain sizes of nanoparticles governs the toxic effect as well as the site of deposition within the respiratory tract of the human body (Witschger and Fabriès 2005).

Insoluble nanoparticles in the alveolar region are generally taken up by macrophages during the process of phagocytosis. However, studies have shown that un-agglomerated polyethylene nanoparticles get deposited in the alveolar region and not phagocyted by the macrophages in contrast to coarser particles having at least 1–3 micrometer size range (Keller et al. 2014). Nanoparticles that are generally deposited in the alveolar region are absorbed by the lung epithelium, which later enter into the bloodstream and lymph vessels and travel to different parts of the body, such as the heart, bone marrow, and spleen (Nurkiewicz et al. 2005). If nanoparticles remain longer in the bloodstream, there are reports that have documented their transportation to the sensory nerve endings followed by the ganglia and the central nervous system by the network of axons (Simko and Mattsson 2014).

Silver nanoparticles has also been reported to closely affect the proper functioning of the respiratory system. Studies have shown that silver nanoparticles not only effect the macrophage cells present in the alveolar region but also the epithelial cells of the lungs (Soto et al. 2007). These nanoparticles can be taken into the respiratory tract by both instillation and inhalation and have the capability to preserve itself in the tract for up to 7 days. Toxicity of silver nanoparticles in the human body spreads by introduction of oxidative stress that eventually leads to cell death and by inhibition of cellular enzymes by interfering with free thiol groups (Völker et al. 2013).

A summary of exposure routes of humans to nanomaterials and their general health effects is presented in Fig. 3.

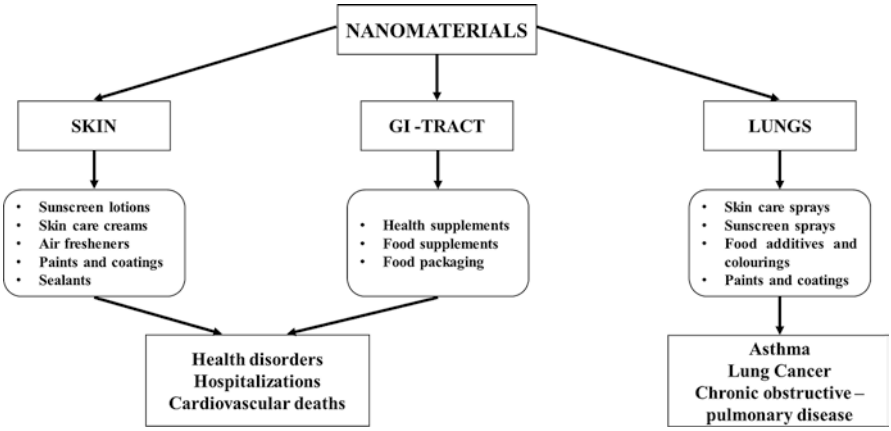

Fig. 3 Summary of effects of nanomaterials on human health

4 Effects of Nanomaterials on Human Health

With the advancement of nanotechnology and the application of nanomaterials in a wide range of applications, it has become very important to know the various health risks nanomaterials can pose to workers, consumers, and also the environment. One of the main reasons to understand the importance of nanotoxicity towards human health is because nanomaterials behave in an unpredictable manner causing adverse effects to human well-being. Nanotoxicity can be introduced to the human body by a number of physiochemical properties such as particle size, shape, chemical composition, surface coatings, etc. along with various circumstantial interactions of nanomaterials with the human body, for instance, mass of administered nanoparticle, mode of exposure, etc.

Though there are not many evidences as to which factor really plays an important role in toxicity of nanoparticles, researchers have always been keen in studying the same. Sly and Schüepp have reported that nanoparticles can interact with a complex network of immune cells located inside and beneath the epithelial surfaces of human cells, and these nanoparticle therefore can act as allergens during the neonatal period thereby activating the immune system to cause allergic inflammation in later life stages (Sly and Schüepp 2012). Liou et al. studied the various detrimental cardiovascular consequences due to exposure of nanoparticles in numerous epidemiological studies (Liu et al. 2009). Wang et al. reported that titanium dioxide doped with nano-cerium elements prompts apoptosis of Bel-7402 human hepatoma cells in the presence of visible light (Wang et al. 2007). Dunford and Salinaro reported that nanoparticles used in sunscreens such as zinc oxide and titanium dioxide have the ability to cause oxidative damage to DNA in vitro and in cultured human fibroblasts (Dunford et al. 1997). Studies conducted by Hougaard et al. concluded that synthetically produced nanoparticles such as ultrafine particles of C60 fullerenes can accumulate in the liver, when inhaled, because of its increased access in the

bloodstream and can be dispersed within different organs easily (Hougaard et al. 2015).

Inorganic nanoparticles are generally composed of metal and metal oxide nanoparticles. Such nanoparticles are the most commonly studied nanomaterial as it can be easily synthesized at low cost and mass-produced with applications in numerous fields. Exceptional properties exhibited by inorganic nanoparticles include extraordinary electrical, optical, and mechanical properties in comparison to their bulk counterparts. The global market share of metal and metal oxide nanoparticles in the year 2016 was recorded to be nearly $14,000 million, and the growth of this market has been estimated to grow up to $51,000 million by 2026 with a CAGR of 13.9% (Metal and Metal Oxide Nanoparticles Market 2017). Another common class of nanoparticles studied are organic nanoparticles that are solid particles made up of organic compounds such as dendrimers, micelles, or liposomes. This category of nanoparticles are used in a wide spectrum of application ranging from photonics, electronics, sensors, medicine, biotechnology, etc. The most important usage of organic nanoparticles is in the field of targeted drug delivery. Inorganic and organic nanomaterials form a significant share of all living beings; thus, different methodologies in regard to their synthesis and stability have been intensively studies, but their toxic effects, fate, and ecological properties still need to be explored (Sharma et al. 2015). The most common mechanism of toxicity induced by inorganic nanoparticles is cell death upon the generation of reactive oxygen species.

4.1 Silver Nanoparticles

Silver is one of the mostly commonly used metal nanoparticles owing to its excellent antimicrobial and antibacterial properties along with other uses in medical fields such as to treat infections, wound dressings, and for various drug delivery systems. Silver nanoparticles have been utilized in the production of numerous consumer products such as ATM buttons, bus railings, several kitchen appliances, filtration products of automobiles, food supplements, cosmetics, sports goods, etc. Owing to such as large market occupied by silver nanoparticle products, global economy has been estimated to grow from $1 billion in 2015 to $3 billion in 2024 with a CAGR of 13% (Unveiling silver nanoparticles market trends in terms of the application spectrum: F&B sector to emerge as a remunerative growth avenue over 2018).

As silver nanoparticles are mostly utilized in the health care and pharmaceutical industry, therefore it is very important to study about the various toxic effects silver nanoparticles can have on human health. Silver nanoparticles have been reported to cause DNA damage, oxidative stress, and apoptosis when in contact with biological systems and human cells (Kim and Ryu 2013). In vitro studies concerning the toxicity of silver nanoparticles reported that size is a key factor that can affect the viability of human cells (Gliga et al. 2014). On other hand, in vivo studies also showed the

toxicity of silver nanoparticles to human beings at different levels of inhalation and oral exposure. From a study conducted on rats to study the inflammatory responses in pulmonary organs, it was reported that minute volume and tidal volume significantly decreased during a period of 90 days of inhalation of different concentrations of silver nanoparticles (Sung et al. 2008). Exposure of silver nanoparticles to mammalian cells leads to a condition of argyria (discoloration of the skin); however, toxicity by oral exposure of silver nanoparticles is relatively low, but prolonged exposure can pose health risk.

4.2 Gold Nanoparticles

Gold is the oldest noble metal known to humankind, which in the nano-form shows excellent electrical and optical properties and biocompatibility that has been used for a wide range of applications in the biomedical fields. The excellent optical properties of gold nanoparticles is because of its ability to strongly absorb and scatter light resulting from the phenomenon of "surface plasmon resonance." Surface plasmon resonance is a phenomenon in which the electrons in the conduction band oscillate together and in resonance under the influence of a suitable frequency. Gold nanoparticles are the most abundantly used metal nanoparticle in the field of therapeutics and diagnostics ranging from biosensors, targeted drug delivery, bio-imaging, and monitoring of tissues, cells, etc. According to reports of the Transparency Market Research, the global economy of gold nanoparticles will show a hike with CAGR of 15% from 2017 to 2026 (Gold Nanoparticles Market 2018). It is because of the increase in the usage of gold nanoparticles in the production of consumer and industrial products all around the globe that has led to increase in the concern about its interaction with different biological systems and the possible toxic effects it can have on human health.

Gold in its bulk form is considered as an inert metal; however size-dependent toxic behavior of gold nanoparticles has been constantly reported. It has been reported that as the diameter of gold nanoparticles decreased from 100 to 50 to 15 nm, the rate of their absorption through the cell in the intestinal epithelium increased; however, their accumulation in the epithelial cells decreased. Accumulation and deposition of gold nanoparticles in the intestinal epithelial cells lead to the depolarization of mitochondria membranes and eventually instigating cytotoxicity (Yao et al. 2015). Reports have also shown that gold nanoparticles tend to influence the lysosome-based degradative pathway in the human body that plays a major role in maintaining homeostasis in the cells (Andón and Fadeel 2012). In vivo studies of toxicity of gold nanoparticles showed that the main targets of attack have been the spleen and liver. A study conducted to investigate the bio-distribution of intravenously injected gold nanoparticles in rats for 24 hours showed that particles having diameters of 10, 50, 100, and 250 nm tend to accumulate most in the spleen and liver (Jong et al. 2008b). Studies have

reported that accumulation and deposition of gold nanoparticles within the bloodstream is directly dependent on the dosage of nanoparticles that have been intravenously injected into the body and can cause acute inflammation and apoptosis of the cells in the liver (Cho et al. 2009). In conclusion, the toxicity of gold nanoparticles is extremely size specific due to the intercalation with the DNA in the nucleus of cells.

4.3 Iron Oxide Nanoparticles

Iron oxide nanoparticles are one of the most important transition metal oxides that have been used in a variety of industrial and commercial applications. Some of the commercial applications in which various properties of iron oxide nanoparticles have been exploited include pigments, coatings, catalysts, lubricants, gas sensors, etc. Iron oxide nanoparticles have potential commercially applications in magnetic recording devices, data storage, wastewater treatment, etc. Through the ongoing development and continuous research in improving the properties of iron oxide nanoparticles to utilize it in numerous applications, the global market of iron oxide nanoparticles has been estimated to rise at a CAGR of 21% between the years 2016 and 2022 (OrbisResearch 2017).

Several in vitro and in vivo studies have been carried out to study the toxic behavior of iron oxide nanoparticles when in contact with human cells and various biological systems. Reports have shown that either bare or surface-coated nanoparticles show no or minimal toxic effects unless they administered in high dosage (Singh et al. 2010). In a study conducted to test the toxicity of iron oxide nanoparticles, citrate-coated superparamagnetic iron oxide nanoparticles with a diameter of 5 nm were exposed to macrophage cells of rats. It was observed that exposure of iron oxide nanoparticles led to a significant increase in the levels of protein carbonyls that eventually induced cytotoxicity (Stroh et al. 2004). When iron oxide nanoparticles were intravenously introduced into rat cells, a transient increase of toxic effects in the lever was observed. Oxidative stress due to difference in lipid hydroperoxide levels was also observed in different organs, such as the kidney and spleen, along with the liver. However, structural analysis of the liver, kidney, and spleen samples on day 1 and day 7 showed no apparent abnormal changes (Jain et al. 2008). On a 28-day study conducted to evaluate size-dependent toxic effects of iron oxide nanoparticles, a dose-dependent toxic behavior was observed for smaller iron oxide nanoparticles and not for larger particles (Kumari et al. 2012). In conclusion, iron oxide nanoparticles show low toxicity in in vitro and in vivo conditions. However, inflammation and oxidative stress has also been observed on continued exposure of these nanoparticles to human health.

4.4 Titania Nanoparticles

Titanium dioxide/titania nanoparticles are n-type semiconductors that have a wide band gap with extraordinary photocatalytic, self-cleaning, antibacterial, and biocompatible properties. Simple, easy, and low-cost production of titania nanoparticles are advantages in addition to its excellent physical and chemical properties. Titania nanoparticles have occupied a large share in the global market with many developing and developed countries utilizing it in a wide range of applications owing to its low carcinogenic characteristics and strong catalytic properties. Countries like the United States and China have occupied, respectively, 25% and 53% of the total share of products being manufactured using titania nanoparticles along with other countries like Australia, Canada, France, Germany, India, Japan, Spain, and Turkey (StatNano 2016 2017). The global market of titania-based products that were commercially available was recorded to be $13.3 billion in 2015, which has been anticipated to grow at a CAGR of 8.9% from 2016 to 2025 (Titanium Dioxide Market Analysis By Application 2015).

The increase in demand of titania nanoparticle-based products in the market has raised a major concern regarding the toxicity of titania nanoparticles to human health. Wide ranges of toxicity studies have been performed to evaluate in vitro and in vivo toxicity of titania nanoparticles to human cells. Size and crystal structure of titania nanoparticles play key factors in affecting nanotoxicity. It has been reported that the smaller the size of titania nanoparticles and the greater the content of anatase structure, the higher will be the ability to induce toxicity into different biological systems (Simon-Deckers et al. 2008). Reports have shown that titania nanoparticles having a mixture of anatase and rutile phases induced highest toxicity in comparison to pure forms to anatase and rutile (Ekstrand-Hammarström et al. 2012). In an experimental study, in which rats were exposed to titania nanoparticles through inhalation of titania nanoparticles with a diameter of 21 nm and 250 nm for a period of 12 weeks, it was observed that titania nanoparticles induced high toxic inflammatory reactions in the pulmonary system. It was also observed that smaller titania nanoparticles remained accumulated in the lungs for a long period of 501 days, thereby indicating that clearance rate of titania nanoparticles is extremely slow (Ferin et al. 1992).

Titania nanoparticles are widely utilized in sunscreens. According to a report established by the European Commission – Scientific Committee on Consumer Safety – the use of titania nanoparticles as UV filters in sunscreens up to a concentration of 25% with no significantly high photocatalytic activity can be considered to not cause any risk adverse toxic effects to humans under healthy or sunburnt skin. However, no such regulations were formulated for the use and safety of titania nanoparticles in sunscreen spray products; therefore inhalation of titania nanoparticles must be avoided (European Commission 2013).

4.5 Zinc Oxide Nanoparticles

Zinc oxide nanoparticles are another class of semiconductor nanoparticles that have been studied and used in a wide range of applications owing to its size-dependent excellent electrical and optical properties. Zinc oxide nanoparticles are also widely

used in sunscreens and paint industries as they are optically transparent and still can absorb ultraviolet light. In the year 2015, the global economy of zinc oxide nanoparticles was reported to be $2099 million and is expected to rise up to $7677 million by 2022 with a CAGR of 20.4% (Nano Zinc Oxide Market by Application 2016). Other applications of zinc oxide nanoparticles include photocatalytic degradation of environmental pollutants, transparent electronics, sensors, solar cells, drug delivery systems, etc.

Increased production of zinc oxide and its application in various fields has increased the need for toxicity study of them. There are reports that state that in comparison to different metal oxides being utilized in various applications, zinc oxide nanoparticles pose the maximum threat to human cells. In a study conducted on four diverse dyes that were induced with 20 nm zinc oxide nanoparticle, lowered that potential of mitochondrial membrane because of Ca^{2+} flux and eventually leading to the loss in the integrity of the membrane (George et al. 2009). The most important factor affecting the toxic behavior of zinc oxide nanoparticle is the concentration of zinc ions that interact with different cell types. Toxicity of zinc oxide nanoparticles can spread across human bodies via in vivo routes as well. Introduction of nano- and micro-sized zinc oxide nanoparticles into the intratracheal tract of rats showed toxicity with a reversibility in the inflammation that was at its maximum at 24 hours and resolved within 4 weeks of introduction (Sayes et al. 2007).

Investigation of dermal effects and penetration property of zinc oxide nanoparticles into skin cells is an important concern because they have been extensively used in sunscreens. Reports have shown that zinc oxide nanoparticles do not enter beyond the stratum corneum of human skin cells. The maximum zinc oxide nanoparticles can penetrate are the upper layers of epidermis both for UVB-damaged and healthy skin cells, though the penetration effect is enhanced for UVB-damaged skin (Monteiro-Riviere et al. 2011). However, there is no literature available on the toxicity of zinc oxide nanoparticles utilized in spray sunscreens. According to a report published under European Commission (Scientific Committee on Consumer Safety), the no-observed-adverse-effect level (NOAEL) for the oral consumption of drugs containing zinc oxide nanoparticles is not more than 50 mg/day (European Commission 2012).

4.6 Dendrimer Nanoparticles

Dendrimer nanoparticles are a class of polymer nanoparticles that have branching and tree-like structure. Dendrimer nanoparticles can be easily functionalized and have unique pharmacokinetic properties, which makes such nanoparticles exceptional for medical applications such as targeted drug delivery, bio-imaging, photodynamic therapy, etc. Since dendrimer nanoparticles are majorly used in biomedical applications, it is very important to understand and study about various toxic effects dendrimers can have on human health.

Many experiments have been carried out using various cell lines, numerous incubation times, and different assay methodologies to study the toxic behavior of dendrimer nanoparticles. Dendrimers having $–NH_2$ at its terminals have been reported to show generation-dependent cytotoxicity. It has been reported that PAMAM dendrimers when incubated with lung fibroblasts of a Chinese hamster for a period of 24 h showed in a decrease in the viability of cells (Roberts et al. 1996). Different concentrations affected different generations of dendrimers, for instance, the concentration that was required for 90% cell death for 3rd generation was 1 nano molar, while for 5th generation 10 micro molar was required. Cytotoxic effects caused by dendrimers is also dependent on the property of its core and the nature of functional groups present on its surface. It has been reported that cationic dendrimers having groups such as amines, guanidine, sulphonates, carbonyls, and phosphonates on the surface showed higher cytotoxic effects as compared to anionic dendrimers (Chen et al. 2004).

Many researches are still trying to study the toxic behavior of various other organic nanoparticles such as liposomes, chitosan nanoparticles, etc. against human health; however, deficient information regarding toxicity of organic nanoparticles is available because of their unconventional route of interaction with human cells and limited information related to assessment and evaluation of nanotoxicity**.**

4.7 Carbon Nanotubes

Carbon nanotubes are the most novel class of carbon nanomaterials that have numerous unique and useful properties. Carbon nanotubes are composed of carbon atoms arranged in a hexagonal manner in the form of hollow cylinders that can have a diameter in the range of 0.7 nm up to some mm in length. These nanotubes can either be single layered or multilayered and are extremely very chemically and thermally stable. Synthesis of carbon nanotubes is generally carried out with the utilization of a metal catalyst, whose quantity mainly depends on the properties required from the final product. Carbon nanotubes are considered as excellent material for the production of various composite materials as the resulting nanomaterial has been reported to have exceptional strength and can be utilized in a number of applications such as for building spacecraft, combat jackets, space elevators, etc.

However, many toxic effects have been associated with the use of carbon nanotubes. Carbon nanotubes have been reported to induce fibroblast and T-lymphocyte apoptosis in human cells therefore posing a serious threat to human health as well as show cytotoxicity in the alveolar macrophages (Jia et al. 2005). It has been reported that hydroxylated single-walled carbon nanotubes when administered to mice by incorporating it into their food and with the help of an external food pump spread to almost all the organs and tissues expect for the brain (Wang et al. 2004). It has also been reported that lysine-functionalized single-walled carbon nanotubes transferred into the fibroblast cells of both humans and mouse. They then tend to pass through the cell membrane and get deposited in the cell and the nucleus

(Pantarotto et al. 2004). Single-walled carbon nanotubes have also been reported to reduce the rate cell proliferation, initiate apoptosis, and decrease adherence of human embryonic kidney cells in vitro (Cui et al. 2005). Reports have also shown that multiwalled carbon nanotubes can also penetrate into the cell membrane and can lead to a decline in cell viability and cause an increase in the inflammation marker: interleukin 8 (Monteiro-Riviere et al. 2005).

4.8 Fullerenes

Fullerenes are a class of carbon nanomaterials having similar structure to that of graphene but in a rolled up closed-cage forming a hollow sphere, tube, or ellipsoids. These are commonly known as buckyballs, as they resemble the shape of a football ball. Fullerenes are utilized in numerous fields such as in energy-related applications, cosmetic industry, medical (targeted drug delivery), and polymer applications because of their unique properties like antioxidants and potential of radical scavenging along with excellent chemical, electrical, conductive, and thermal characteristics. With its extraordinary properties and numerous applications, the global economy of fullerenes are expected to increase at a CAGR of 13.96% up to 2019 (Global Fullerene Market 2015 – 2019 2015).

Functionalization of fullerenes is generally done using different hydroxyl groups; though it has excellent improved properties like solubility and high cellular interaction in comparison to pristine fullerene, its toxicity is also an important aspect that has been displaying many harmful and hazardous heath issues. Fullerenes have been reported to cause toxic effects to pulmonary, reproductive, cardiac, renal, and cellular functions of the human body (Injac et al. 2013). Various studies have shown that pristine fullerenes do not show any kind of toxicity (either acute or sub-chronic) to humans nor animals cells (Nelson et al. 1993). However non-covalently functionalized fullerenes have been reported to show some kind of toxicity to mammalian cells. It has been reported that PVP-fullerene complexes tend to bind differently with biological systems as compared to pristine fullerene, which eventually leads to generation of various toxic effects. Reports have shown that repeated exposure of PVP-fullerene complex showed acute and short-term toxic effects on various tissues of rats and guinea pigs (Satoh et al. 1995). Covalently functionalized fullerenes also tend to cause toxic effects on human beings, which has been confirmed by many in vitro and in vivo studies.

4.9 Nanospheres, Nanoshells, and Nanocapsules

Nanospheres, nanoshells, and nanocapsules are described as nanomaterials encapsulated within insoluble organic polymers with an attempt to allow incorporation with other substances (often drugs and medications). The exterior coating of such nanomaterials can be fine-tuned to selectively interact with certain required sites of the human body.

It has been reported that hydrogel nanosphere interact with the cells of the human body in an reversible manner to produce alteration in the electrical resistance of the epithelial cells thereby showcasing in vitro cytotoxicity (Torres-Lugo et al. 2002). Reports have shown that nanocapsules with a lipid core and shell comprising 2-hydroxy-polyethylene glycol stearate and lecithin stained with technetium-99 and iodine-125 remained deposited in the blood of rats much longer than expected. The nanomaterial was found to be distributed in the animal's stomach, intestines, and livers but not in the cerebral regions (Cahouet et al. 2002). There have been many reports showing different cytotoxic effects of nanospheres, nanoshells, and nanocapsules affecting different parts of the human body functions such as enzyme leakage and disruption in the mitochondrial function and DNA and other biomolecules (Liu et al. 2013).

4.10 Quantum Dots

Quantum dots are nanoparticles having a diameter in the range of 1–10 nm. Quantum dots are mainly used in medical applications such as tagging of biomolecules and in vivo imaging because of their unique fluorescence property. Increased usage in varied applications of quantum dots has also increased the concern related to its toxic effects on human health.

It has been reported that zinc sulfide and cadmium selenide quantum dots coated with sheep serum albumin and mercaptopropionic acid are much more toxic for human fibroblasts and tumor cells (Shiohara et al. 2004). These quantum dots have also been reported to cause cervical cancer and human hepatocytes. A study conducted by incubating double-stranded DNA in cadmium selenide solution encapsulated with zinc sulfide functionalized with surface biotin under ultraviolet radiation showed that the level of toxicity and alteration in the DNA was dependent on the application of ultraviolet radiation (Kirchner et al. 2005). It has been reported that cadmium telluride quantum dots have the ability to cause disruption of mitochondrial membrane and increase the level of intracellular calcium, impair cellular respiration, and decrease the synthesis of ATP causing very detrimental health effects (Green and Howman 2005).

5 Effects of Nanomaterials on the Environment

5.1 Nanomaterials and Air Pollution

The fate of nanomaterials and ultrafine particles in the contribution of airborne diseases is an important topic of concern. As nanoparticles have extremely small size and high mobility, they tend to mix more rapidly with the aerosol systems and can be easily exposed to sunlight and ultraviolet radiation at a much larger degree as comparted to other environmental systems.

Exposure of nanoparticles to air and sunlight increases the possibility of them to be photochemically transformed, causing many hazardous environmental conditions. Another important factor governing the deposition and settling of toxic nanoparticles in the air is gravitational velocity, which is proportional to the morphology and dimensions of the particle. Therefore, smaller particle will deposit in air at a much slower rate in comparison to larger particles. Agglomeration also plays an important role as it enhances the deposition of nanoparticles in air. It is essential to understand the source, fate, and different physical and chemical properties of nanomaterials present in the environment. It is also important to know about their origin, medium, and nature of interaction with other nanoparticles and environmental pollutants.

Nanomaterials present in the atmosphere are majorly from three sources (Nguyen et al. 2015a):

1. *Primary emission*: Nanomaterials in this category are mainly from the openly released pollutants from industrial combustion and car exhausts.
2. *Secondary emission*: Nanomaterials in this category are those produced from the compression of low volatile vapors from the oxidation of the gasses present in the atmospheric.
3. *Tertiary emission*: Nanomaterials in this category are obtained from the exhaust of diesel dilution.

The risk associated with the presence of nanomaterials being present in the atmosphere is very gruesome, yet not much knowledge or literature is available due to the lack of assessment tool to distinguish between nanomaterials and the naturally present particles in air (Nguyen et al. 2015a). However, from the existing literature, it is reported that ultrafine particles and nanomaterials present in the atmosphere undergo several processes in the air and lead to numerous environmental as well as health issues. Nanomaterials present in the air can be produced by condensation of low volatile compounds or undergo reduction in size by evaporation of adsorbed water molecules as a consequence of which the particle size distribution deviated from the overall concentration. Nanomaterials in the atmosphere can also combine with the already present particles and increase in the dimension causing an overall decrease in the original concentration (Baalousha and Lead 2009). Also sometimes nanoparticles have the possibility to go completely missing from the atmosphere either by dry or wet deposition process therefore causing a decline in the concentration and change in the particle size distribution of atmospheric particle thereby causing changes in the atmospheric conditions (Gidhagen et al. 2004).

5.2 Nanomaterials and Soil Pollution

Soil is a mixture and complex interface between inorganic and organic matter along with some gasses, water, solid matter, and organisms. The extremely small size of nanoparticles permits it to penetrate and pass easily through the pores present in the

surface of soils. These nanoparticles can also stick to the surface of soil particles owing to their high surface area and become immobilized which can eventually cause soil pollution. Large agglomerated nanoparticles tend to be immobilized within the smaller pores of soil during different process such as sedimentation, filtration, and straining.

There have been studies conducted that report the transport and fate of nanomaterials in the naturally occurring porous nature of the soil environment which concludes that movement of nanomaterials in the soil is at a moderately fast speed and is mainly dependent on the size, shape, and type of nanomaterial under consideration (Clarke et al. 2004). The probable mechanism of nanomaterials spreading its toxic effects within the soil environment include oxidation of proteins, interruption of energy transduction, disruption of membrane potential, genotoxicity, formation of reactive oxygen species, and release of various toxic constituents (Li et al. 2006). Toxicity of nanomaterials in soil depends on many factors such as surface charge, high surface-to-volume ratio, hydrophobicity, and presence of different lipophilic groups that may allow them to connect with the proteins and membranes thereby causing inhibition of enzymatic activity, bioaccumulation, and variation in chemical composition (Zharov et al. 2006). Numerous properties of soil and nanoparticles together interact to affect the transport and mediation of nanomaterials, which effect soil conditions and cause soil pollutions. Properties include type of organism present, nature of soil constituents, various physical and chemical properties of nanomaterials, and interaction between nanoparticle and the natural colloid materials.

5.3 Nanomaterials and Water Pollution

Water is the most abundant and important resource present on the planet earth. However, with the advancement in technology and utilization of nanomaterials in a variety of applications, the disposal of such materials into water bodies has become a common practice. Nanomaterials interact with the natural constituents of the water body and adversely affect the natural habitat of the aquatic ecosystem. Fate of nanomaterials in the aquatic environment greatly depends on the interaction between nanoparticles and the different biotic and abiotic components.

In an experiment conducted to study the determining factor that spreads toxicity of nanoparticles in water bodies, agglomeration, and sedimentation of citrate and polyvinylpyrrolidone, capped silver nanoparticles in calcium chloride solution were studied. It was concluded that as the ionic strength of the solution increased, citrate-capped silver nanoparticles aggregated more rapidly and settled down at a faster rate in comparison to polyvinylpyrrolidone-capped silver nanoparticle, which on the other hand did not aggregate at all. This was attributed to the fact that polyvinylpyrrolidone coating created steric hindrance even at a strong ionic strength of 10 mM calcium chloride. It was also observed that polyvinylpyrrolidone-coated silver nanoparticles sedimented within a week without aggregating confirming that particle size and the type of coating used for nanoparticles played major roles in determining the fate of nanoparticles in water (Jafar and Hamzeh 2013).

Nanomaterials released into water bodies can affect the ecology at different trophic levels. There are many reports stating the ill effects of different types of nanomaterials to different marine organisms present in water bodies; however their mode of action is still unclear (Jang et al. 2014). The process of sedimentation and aggregation has been reported to carry nanoparticles into different levels of trophic levels of the water ecosystem, resulting in bioaccumulation of the toxic deposits subsequently polluting the aquatic ecosystem (Rocha et al. 2015).

6 Mechanism of Nanotoxicity

A number of properties of nanomaterials based on their physical and chemical features can affect the toxicity caused by nanomaterials such as their size, shape, surface area, etc. One of the most important characteristics of nanomaterials to cause toxicity is its size. The smaller the size of the nanoparticle, the easier it becomes for it to penetrate into the epithelial and endothelial cells of the body which are then transported into the lymph and bloodstream to different organs such as the brain, liver, heart, kidney, etc.

Various other mechanisms reported for the entry of nanomaterials into the body and bloodstream include the trans-cytosis mechanism, diffusion mechanism through the cell membrane, ingestion (Tiede et al. 2016), and penetration through the skin (Holsapple et al. 2005). Nanoparticles along with microparticles of greater size can enter into the body through the skin when loose or bent (Ryman-Rasmussen et al. 2006b).

Researchers have conducted numerous experiments to study the toxicity of nanomaterials in the human body and have concluded that nanoparticles increase the aggregation of platelet in the bloodstream thereby causing thrombosis (Tinkle 2003). Neurodegenerative disorders, inflammation of the upper and lower respiratory tracts, and myocardial infarction are some ways by which toxicity of nanomaterials spread and lead to discomforts in the human body (Radomski et al. 2005). Nanoparticles also have the tendency to enter into cell organelles such as mitochondria and nuclei owing to its extremely small size, which indeed can modify cell metabolism and eventually cause DNA lesions, mutations, and cell death (Madl et al. 2014).

Quantum dots are the smallest class of nanomaterials that have a diameter of up to 10 nm; studies have been reported showing the leakage of the metal ions contained within their cores such as cadmium, arsenic, lead, and mercury when oxidized by various environmental agents. Quantum dots within body can be absorbed by different cell organelles and have been reported to cause conformational changes and dysfunction (Barua and Mitragotri 2014). Quantum dot penetration also increases the fluidity of the cell membrane eventually causing cell death (Nguyen et al. 2015b). When cadmium-based quantum enter into the bloodstream and reach different organs and organelles, it tends to release Cd^{2+} ions resulting in oxidative stress of the cells (Wang et al. 2012a). Nanoparticles having size of about 50 nm

when is exposed to the tissues of the respiratory system (especially the lung tissues), causes the aperture of the type I alveolar membrane ultimately causing cell necrosis because of the release of lactate dehydrogenase (Singh et al. 2012). One of the most common mechanisms of toxicity of nanoparticles affecting human cells is the formation of reactive oxygen species (ROS), which is induced by peroxidation of the lipid membrane that may lead to the loss of the flexibility of the membrane, irregularly increase the fluidity of the cell, and inevitably result in cell death.

Nanoparticle interaction with the cytoskeleton has also been reported to damage the same. For instance, TiO_2 NP instigate structural changes in tubulin, thereby hindering its polymerization (Ruenraroengsak et al. 2012), which intrudes various cell procedures such as intracellular transport, cell division, and cell migration. Nanoparticle cytotoxicity may obstruct cell differentiation and protein synthesis and also activate pro-inflammatory genes and synthesis of various inflammatory mediators. The general mechanism of nanotoxicity on human cells is illustrated in Fig. 4. Studies have also showed that typical protective and defensive mechanisms of the cell do not affect nanoparticles; however, macrophage uptake of large PEGylated is more effective than the uptake of small particles, which prompts aggregation of nanoparticles within the body (Mao et al. 2015). Superparamagnetic iron oxide nanoparticles have been exhibited to interrupt or completely destroy osteogenic differentiation of undifferentiated cells and activate the synthesis of signal molecules, tumor-causing antigens, etc. (Walkey et al. 2012). Moreover, interaction of nanoparticles with the cell improves the expression of the genes that are accountable for the development of lysosomes (Kostura et al. 2004), aggravates their working (Kedziorek et al. 2010), and represses protein synthesis (Puppi et al. 2011).

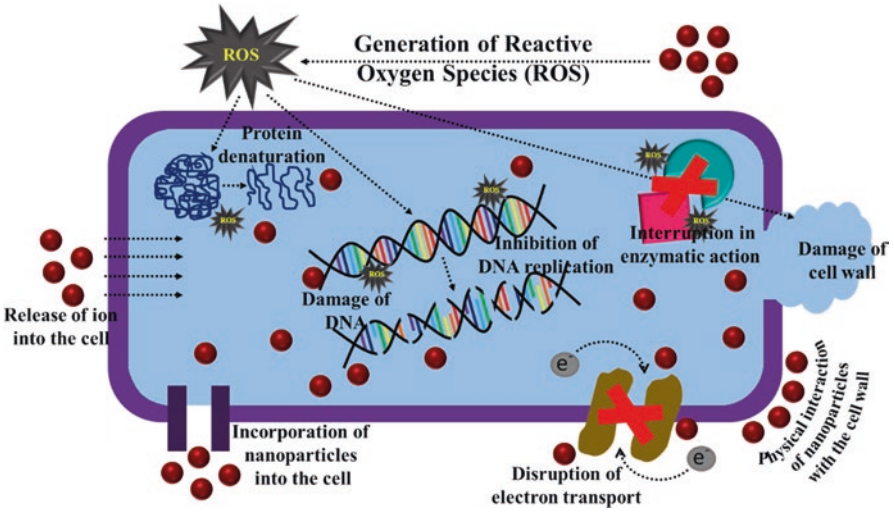

Fig. 4 Mechanism of nanotoxicity on human cells

An investigation on the toxic impact of nanoparticle of various compositions and conformations on lung epithelial cells and human tumor cell lines has demonstrated that nanoparticles stimulate the synthesis of inflammation mediators, e.g., interleukin 8 (Wang et al. 2017). According to Park, who studied the expression of pro-inflammatory cytokines in vitro and in vivo, the expressions of interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNFα) are enhanced in response to silicon nanoparticles (Choi et al. 2009).

7 Factors Affecting Nanotoxicity

A number of physical and chemical properties of nanomaterials can introduce toxicity into the human body such as size, shape, surface area, aspect ratio, surface charge, aggregation and agglomeration of nanoparticles, chemical composition, surface coating, porosity and solubility, and protein corona.

7.1 Size and Surface Area

Size is one of the most important and characteristic features of nanomaterials that gives it unique properties as compared to its bulk counterparts. The size and surface area of nanoparticles play a very essential role in underlying the toxicity mechanism when in contact with biological molecules. Decreasing the size of the nanomaterial increases its surface area thus making it more reactive to itself and its surrounding environment. The size of an average nanoparticle (1–100 nm) is closely related to the size of biomolecules such as the diameter of the DNA helix (~2 nm) and a protein globule (2–10 nm) and the thickness of cell membranes (~10 nm) which lets it easily penetrate into the cell and cell organelles thereby causing conformational changes of various biomolecules in the human body and thus hindering with vital biological functions (Park and Park 2009).

Based on a size-dependent study of toxicity of gold nanoparticle conducted by Pan et al., it was concluded that the toxicity was inversely dependent on the particle size of the nanoparticle in the range of 0.8–15 nm. Gold nanoparticles with a particle size of 1.4 nm were observed to be much more toxic to epithelial cells, connective tissues, and fibroblasts as compared to particles with a smaller size range (0.8 nm, 1.2 nm) and larger dimension (1.8 nm, 15 nm). Difference in the size of gold nanoparticle caused different toxic effects, for instance, gold nanoparticles with 1.4 nm caused cell necrosis, while gold nanoparticle with a size of 1.2 nm mainly led to apoptosis (Aggarwal et al. 2009).

The size of the nanoparticles affects the absorption, metabolism, distribution, and excretion from the body. Taking into account the respiratory system of the human body, the lungs play a vital role in absorption and distribution of various nanoparticles within the body; therefore, the size of nanoparticles plays a very

crucial role as different sizes of the same material might show different respiratory health effects and toxicity concerns. It has been reported that inhaled ultrafine nanoparticles with a diameter less than 100 nm deposit in all regions of the lungs, while nanoparticles with a size less than 10 nm deposit in the tracheobronchial region; however the smallest nanoparticles in the range of 10–20 nm deposit in the alveolar region (Pan et al. 2007).

7.2 Shape and Aspect Ratio

Synthesis of nanoparticles results in a variety of shapes such as spheres, rods, tubes, sheets, cubes, cylinders, etc. Toxicity of nanomaterials is also a shape-dependent phenomenon as it stimulates the membrane wrapping events in vivo during endocytosis or phagocytosis. Spherical nanoparticles are the most commonly synthesized shape; they have been reported to be most likely to undergo cell endocytosis more effectively and at a faster rate as compared to nanofibers and nanotubes (Champion and Mitragotri 2006); however, spherical nanoparticles are less toxic regardless of their heterogeneity or homogeneity (Lee et al. 2007). Studies have reported different morphologies of hydroxyapatite nanoparticles (spherical, rod-shaped, needle-shaped, and platelike) have different toxicity effects on BEAS-2B-cultured cells. Platelike and needlelike nanoparticles tend to cause death of the cultured cells at a much faster rate and percentage in comparison with the conventional spherical or rod-shaped nanoparticles (Zhao et al. 2013).

Aspect ratio is another very important characteristic of nanomaterials. It is the ratio of length to diameter (width) of a nanoparticle. Toxicity of nanomaterials also varies with their varying aspect ratios. Huang et al. studied the biocompatibility, distribution, and clearance properties of silica nanoparticles with a diameter of 70 nm of two different aspect ratios (5 and 1.5). Results concluded that silica nanoparticles having higher aspect ratio were found distributed in the spleen, while nanoparticles with lower aspect ratio were found trapped in the liver (all experiments were carried out by intravenously injecting the nanoparticle solution into laboratory mice). Excretion of nanoparticles with higher aspect ratio was done through urine or feces at a slower rate as they remained for a longer period in the blood, compared to nanoparticles with smaller aspect ratio (Huang et al. 2011).

7.3 Surface Charge and Chemical Composition

Surface charge is an important factor affecting nanotoxicity as it greatly regulates the interaction of nanoparticles with different bio-systems present in the human body. Surface charge determines the selective adsorption of nanomaterials, plasma protein binding, and transmembrane permeability with biomolecules. It has been reported that positively charged nanoparticles have better capability of cellular

uptake as compared to negatively charged nanoparticles because of the enhanced property of opsonization by their plasma proteins (Goodman et al. 2004).

Surface charge of nanoparticles plays an important role in determining the colloidal behavior and its influence with the response of an organism towards nanoparticle uptake by changing the shape and size due to agglomeration or aggregation of the particles resulting in various toxic effects. For example, reports have shown that positively charged PAMAM dendrimers demonstrate toxic effects towards zebrafish and mice embryos, while anionic PAMAM dendrimers show no toxic effects at all (Heiden et al. 2007). It has also been reported that surface charge of nanoparticles modifies the blood-brain integrity and transmembrane permeability; experiments conducted show that negatively charged nanoparticles with a size range of 50–500 nm have the ability to spread throughout the skin, while positively charged or neutral nanoparticles of the same dimensions show no such toxic effect. This is mainly because nanoparticles having the size 50 nm have large specific area, while nanoparticles of 500 nm size have high charge concentration and have the ability to move past the skin barrier (Kohli and Alpar 2004).

Apart from size, shape, and surface area, chemical composition of the nanoparticles with the same dimensions also contribute to the toxic effects produced by them. A study conducted on toxic effects of nano-silver, nano-copper, and nano-titania on zebrafish, daphnids, and algal species has concluded that nano-silver and nano-copper produced toxic effects, while on the other hand, nano-titania with all the same dimensions did not produce any toxic effect at all (Griffitt et al. 2008).

7.4 Surface Coating and Protein Corona

Nanomaterials are usually surface modified and functionalized to improve their properties such as chemical reactivity, electrical, optical, and magnetic, to improve their cellular uptake, distribution, and accumulation and similarly to reduce its toxicity to an excessive limit. However, there have been reports stating that surface-modified nanoparticles tend to increase the presence of oxygen, oxygen radicals, and transition metals, thereby leading to generation of reactive oxygen species thus inducing inflammatory and toxic effects onto biological systems (Risom et al. 2005). For instance, studies have reported that cytotoxicity of silica nanoparticles increased in the presence of surface radicals generating reactive oxygen species inducing toxic effects**.**

Occasionally nanoparticles are functionalized with ligands in order to aid selective drug delivery to targeted regions in the body. When these functionalized nanoparticles stream into the blood, proteins present in the plasma bind to their surfaces forming either hard or soft protein corona (depending on the type of protein that binds to the surface) that affects the nanoparticle uptake and efficiency. Protein corona can induce toxic effects and sometimes perform advantageous functions, for example, multiwalled carbon nanotubes bound with pulmonary surfactant proteins A and D can cause lung infections and emphysema, and correspondingly

single-walled carbon nanotubes and alpha-chymotrypsin complex inhibits enzymatic activity (Salvador-Morales et al. 2007).

7.5 Porosity and Solubility

Nonporous nanoparticles tend to be more toxic in nature in comparison to porous nanoparticles. It has been reported that porous silica nanoparticles show excellent biocompatibility properties, and the hemolytic activity of the same is much lower in comparison to nonporous silica nanoparticles (Slowing et al. 2009). Solubility is another factor that greatly affects the toxic behavior displayed by different nanoparticles (Brunner et al. 2006). Dissolution of nanoparticles leads to the breakdown of the crystal structure of the nanoparticles causing defect state formation within the surface because of which generation of reactive oxygen species takes place therefore inducing toxic effects. Highly soluble nanoparticles can cause acute toxicity to human health, and low solubility nanoparticles have been reported to be carcinogenic in nature (Ferrari 2008).

8 Methods for Detection of Nanotoxicity

Assessment of toxicity of nanomaterials has become a major issue of concern, but it is one of most challenging branches of nanotechnology as there possess many factors of nanomaterials that hinder with the normal functioning of the human body and also the environmental system. Thus, the need of the hour is the development of various analytical techniques to identify the potential level and the mechanism of toxicity of nanomaterials.

8.1 Analytical Techniques

General nanotoxicity assessments starts with different techniques involved in the characterization of nanoparticles. Different characterization techniques help us to identify some of the basic parameters leading to toxicity of nanomaterials such as size, shape, surface charge, chemical composition, availability and nature of nanocoating, etc. These factors not only help one to understand the factors that contribute to toxicity of nanomaterials but also help in determining the transport and fate of nanomaterials in human body as well as the environment**.**

General techniques are used to characterize nanoparticles for its physical and chemical properties. Scanning electron microscope (SEM), transmission electron microscope (TEM), and dynamic light scattering (DLS) characterize nanoparticles based on its size distribution (Murdock et al. 2008). DLS and zeta potential analysis

techniques also help in identifying various hydrodynamic properties such as surface charge and agglomeration of particles (Powers et al. 2006). X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and Brunauer-Emmett-Teller (BET) nitrogen adsorption techniques allow detailed analysis of crystal size, crystal phase, specific surface area, nature of surface coatings, and chemical composition of nanomaterials (Sapsford et al. 2011). Some metal and metal oxide nanoparticles tend to dissolve slowly over a period of time, releasing different toxic ions. Such dissolved ions can be quantified using techniques such as atomic adsorption spectroscopy (AAS), atomic emission spectroscopy (AES), and inductively coupled plasma mass spectrometry (ICP-MS). The description of the abovementioned analytical techniques is beyond the scope of this subject.

8.2 Assays for Detection of Toxicity

8.2.1 Colorimetric Assays

The metabolic activity of the cells (viable or nonviable) leads to the release of several enzymes. The basic mechanism of different colorimetric assays is that different dyes used form complexes with enzymes released from the cells or with the DNA. These complexes generate different colors, and the intensity of the color helps in the further quantification of the number of viable or the nonviable cells. A general protocol followed for colorimetric assay is that a given amount of cell suspension (counting of cells is carried using the hemocytometer and binocular microscope) is mixed with a small quantity of dye.

The percentage of toxic cells is then calculated using the following formula:

$$\text{percentage of toxic cells} = \frac{\text{total number of toxic cells per mL of aliquot}}{\text{total number of cells per mL of aliquot}} \times 100$$

Microculture Tetrazolium Assay

Microculture tetrazolium assay is a type of colorimetric detection technique that is utilized to qualitatively measure cytotoxicity. There are different types of tetrazolium salts that are used in this technique, such as the following:

- 3-(4,5-Dimethyl-2-thiazolyl)- 2,5-diphenyl-2H-tetrazolium bromide (MTT)
- 3-(4,5-Dimethyl-2-thiazolyl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)
- 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-1)
- 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-8)

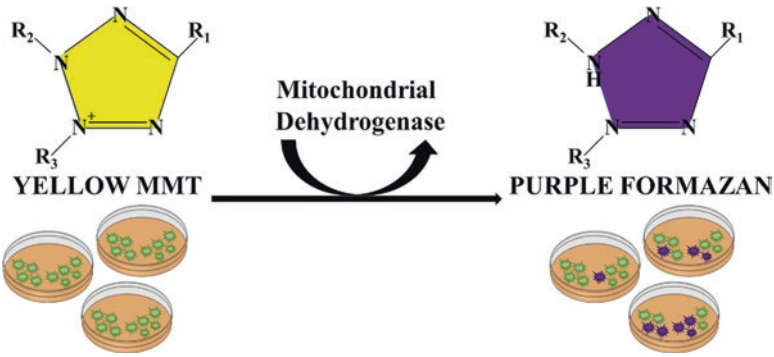

Fig. 5 Working principle of MTT assay

This nonradioactive detection methodology works on the principle of variation of metabolic activity of mitochondria to evaluate the toxicity of the cell. Figure 5 represents the mechanism of evaluation of nanotoxicity by MTT assay. A normal functioning cell has mitochondria that is responsible for the proper functioning of different metabolic activities within the cell along with the release of necessary enzymes such as NADPH oxidoreductase enzyme. This enzyme penetrates out of the cell membrane and reacts with the tetrazolium dye to form a complex that is purple in color, which is insoluble in formazan; further, this compound is solubilized using dimethyl sulfoxide (DMSO). The variation in the intensity of the color can be confirmed by spectrophotometer to evaluate the amount of toxic cells present (Mosmann 1983).

The drawback associated with this technique is the limit of evaluating the exact number of toxic cells along with other disadvantages such as it is not sensitive to human cell lines and poses a threat to the individual carrying out the experiments because of the usage of DMSO. Therefore, numerous modifications have been employed: utilization of tetrazolium derivatives such as XTT, WST-1, WST-8, etc. reacted with the cells to produce water and soluble formazan in order to reduce the toxic effect of the technique.

Flow Cytometry

Flow cytometry is the most accurate and precise cell viability technique available in which a suspension containing the cells is made to pass through a small slit which only allows the passage of particles in the range of 0.2–150 micrometers. Laser lights with a definite wavelength is then allowed to pass through the steamed liquid solution, which then scatters light based on the interaction with the cells. There are two distinct types of light scattered: side scattered light and forward scattered light. Different detectors placed at specific positions detect each of these lights, which are subsequently converted to voltage signals. Therefore, the data obtained from the

scattered light are used to determine different information regarding the presence and the amount of DNA/RNA present, various enzymatic activities taking place, etc. (Darzynkiewicz et al. 1992).

To calculate the number of toxic cells within the solution, a small amount of propidium iodide (nuclear stain dye) is added with the cell suspension. This dye is utilized to quantify apoptosis after breakage of the cell membrane. The dye binds to the double-stranded DNA of nontoxic cells and forms a complex that is fluorescent in nature. This complex is then excited using a wavelength of 488 nm that gives an emission at 617 nm. The higher is the number of fluorescent complexes in the cell suspension, the higher will be the output intensity (Jones and Senft 1985).

The only drawbacks associated with technique are that it requires the utilization of a very complex and expensive instrument with a high maintenance cost and the time taken to carry out a single test is very long.

Trypan Blue Exclusion Assay

Trypan blue exclusion assay utilizes a diazo dye named trypan blue dye (Fig. 6). In this technique, the suspension containing cells is treated with trypan blue dye, which is then visually analyzed whether the cells in the suspension have taken up the dye or not. Cells that are dead absorb the dye and show a color change from white to blue, while live cells have a clear cytoplasm (Strober 1997).

The advantage of this technique is that it helps in easy identification of live and dead cells in contrast to different tedious techniques that involve manually counting the number of cells under a microscope. One of the recent advancements in taking measurements using trypan blue dye includes the introduction of a high-precision flow cytometry for easy measurements. The dye interacts with the cytoplasmic proteins to form complexes that when exposed to green light emit a deep red color at 660 nm, which is easily detected by a flow cytometry.

Lactate Dehydrogenase Assay

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme that is released after the breakage of plasma membrane due to various reasons such as apoptosis and necrosis. The release of LDH is exploited in the working of lactate dehydrogenase assays.

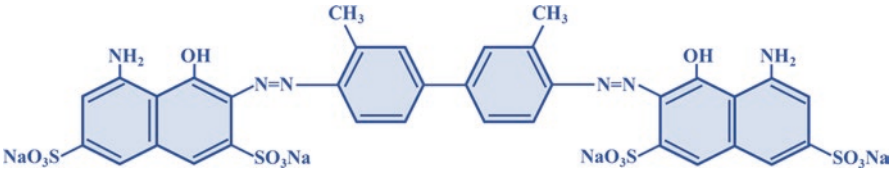

Fig. 6 Structure of trypan blue dye

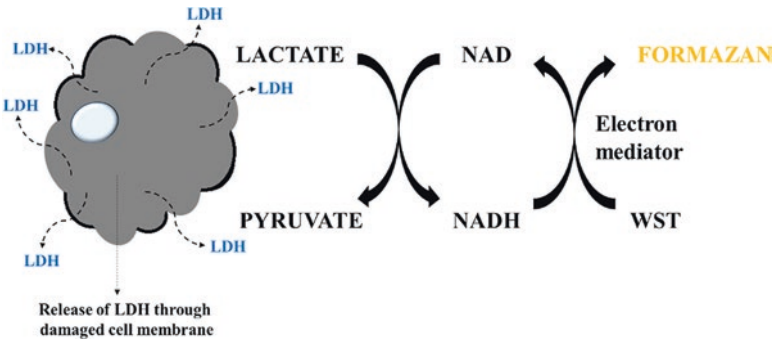

Fig. 7 Mechanism of LDH assay

Upon plasma membrane breakage, LDH releases nicotinamide adenine dinucleotide (NADH) which forms a yellow-colored complex when treated with tetrazolium salt; thus quantification of living cells is done using the amount of formazan complex formed (Chan et al. 2013). The mechanism of lactate dehydrogenase assay is illustrated in Fig. 7. The drawbacks associated with this technique is that not most of the times, cell death occurs due to release of cytosolic enzymes, as there have been reports, which shows that damage or rupture of the membrane can occur eventually. Therefore, under such circumstances, different assays must be employed in order to get accurate results.

8.2.2 Apoptosis-Detecting Assays

Apoptosis is a type of cell death in which the body's cells are programmed to undergo cell death, which is commonly characterized by the condensation of chromatin and nucleus, DNA fragmentation, and formation of a bulge on the surface of the cytoplasm. The most commonly used techniques to quantify apoptosis are Apostain technique, Lamin-B technique, and TUNEL technique.

Apostain Technique

Apostain is a special monoclonal anti-single-stranded DNA present in mouse. This technique is utilized for the early detection of caspase 3, a protein found in the cytoplasm of cells that suffers apoptosis. Cells undergoing apoptosis become brown in color when stained, while live cells remain blue when observed under a light microscope. The advantages of this technique are that it utilizes simple light microscope for detection and also it is highly sensitive and specific and does not require any specialized training and any kind of processing of the DNA (Enari et al. 1998).

Lamin-B Technique

Lamin-B technique is also an early-stage detection of apoptosis technique. In this technique, a nuclear lamin [Lamin-A (acidic); Lamin-B (neutral)] is utilized that has a mesh-like assembly that is present between the heterochromatin and the inner nuclear membrane. Different functions taking place within the nuclear such as chromatin organization, DNA replication, etc. are a result of process undertaken by the nuclear lamins. During the initial stages of apoptosis, a cascade of caspases are released. A caspase responsible for the cleavage of lamin is caspase 6 that triggers the mutation of both Lamin-A and Lamin-B, which eventually triggers DNA fragmentation and chromatin condensation. Therefore, with the utilization of immunohistochemistry, Lamin-B can be identified with the help of antigen (Worman et al. 1988).

TUNEL Technique

Terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) technique works in the principle of detection of DNA fragments. In this technique, a fluorescent dye incorporated into the dUTP nucleotide with the assay molecules subsequently the cells that have fragmented DNA binds with the fluorescent markers and assay molecules, which are further detected using a fluorescent microscope or immunohistochemical stain. The disadvantages associated with this technique are that it fails to differentiate between necrosis and apoptosis and can only detect the final stage of cell damage (Charriaut-Marlangue and Ben-Ari 1995).

8.2.3 Cell Proliferation Assays

Cell proliferation assays are techniques used to determine cell viability after its exposure to various toxins. Different methodologies employed to analyze cell proliferation are flow cytometry, immunohistochemical, histochemical, and protein proliferation techniques. In this technique, DNA of the cells to be studied are stained with either radioactive or fluorescent materials, for instance, thymidine or bromodeoxyuridine, which are then observed using various fluorescence techniques (Fig. 8). The disadvantage of using radioactive thymidine is that it can damage or mutate the DNA, with regard to which generally nonradioactive markers such as bromodeoxyuridine are used. The most advanced cell proliferation technique is protein proliferation. This technique identifies protein proliferation with an attempt to distinguish cells that undergo proliferation from the non-proliferated cells using primary antibodies against different antigens that are generally expressed during proliferation (Ahmed et al. 1994). Commonly used antigens for this technique include Ki67, PCNA, and MCM-2.

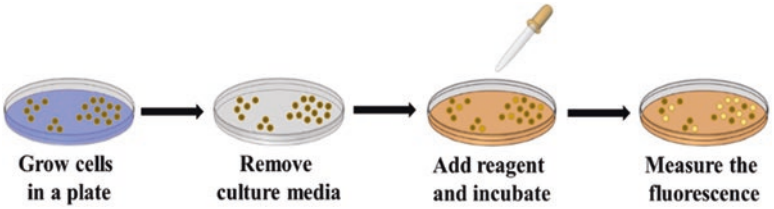

Fig. 8 Methodology of cell proliferation assay

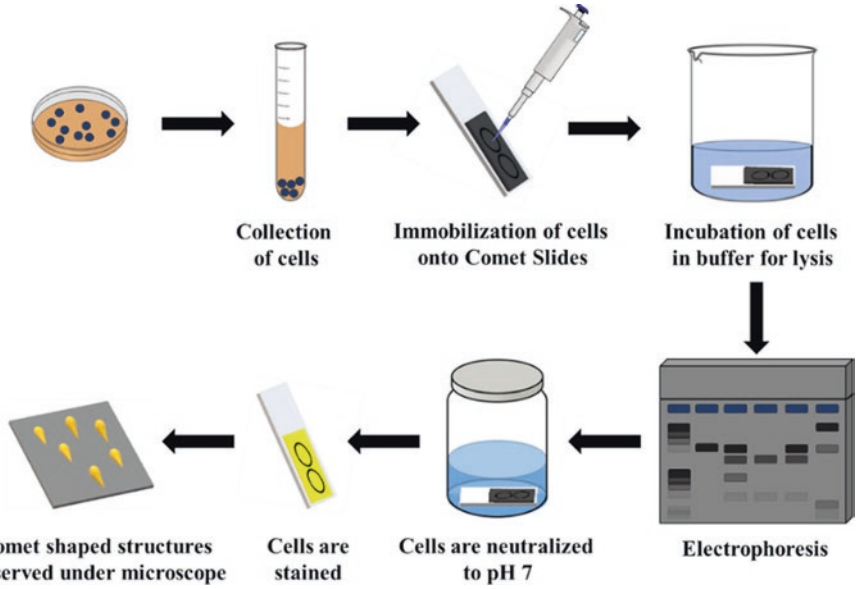

Fig. 9 Steps involved in comet assay

8.2.4 Comet Assay

The single cell gel electrophoresis (SCGE) assay or the comet assay is one of the most common techniques used for in vitro detection of fragments of single-stranded DNA, labile alkali sites, etc. The comet assay is based on the principle of gel electrophoresis, where negatively charged DNA fragments travel across agarose gel on the application of an electric field. Consequent electrophoresis at high pH results in the formation of a comet-like structure; the whole head is composed of intact DNA, while the tail contains broken DNA fragments (comet-like structure formation can be attributed to the attraction of the broken DNA fragments towards the anode). These comet-like structures are stained with the help of fluorescent markers and analyzed under fluorescent microscope (Fig. 9). The ratio of DNA fragments present in the head to the fragments in the tail gives an account on the quantity of damaged fragments (Murugadas et al. 2016).

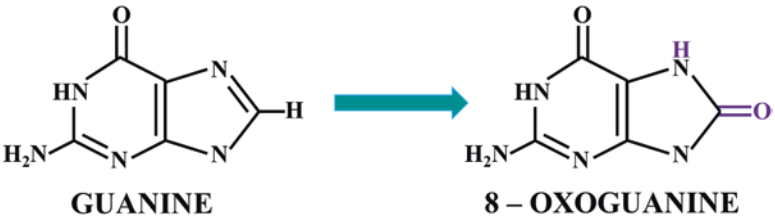

Fig. 10 Structural changes occurring on reaction of cells with nanomaterials

8.2.5 Measurement of Oxidized Guanine Bases

Production of reactive oxygen species is considered as one of the most common mechanisms of toxicity caused by nanomaterials, and oxidative DNA damage is considered as the main cause of cell death. Reactive oxygen species such as singlet oxygen, hydrogen peroxide, and hydroxyl radicals produce superoxide radicals. These superoxide radicals are inactive against DNA but indirectly lead to the oxidation of guanine base pairs and eventually cause rupture of DNA strands. After the oxidation of guanine base pairs, different bases such as 8-hydroxydeoxyguanosine (8-OHdG) and 7,8-dihydro-oxodeoxyguanine (oxo-dG) are produced that can be detected using high-performance liquid chromatography coupled with electrochemical detection (HPLC-ECD). Structural variation of guanine to 8-oxoguanine is represented in Fig. 10. One of the demerits of this technique is that if the bases experience incomplete enzymatic hydrolysis, then it can interfere with action of nucleases and deglycosylation of 8-OHdG thus causing difficulty in analysis (Harparkash and Halliwell 1996).

8.2.6 Chromosomal Aberrations Assays

Genotoxicity of biomaterials can also be assessed by evaluating the chromosomal aberrations. Chromosomal aberrations can be analyzed using different techniques such as micronucleus assay for chromosomal aberrations and trypsin staining. The techniques work on the principle of analysis of the integrity of chromosomes**.**

Micronucleus Assay for Chromosomal Aberrations

Micronucleus assay for chromosomal aberrations work on the principle of analysis of micronuclei that are present inside the cell. Micronuclei are chromatin-containing small bodies that are composed of either partial or whole chromosomes, which leave the nucleic after being exposed to different toxic materials.

Trypsin Staining

Trypsin staining is an assaying methodology that helps in imagining different structural features of chromosomes. When chromosomes are stained with Giemsa, it turns into purple in color. In order to enhance the analysis, chromosomes are initially treated with proteolytic enzyme trypsin and then stained with Giemsa with an attempt to increase G-banding and improve visualization (Barcia 2007).

8.3 Assessment of Nanotoxicity Through Electrochemistry

8.3.1 Carbon-Fiber Microelectrode (CFME) Amperometry

CFME amperometry have been developed to monitor subtle changes in various physiological and neurological processes due to toxicity of different nanoparticles in biological systems of organisms. This technique has many advantages such as the small size of microelectrodes (5–10 micrometer) can be utilized various biochemical changes and exocytosis in single cells as well as in cells that have been damaged due to exposure of toxic nanomaterials. In order to increase the specificity of this technique, electrodes can be modified via functionalization or selective surface coatings.

In order to assess toxicity of nanomaterials, CFME amperometry has been used to estimate the impact of various nanoparticles such as silver, gold, titania, silica, etc. on the impact of serotonin release during exocytosis in cells. Researchers have also reported the use of this technique to study toxic effects of copper oxide nanoparticles and nickel nanoparticles on the intestinal release of serotonin in developing embryos of zebrafish (Özel et al. 2013). Other types of biomolecules in organisms such as dopamine, glucose, hydrogen peroxide, etc. have also been studies using this technique.

8.3.2 Electrochemical Evaluation of Oxidative and Nitrosative Stress Response

Toxicity of nanoparticles can cause production of reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that have been reported to damage DNA, proteins, and lipids. However, the extent of damage caused varied with the type, size, shape, and surface coatings along with different experimental conditions of nanoparticles. Study of such conditions to identify and evaluate the type and extent of damage caused by the production of ROS/RNS by toxic nanoparticles has been carried out using various electrochemical techniques. Gold nanoparticles have been reported to cause oxidative stress in rats thus leading to impairment of the antioxidant enzyme glutathione peroxide present in the brain (Xiong et al. 2011). It has been reported that titania nanoparticles and zinc oxide nanoparticles tend to increase

the production of hydroxyl radical thereby causing oxidative mutation in zebrafish (Wang et al. 2012b).

Nanoparticles tend to either produce excessive amount of superoxide or nitrous oxide or forage or inhibit ROS/RNS. However, detection of such species is very difficult because of their extremely short lifetime; thus advanced techniques have been developed such as electrochemical studies to monitor the free radicals produced under the influence of nanoparticles. An advantage of electrochemical measurements is that it provides real-time release profile of the radical ions produced exactly at the nanoparticle exposure environment to various biological cells.

Reports have shown that Pt/Pt black-coated electrodes allowed quantitative determination of ROS and RNS inside macrophages using voltammetry studies. Cytochrome c-based sensors have been utilized to evaluate and monitor the real-time production of ROS species in the brain slices that were exposed to cerium dioxide nanoparticles (Giaever and Keese 1991).

8.3.3 Electrochemical Impedance Spectroscopy for the Assessment of Nano Effects on Cells

Electrochemical impedance spectroscopy (EIS) is a biophysical assessment procedure that measures the changes in resistance occurring at electrode surfaces. The working principle of EIS techniques is that electrodes immobilized with different cells alter the capacitance and electrode transfer resistance at the surface thereby allowing analysis of changes at the interface occurring because of different properties of the cell. The advantage of using EIS techniques is that it is a noninvasive and label-free technique used to examine motility of cell and study the kinetic behavior of the cell. Reports have shown the use of EIS technique to study the toxicity of different metal oxide nanoparticles, graphene nanomaterials, and carbon nanotubes as well as established as a stable platform for whole-cell-based toxicity study of nanomaterials. It has been reported that graphene nanoflakes showed size-dependent toxicity on various cell line studies using EIS technique (Hondroulis et al. 2012).

9 Regulations on the Use of Nanomaterials

The increasing negative effects of nanomaterials on human and the environment have led to the increase in a new subtopic in the branch of nanotechnology, known as “nanotoxicology.” The issue of toxicity of nanomaterials has been a relatively new topic of concern with regard to nanotechnology, and this is because of the lack of techniques and experimental conditions in order to evaluate and monitor nanotoxicity. With the increase in concerns related to nanotoxicity, many global organizations have taken up the initiative to spread awareness and to design various strategies to assess the toxic effect of nanomaterials on human health and environment.

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental association wherein representatives of 34 developed countries from Asia, Europe, North and South America, and Pacific region, along with the European Commission (EC), associate to deliberate on issues, work in harmony, and formulate policies on issues that have a global concern. One of the important roles performed by OECD is the evaluation of risk associated with the usage of nanomaterials for various applications by means of exchange of information and determination of opportunities to strengthen and reinforce already existing nanotoxicity detection techniques. Various other responsibilities of OECD include promotion of policies and latest technologies for sustainable development for better living standards along with a strong, clean, and fair economy.

The Nanotechnology Research Center, established in the year 2004 under the National Institute for Occupational Safety and Health (NIOSH), is an organization that works and discusses issues related to different occupational safety and numerous health concerns because of the use of nanomaterials. It also works on conducting detailed and exhaustive research and planning on the development and commercialization of nanotechnology and its related products in collaboration with various public and private sectors in the United States and other countries.

An EC-funded organization, the NanoSafety Cluster, has acknowledged the necessity for a computational infrastructure for data management of toxicity of nanomaterials. NanoSafety Cluster plans to maximize and utilize all the collaborations of different projects that had undertaken in the past, ongoing, as well as future planned projects. Different issues covered under these projects include toxicity of nanomaterials to human health and environment, various exposure routes and their respective assessment techniques, mechanisms of interactions, etc. Presently almost 40 projects have been dealing with alarming concern under the umbrella of nanotoxicity. As per a report in the year 2014, NanoSafety Cluster piloted a "Key Global Nano Safety Database Survey," which was first of its kind to accumulate, organize, and share all recent information regarding safety of nanomaterials that was released worldwide. Since then all the resulting lists have been continuously updated with innovative and pioneering insights along with various advancements in the field (Oomen et al. 2014). The Strategic Research Agenda (SRA) is a suborganization of the European NanoSafety Cluster establishment that works on the production of safe and sustainable commercial products using nanomaterials.

Various other organizations working to investigate, evaluate, monitor, and find solutions of nanotoxicity include Federal Institute for Risk Assessment, Federal Ministry of Education and Research, Federal Institute of Occupational Safety and Health, Federal Research Institute of Nutrition and Food, Federal Environment Agency, etc.

Many international organizations have addressed the issue of nanotoxicity and have been working on the same. However, there are no policies or regulations regarding the concern of use and exposure of nanomaterials to humans and the environment in India (Adholeya et al. 2017). The various goals and duties of international organizations have been summarized in Table 3.

Table 3 International organizations involved in the regulation of nanomaterial usage

S. no.	Organization	Goals
1	Organisation for Economic Co-operation and Development (OECD)	Evaluation of toxicity of different nanomaterials through exchange of information and identification of prospects for enhancement of various toxicity detection techniques
2	National Institute for Occupational Safety and Health (NIOSH)	Commercialization of safe and sustainable nanomaterial products
3	EU NanoSafety Cluster	Implementation of projects in all relative nanotoxicology fields
		Organization of workshops to educate general masses and especially workers in the nanotechnology field regarding various toxic effects
4	Federal Institute for Materials Research and Testing	Two main projects taken up:
		NanoDefine (FP7): The objective of this project is to support legislation regarding use of nanomaterials and on availability of different detection techniques relative to nanotoxicity
		NanoValid (FP7): The objective of this project is to develop new reference methods and certified reference materials for detection, characterization, and toxicity documentation of nanomaterials
5	Federal Ministry of Education and Research	*NanoCare*: Deals with the safe usage of nanomaterials
		Studying the toxic effects of manufactured nanomaterials on human health and the environment
6	Federal Environment Agency	*NanoCare*: Deals with the safe usage of nanomaterials
		Inspecting the toxic effects of nanomaterials on environment and human health
7	Federal Institute for Risk Assessment	Demonstration and establishment of new technologies on the basis of various studies and research
		Establishment of "safe by design" products that are manufactured using nanomaterials
		Classification and identification of different classes of nanomaterials based on their origin and toxicity
		Grouping of nanomaterials safely usable by consumers and workers along with reduced environmental and human health risk
8	Federal Institute of Occupational Safety and Health	Grouping of nanomaterials safely usable by consumers and workers along with reduced environmental and human health risk
9	Federal Research Institute of Nutrition and Food	Characterization and detection of nanomaterials used in different industries such as food, textiles, etc.
		Carrying out research on bioactive compounds to be utilized as nano-carriers
		Study of interaction and the mechanism of engineered nanomaterials with food matrices

(continued)

Table 3 (continued)

S. no.	Organization	Goals
10	Modena Cost	Synthesis of nanomaterials with controlled shape, size, composition, etc.
		Development of strategies to immobilize nanomaterials onto different matrices with minimum toxic effect and maximum desired properties and documentation of significant information for quantitative nanostructure-toxicity relationships (QNTR) modelling

References

Klefenz, Heinrich. "Nanobiotechnology: from molecules to systems." *Engineering in life sciences* 4.3 (2004): 211-218.

Takagahara, Toshihide, and Kyozaburo Takeda. "Theory of the quantum confinement effect on excitons in quantum dots of indirect-gap materials." *Physical Review B* 46.23 (1992): 15578.

Hahn, Horst. "Gas phase synthesis of nanocrystalline materials." *Nanostructured Materials* 9.1-8 (1997): 3-12.

Daniel, Marie-Christine, and Didier Astruc. "Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology." *Chemical reviews* 104.1 (2004): 293-346.

Singh, Saurabh, et al. "Applications of nanotechnology in agricultural and their role in disease management." *Res J Nanosci Nanotechnol* 5.1 (2015): 1-5.

Holzinger, Michael, Alan Le Goff, and Serge Cosnier. "Nanomaterials for biosensing applications: a review." *Frontiers in chemistry* 2 (2014): 63.

Mousavi, S. Zeinab, Shohreh Nafisi, and Howard I. Maibach. "Fullerene nanoparticle in dermatological and cosmetic applications." *Nanomedicine: Nanotechnology, Biology and Medicine* 13.3 (2017): 1071-1087.

Priyadarsini, Subhashree, Sumit Mukherjee, and Monalisa Mishra. "Nanoparticles used in dentistry; a review." *Journal of oral biology and craniofacial research* (2017).

De Jong, Wim H., and Paul JA Borm. "Drug delivery and nanoparticles: applications and hazards." *International journal of nanomedicine* 3.2 (2008a): 133.

Bahiraei, Mehdi, and Saeed Heshmatian. "Electronics cooling with nanofluids: A critical review." *Energy Conversion and Management* 172 (2018): 438-456.

Dong, Fan, et al. "Nanomaterials for environmental applications." *Journal of Nanomaterials* 2014 (2014).

Berekaa, Mahmoud M. "Nanotechnology in food industry; advances in food processing, packaging and food safety." *Int. J. Curr. Microbiol. App. Sci* 4.5 (2015): 345-357.

Hwang, Jangsun, et al. "Engineered nanomaterials for their applications in theragnostics." *Journal of Industrial and Engineering Chemistry* (2018).

Lu, Feng, and Didier Astruc. "Nanomaterials for removal of toxic elements from water." *Coordination Chemistry Reviews*356 (2018): 147-164.

BccResearch. Nanotechnology sees big growth in products and applications. Globe Newswire. 2017. https://globenewswire.com/news-release/2017/01/17/906164/0/en/Nanotechnology-Sees-Big-Growth-in-Products-and-Applications-Reports-BCC-Research.html

Dickson, D. “Advanced Manufacturing in a highly connected world.” *Deloitte Touche Tohmatsu Limited* (2015).

Inshakova, Elena, and Oleg Inshakov. “World market for nanomaterials: structure and trends.” *MATEC Web of Conferences*. Vol. 129. EDP Sciences, 2017.

Market, Vertical Farming. "Global Opportunity Analysis and Industry Forecast, 2017-2023." (2016a).

Nanomaterials Market – Global Trends, Investment Analysis and Future Scope to 2022. *Mordor Intelligence, Hydrabad.* (2011)

Abraham, Thomas. "Nanotechnology and Nano Materials: Types, Current/Emerging Applications and Global Markets" *Innovative Research and Products, Inc.* (2011)

Market, Vertical Farming. "Global Opportunity Analysis and Industry Forecast, 2014-2022." (2016b).

Silver Nanoparticles Market Size by Application, Industry Analysis Report, Regional Outlook, Growth Potential, Price Trends, Competitive Market Share & Forecast, 2016–2024. *Global Market Insights Inc.* (2017)

Nanoclay market – Global Industry analysis, Size, Share, Growth, Trends and Forecast 2015-2023. *Transparency Market Research.* (2016)

Production and Applications of Carbon nanotubes, Carbon nanofibers, Fullerenes, Graphene and Nanodiamonds: A Global Technology Survey and Market Analysis, *Innovative Research and Products, Inc,* (2011)

Shvedova, Anna, Antonio Pietroiusti, and Valerian Kagan. "Nanotoxicology ten years later: lights and shadows." (2016): 1-2.

Civeira, Matheus S., et al. "The properties of the nano-minerals and hazardous elements: potential environmental impacts of Brazilian coal waste fire." *Science of the Total Environment* 544 (2016): 892-900.

Leo, Bey Fen, et al. "The stability of silver nanoparticles in a model of pulmonary surfactant." *Environmental science & technology* 47.19 (2013): 11232-11240.

Buzea, Cristina, Ivan I. Pacheco, and Kevin Robbie. "Nanomaterials and nanoparticles: sources and toxicity." *Biointerphases* 2.4 (2007): MR17-MR71.

Mackevica, Aiga, and Steffen Foss Hansen. "Release of nanomaterials from solid nanocomposites and consumer exposure assessment–a forward-looking review." *Nanotoxicology* 10.6 (2016): 641-653.

Warheit, David B., and E. Maria Donner. "Risk assessment strategies for nanoscale and fine-sized titanium dioxide particles: recognizing hazard and exposure issues." *Food and chemical toxicology* 85 (2015): 138-147.

Hagens, Werner I., et al. "What do we (need to) know about the kinetic properties of nanoparticles in the body?." *Regulatory toxicology and pharmacology* 49.3 (2007): 217-229.

Shakeel, Muhammad, et al. "Toxicity of nano-titanium dioxide (TiO 2-NP) through various routes of exposure: a review." *Biological trace element research* 172.1 (2016): 1-36.

Ryman-Rasmussen, Jessica P., Jim E. Riviere, and Nancy A. Monteiro-Riviere. "Penetration of intact skin by quantum dots with diverse physicochemical properties." *Toxicological sciences* 91.1 (2006a): 159-165.

Gopee, Neera V., et al. "Migration of intradermally injected quantum dots to sentinel organs in mice." *Toxicological Sciences* 98.1 (2007): 249-257.

Rouse, Jillian G., et al. "Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin." *Nano Letters* 7.1 (2007): 155-160.

Haliullin, T. O., et al. "Hygienic evaluation of multilayer carbon nanotubes." *Meditsina truda i promyshlennaia ekologiia* 7 (2015): 37-42.

Som, Claudia, et al. "Environmental and health effects of nanomaterials in nanotextiles and facade coatings." *Environment international* 37.6 (2011): 1131-1142.

Chen, Zhen, et al. "Acute toxicological effects of copper nanoparticles in vivo." *Toxicology letters* 163.2 (2006): 109-120.

Bergin, Ingrid L., and Frank A. Witzmann. "Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps." *International journal of biomedical nanoscience and nanotechnology* 3.1-2 (2013): 163-210.

Pelclova, D., et al. "Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO2 (nano) particles." *Occup Environ Med* (2015): oemed-2015.

Witschger, Oliver, and Jean-François Fabriès. *Particules ultra-fines et santé au travail: 1-Caractéristiques et effets potentiels sur la santé*. Institut national de sécurité, 2005.

Keller, Jana, et al. "Time course of lung retention and toxicity of inhaled particles: short-term exposure to nano-Ceria." *Archives of toxicology* 88.11 (2014): 2033-2059.

Nurkiewicz, Timothy R., et al. "Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure." *Environmental health perspectives* 114.3 (2005): 412-419.

Simko, M., and Mats-Olof Mattsson. "Interactions between nanosized materials and the brain." *Current medicinal chemistry* 21.37 (2014): 4200-4214.

Soto, Karla, K. M. Garza, and L. E. Murr. "Cytotoxic effects of aggregated nanomaterials." *Acta Biomaterialia* 3.3 (2007): 351-358.

Völker, Carolin, Matthias Oetken, and Jörg Oehlmann. "The biological effects and possible modes of action of nanosilver." *Reviews of Environmental Contamination and Toxicology Volume 223*. Springer, New York, NY, 2013. 81-106.

Sly, Peter D., and Karen Schüepp. "Nanoparticles and children's lungs: is there a need for caution?." *Paediatric respiratory reviews* 13.2 (2012): 71-72.

Liu, Zhuang, et al. "Carbon nanotubes in biology and medicine: in vitro and in vivo detection, imaging and drug delivery." *Nano research* 2.2 (2009): 85-120.

Wang, Long, et al. "Nano-cerium-element-doped titanium dioxide induces apoptosis of Bel 7402 human hepatoma cells in the presence of visible light." *World Journal of Gastroenterology: WJG* 13.29 (2007): 4011.

Dunford, Rosemary, et al. "Chemical oxidation and DNA damage catalyzed by inorganic sunscreen ingredients." *FEBS letters* 418.1-2 (1997): 87-90.

Hougaard, Karin Sørig, et al. "A perspective on the developmental toxicity of inhaled nanoparticles." *Reproductive Toxicology* 56 (2015): 118-140.

Metal & Metal Oxide Nanoparticles Market: Gold Nanoparticles Continue to Shine in Terms of Value Owing to Significant Market Demand: Global Industry Analysis and opportunity assessment, 2016 – 2026. *Future Market Insights.(*2017*)*

Sharma, Virender K., et al. "Natural inorganic nanoparticles–formation, fate, and toxicity in the environment." *Chemical Society Reviews* 44.23 (2015): 8410-8423.

Unveiling silver nanoparticles market trends in terms of the application spectrum: F&B sector to emerge as a remunerative growth avenue over 2018 – 2024. *Fractovia.*(2018)

Kim, Soohee, and Doug-Young Ryu. "Silver nanoparticle-induced oxidative stress, genotoxicity and apoptosis in cultured cells and animal tissues." *Journal of Applied Toxicology* 33.2 (2013): 78-89.

Gliga, Anda R., et al. "Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release." *Particle and fibre toxicology*11.1 (2014): 11.

Sung, Jae Hyuck, et al. "Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparticles." *Inhalation toxicology* 20.6 (2008): 567-574.

Gold Nanoparticles Market (End-Users – Healthcare, Electronics, Chemicals, and Other End-Users; Applications – Imaging, Targeted Drug Delivery, Proton Therapy, In-Vitro Assays, Sensors, Probes, Catalysis, and Other Applications) – Global Industry Analysis, Size, Share, Growth, Trends, and Forecast 2017 – 2026. *Transparency Market Research.* (2018)

Yao, Mingfei, et al. "Uptake of gold nanoparticles by intestinal epithelial cells: impact of particle size on their absorption, accumulation, and toxicity." *Journal of agricultural and food chemistry* 63.36 (2015): 8044-8049.

Andón, Fernando Torres, and Bengt Fadeel. "Programmed cell death: molecular mechanisms and implications for safety assessment of nanomaterials." *Accounts of chemical research*46.3 (2012): 733-742.

De Jong, Wim H., et al. "Particle size-dependent organ distribution of gold nanoparticles after intravenous administration." *Biomaterials* 29.12 (2008b): 1912-1919.

Cho, Wan-Seob, et al. "Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles." *Toxicology and applied pharmacology* 236.1 (2009): 16-24.

OrbisResearch. Global Magnetic Nanoparticle Market Research Report and Forecast to 2017-2022. Reuters. (2017) https://www.reuters.com/brandfeatures/venture-capital/article?id=12866

Singh, Neenu, et al. "Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION)." *Nano reviews* 1.1 (2010): 5358.

Stroh, Albrecht, et al. "Iron oxide particles for molecular magnetic resonance imaging cause transient oxidative stress in rat macrophages." *Free Radical Biology and Medicine* 36.8 (2004): 976-984.

Jain, Tapan K., et al. "Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats." *Molecular pharmaceutics* 5.2 (2008): 316-327.

Kumari, Monika, et al. "Repeated oral dose toxicity of iron oxide nanoparticles: biochemical and histopathological alterations in different tissues of rats." *Journal of nanoscience and nanotechnology* 12.3 (2012): 2149-2159.

StatNano 2016 – Status of Nano-science, Technology and Innovation. *Statnano Publication.* (2017).

Titanium Dioxide Market Analysis By Application, By Region And Segment Forecast, 2018 – 2025. *Grand View Research.* (2015).

Simon-Deckers, A., et al. "In vitro investigation of oxide nanoparticle and carbon nanotube toxicity and intracellular accumulation in A549 human pneumocytes." *Toxicology*253.1-3 (2008): 137-146.

Ekstrand-Hammarström, Barbro, et al. "Human primary bronchial epithelial cells respond differently to titanium dioxide nanoparticles than the lung epithelial cell lines A549 and BEAS-2B." *Nanotoxicology* 6.6 (2012): 623-634.

Ferin, Juraj, G. Oberdorster, and D. P. Penney. "Pulmonary retention of ultrafine and fine particles in rats." *Am J Respir Cell Mol Biol* 6.5 (1992): 535-542.

European Commission, 2013. Scientific Committee on Consumer Safety. SCCS/1516/13.

Nano Zinc Oxide Market by Application – Global Opportunity Analysis and Industry Forecast, 2014 – 2022. *Allied Market Research.* (2016).

George, Saji, et al. "Use of a rapid cytotoxicity screening approach to engineer a safer zinc oxide nanoparticle through iron doping." *ACS nano* 4.1 (2009): 15-29.

Sayes, Christie M., Kenneth L. Reed, and David B. Warheit. "Assessing toxicity of fine and nanoparticles: comparing in vitro measurements to in vivo pulmonary toxicity profiles." *Toxicological sciences* 97.1 (2007): 163-180.

Monteiro-Riviere, Nancy A., et al. "Safety evaluation of sunscreen formulations containing titanium dioxide and zinc oxide nanoparticles in UVB sunburned skin: an in vitro and in vivo study." *Toxicological Sciences* 123.1 (2011): 264-280.

European Commission, 2012. Scientific Committee on Consumer Safety. SCCS/1489/12.

Roberts, Jeanette C., Mahesh K. Bhalgat, and Richard T. Zera. "Preliminary biological evaluation of polyamidoamine (PAMAM) StarburstTM dendrimers." *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials and The Japanese Society for Biomaterials* 30.1 (1996): 53-65.

Chen, Hui-Ting, et al. "Cytotoxicity, hemolysis, and acute in vivo toxicity of dendrimers based on melamine, candidate vehicles for drug delivery." *Journal of the American Chemical Society* 126.32 (2004): 10044-10048.

Jia, Guang, et al. "Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene." *Environmental science & technology* 39.5 (2005): 1378-1383.

Wang, Haifang, et al. "Biodistribution of carbon single-wall carbon nanotubes in mice." *Journal of nanoscience and nanotechnology* 4.8 (2004): 1019-1024.

Pantarotto, Davide, et al. "Translocation of bioactive peptides across cell membranes by carbon nanotubes." *Chemical communications* 1 (2004): 16-17.

Cui, Daxiang, et al. "Effect of single wall carbon nanotubes on human HEK293 cells." *Toxicology letters* 155.1 (2005): 73-85.

Monteiro-Riviere, Nancy A., et al. "Multi-walled carbon nanotube interactions with human epidermal keratinocytes." *Toxicology letters* 155.3 (2005): 377-384.

Global Fullerene Market 2015 – 2019. *Prnewswire.* (2015).

Injac, Rade, Matevz Prijatelj, and Borut Strukelj. "Fullerenol nanoparticles: toxicity and antioxidant activity." *Oxidative Stress and Nanotechnology*. Humana Press, Totowa, NJ, 2013. 75-100.

Nelson, Mark A., et al. "Effects of acute and subchronic exposure of topically applied fullerene extracts on the mouse skin." *Toxicology and industrial health* 9.4 (1993): 623-630.

Satoh, Mitsutoshi, et al. "Effects of acute and short-term repeated application of fullerene C60 on agonist-induced responses in various tissues of guinea pig and rat." *General pharmacology* 26.7 (1995): 1533-1538.

Torres-Lugo, Madeline, et al. "Physicochemical behavior and cytotoxic effects of p (methacrylic acid–g-ethylene glycol) nanospheres for oral delivery of proteins." *Journal of controlled release* 80.1-3 (2002): 197-205.

Cahouet, A., et al. "Biodistribution of dual radiolabeled lipidic nanocapsules in the rat using scintigraphy and γ counting." *International journal of pharmaceutics* 242.1-2 (2002): 367-371.

Liu, Huiyu, et al. "Impact of PEGylation on the biological effects and light heat conversion efficiency of gold nanoshells on silica nanorattles." *Biomaterials* 34.28 (2013): 6967-6975.

Shiohara, A., et al. "On the cytotoxicity caused by quantum dots Microbiol Immunol 48: 669–675." *Find this article online*(2004).

Kirchner, Christian, et al. "Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles." *Nano letters* 5.2 (2005): 331-338.

Green, Mark, and Emily Howman. "Semiconductor quantum dots and free radical induced DNA nicking." *Chemical communications* 1 (2005): 121-123.

Nguyen, Kathy C., et al. "Mitochondrial toxicity of cadmium telluride quantum dot nanoparticles in mammalian hepatocytes." *Toxicological sciences* 146.1 (2015a): 31-42.

Baalousha, Mohamed, and Jamie R. Lead. "Overview of nanoscience in the environment." *Environmental and human health impacts of nanotechnology. Wiley-Blackwell Publishing Ltd, Hoboken, NJ* (2009): 1-25.

Gidhagen, Lars, et al. "Model simulations of NO x and ultrafine particles close to a Swedish highway." *Environmental science & technology* 38.24 (2004): 6730-6740.

Clarke, A. G., et al. "A Lagrangian model of the evolution of the particulate size distribution of vehicular emissions." *Science of the total environment* 334 (2004): 197-206.

Li, Xiao-qin, Daniel W. Elliott, and Wei-xian Zhang. "Zero-valent iron nanoparticles for abatement of environmental pollutants: materials and engineering aspects." *Critical reviews in solid state and materials sciences* 31.4 (2006): 111-122.

Zharov, Vladimir P., et al. "Photothermal nanotherapeutics and nanodiagnostics for selective killing of bacteria targeted with gold nanoparticles." *Biophysical journal* 90.2 (2006): 619-627.

Jafar, Gohargani, and Ghasemi Hamzeh. "Ecotoxicity of nanomaterials in soil." *Ann Biol Res* 4.1 (2013): 86-92.

Jang, Min-Hee, et al. "Effect of material properties on stability of silver nanoparticles in water." *Journal of nanoscience and nanotechnology* 14.12 (2014): 9665-9669.

Rocha, Thiago Lopes, et al. "Ecotoxicological impact of engineered nanomaterials in bivalve molluscs: an overview." *Marine environmental research* 111 (2015): 74-88.

Tiede, Karen, et al. "How important is drinking water exposure for the risks of engineered nanoparticles to consumers?." *Nanotoxicology* 10.1 (2016): 102-110.

Holsapple, Michael P., et al. "Research strategies for safety evaluation of nanomaterials, part II: toxicological and safety evaluation of nanomaterials, current challenges and data needs." *Toxicological Sciences* 88.1 (2005): 12-17.

Ryman-Rasmussen, Jessica P., Jim E. Riviere, and Nancy A. Monteiro-Riviere. "Penetration of intact skin by quantum dots with diverse physicochemical properties." *Toxicological sciences* 91.1 (2006b): 159-165.

Tinkle, Sally S., et al. "Skin as a route of exposure and sensitization in chronic beryllium disease." *Environmental health perspectives* 111.9 (2003): 1202.

Radomski, Anna, et al. "Nanoparticle-induced platelet aggregation and vascular thrombosis." *British journal of pharmacology* 146.6 (2005): 882-893.

Madl, Amy K., et al. "Nanoparticles, lung injury, and the role of oxidant stress." *Annual review of physiology* 76 (2014): 447-465.

Barua, Sutapa, and Samir Mitragotri. "Challenges associated with penetration of nanoparticles across cell and tissue barriers: a review of current status and future prospects." *Nano today* 9.2 (2014): 223-243.

Nguyen, Kathy C., et al. "Mitochondrial toxicity of cadmium telluride quantum dot nanoparticles in mammalian hepatocytes." *Toxicological sciences* 146.1 (2015b): 31-42.

Wang, Tiantian, et al. "Cellular uptake of nanoparticles by membrane penetration: a study combining confocal microscopy with FTIR spectroelectrochemistry." *ACS nano* 6.2 (2012a): 1251-1259.

Singh, Braj R., et al. "ROS-mediated apoptotic cell death in prostate cancer LNCaP cells induced by biosurfactant stabilized CdS quantum dots." *Biomaterials* 33.23 (2012): 5753-5767.

Ruenraroengsak, Pakatip, et al. "Respiratory epithelial cytotoxicity and membrane damage (holes) caused by amine-modified nanoparticles." *Nanotoxicology* 6.1 (2012): 94-108.

Mao, Zhilei, et al. "Titanium dioxide nanoparticles alter cellular morphology via disturbing the microtubule dynamics." *Nanoscale* 7.18 (2015): 8466-8475.

Walkey, Carl D., et al. "Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake." *Journal of the American Chemical Society* 134.4 (2012): 2139-2147.

Kostura, Lisa, et al. "Feridex labeling of mesenchymal stem cells inhibits chondrogenesis but not adipogenesis or osteogenesis." *NMR in Biomedicine: An International Journal Devoted to the Development and Application of Magnetic Resonance In Vivo* 17.7 (2004): 513-517.

Kedziorek, Dorota A., et al. "Gene expression profiling reveals early cellular responses to intracellular magnetic labeling with superparamagnetic iron oxide nanoparticles." *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* 63.4 (2010): 1031-1043.

Puppi, Juliana, et al. "Use of a clinically approved iron oxide MRI contrast agent to label human hepatocytes." *Cell transplantation* 20.6 (2011): 963-975.

Wang, Linlin, Chen Hu, and Longquan Shao. "The antimicrobial activity of nanoparticles: present situation and prospects for the future." *International journal of nanomedicine*12 (2017): 1227.

Choi, Soo-Jin, Jae-Min Oh, and Jin-Ho Choy. "Toxicological effects of inorganic nanoparticles on human lung cancer A549 cells." *Journal of Inorganic Biochemistry* 103.3 (2009): 463-471.

Park, Eun-Jung, and Kwangsik Park. "Oxidative stress and pro-inflammatory responses induced by silica nanoparticles in vivo and in vitro." *Toxicology letters* 184.1 (2009): 18-25.

Aggarwal, Parag, et al. "Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy." *Advanced drug delivery reviews*61.6 (2009): 428-437.

Pan, Yu, et al. "Size-dependent cytotoxicity of gold nanoparticles." *Small* 3.11 (2007): 1941-1949.

Champion, Julie A., and Samir Mitragotri. "Role of target geometry in phagocytosis." *Proceedings of the National Academy of Sciences* 103.13 (2006): 4930-4934.

Lee, Mi-Kyung, Soo-Jeong Lim, and Chong-Kook Kim. "Preparation, characterization and in vitro cytotoxicity of paclitaxel-loaded sterically stabilized solid lipid nanoparticles." *Biomaterials* 28.12 (2007): 2137-2146.

Zhao, Xinxin, et al. "Cytotoxicity of hydroxyapatite nanoparticles is shape and cell dependent." *Archives of toxicology* 87.6 (2013): 1037-1052.

Huang, Xinglu, et al. "The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility in vivo." *ACS nano* 5.7 (2011): 5390-5399.

Goodman, Catherine M., et al. "Toxicity of gold nanoparticles functionalized with cationic and anionic side chains." *Bioconjugate chemistry* 15.4 (2004): 897-900.

Heiden, Tisha C. King, et al. "Developmental toxicity of low generation PAMAM dendrimers in zebrafish." *Toxicology and applied pharmacology* 225.1 (2007): 70-79.

Kohli, A. K., and H. O. Alpar. "Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge." *International journal of pharmaceutics* 275.1-2 (2004): 13-17.

Griffitt, Robert J., et al. "Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms." *Environmental Toxicology and Chemistry* 27.9 (2008): 1972-1978.

Risom, Lotte, Peter Møller, and Steffen Loft. "Oxidative stress-induced DNA damage by particulate air pollution." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 592.1 (2005): 119-137.

Salvador-Morales, Carolina, et al. "Binding of pulmonary surfactant proteins to carbon nanotubes; potential for damage to lung immune defense mechanisms." *Carbon* 45.3 (2007): 607-617.

Slowing, Igor I., et al. "Mesoporous silica nanoparticles for reducing hemolytic activity towards mammalian red blood cells." *Small* 5.1 (2009): 57-62.

Brunner, Tobias J., et al. "In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility." *Environmental science & technology* 40.14 (2006): 4374-4381.

Ferrari, Mauro. "Nanogeometry: beyond drug delivery." *Nature Nanotechnology* 3.3 (2008): 131.

Murdock, Richard C., et al. "Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique." *Toxicological sciences* 101.2 (2008): 239-253.

Powers, Kevin W., et al. "Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation." *Toxicological Sciences* 90.2 (2006): 296-303.

Sapsford, Kim E., et al. "Analyzing nanomaterial bioconjugates: a review of current and emerging purification and characterization techniques." *Analytical chemistry* 83.12 (2011): 4453-4488.

Mosmann, Tim. "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays." *Journal of immunological methods* 65.1-2 (1983): 55-63.

Darzynkiewicz, Zbigniew, et al. "Features of apoptotic cells measured by flow cytometry." *Cytometry* 13.8 (1992): 795-808.

Jones, Kenneth H., and James A. Senft. "An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide." *Journal of Histochemistry & Cytochemistry* 33.1 (1985): 77-79.

Strober, Warren. "Trypan blue exclusion test of cell viability." *Current protocols in immunology* 21.1 (1997): A-3B.

Chan, Francis Ka-Ming, Kenta Moriwaki, and María José De Rosa. "Detection of necrosis by release of lactate dehydrogenase activity." *Immune Homeostasis*. Humana Press, Totowa, NJ, 2013. 65-70.

Enari, Masato, et al. "A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD." *Nature* 391.6662 (1998): 43.

Worman, Howard J., et al. "A lamin B receptor in the nuclear envelope." *Proceedings of the National Academy of Sciences*85.22 (1988): 8531-8534.

Charriaut-Marlangue, C., and Y. Ben-Ari. "A cautionary note on the use of the TUNEL stain to determine apoptosis." *Neuroreport* 7.1 (1995): 61-64.

Ahmed, S. Ansar, Robert M. Gogal Jr, and Jane E. Walsh. "A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H] thymidine incorporation assay." *Journal of immunological methods* 170.2 (1994): 211-224.

Murugadas, Anbazhagan, et al. "Hydra as a model organism to decipher the toxic effects of copper oxide nanorod: Eco-toxicogenomics approach." *Scientific reports* 6 (2016): 29663.

Harparkash, K. A. U. R., and Barry Halliwell. "Measurement of oxidized and methylated DNA bases by HPLC with electrochemical detection." *Biochemical Journal*318.1 (1996): 21-23.

Barcia, Juan José. "The Giemsa stain: its history and applications." *International journal of surgical pathology* 15.3 (2007): 292-296.

Özel, Rıfat Emrah, et al. "Effect of cerium oxide nanoparticles on intestinal serotonin in zebrafish." *RSC advances* 3.35 (2013): 15298-15309.

Xiong, Daowen, et al. "Effects of nano-scale TiO2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage." *Science of the Total environment* 409.8 (2011): 1444-1452.

Wang, Yixian, et al. "Nanoelectrodes for determination of reactive oxygen and nitrogen species inside murine macrophages." *Proceedings of the National Academy of Sciences* 109.29 (2012b): 11534-11539.

Giaever, Ivar, and Charles R. Keese. "Micromotion of mammalian cells measured electrically." *Proceedings of the National Academy of Sciences* 88.17 (1991): 7896-7900.

Hondroulis, Evangelia, et al. "Impedance based nanotoxicity assessment of graphene nanomaterials at the cellular and tissue level." *Analytical Letters* 45.2-3 (2012): 272-282.

Oomen, Agnes G., et al. "Concern-driven integrated approaches to nanomaterial testing and assessment–report of the NanoSafety Cluster Working Group 10." *Nanotoxicology*8.3 (2014): 334-348.

Adholeya A, Das R. K, Dubey M. K, Kochar M, Singh R, Regulations of Nanoproducts in Agriculture. 2017 (downloaded on 29th August 2018)

Environmental Nanotoxicology: Features, Application, and Characterization

Rupesh Kumar Basniwal, Anuj Ranjan, and Abhishek Chauhan

Abstract Currently, environmental nanotoxicology is a new and growing field. It deals with the toxicological impact of nanoparticles (NPs) on the environment. As we are not much aware of these nanoparticles, they are continuously adding to the environment through various routes like food, cosmetics, food packaging materials, contaminated water, etc. The toxicity of nanoparticles depends on its size, structural properties, concentrations, and environmental factors like temperature, pH, ionic strength, salinity, and organic matter. Assessing the state of knowledge about the toxicity of these nanoparticles is important for the environment and for human welfare. In this chapter, we have discussed features, characterization, applications, environmental routes for dispersion, nanotoxicity, ecotoxicity, and genotoxicity of nanomaterials.

Keywords Nanoparticles · Nanotoxicity · Ecotoxicity · Genotoxicity · Environmental routes

1 Introduction

As per researchers, the nanoparticle-based market has been increased up to 30 folds, and currently, it is around $1 trillion (Vance et al. 2015 and Toensmeier 2004). Metal nanoparticles (specifically, carbon and silver nanoparticles) represent the largest and fastest-growing group of nanoparticles. Escalating production of nanoparticles for various applications presents a serious threat to humans as well as to the environment. The management of these nanoparticles is necessary for human welfare. For this purpose, knowing salient features, characterization, production,

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_6

and commercial application studies is necessary for nanomaterials. Many researchers are fundamentally in contradiction of using nanomaterials for both human medicine and environment, while others are in favor because they believe that their side effects are acceptable (Borm 2002; Colvin 2003). Estimation of the pros and cons of nanoparticles on human health is very difficult because of the presence of plenty of amounts and types of nanomaterial for different applications. This can be done through intensive research (with scientific facts) and by using modern instrumental techniques and approaches.

The nanomaterials show different behaviors as compared to their bulk material, and this is because of their small size, i.e., less than 100 nm. On this size, they represent extraordinary qualities like higher electrical conductivity, reactivity, stability, and color ability (Toensmeier 2004). Carbon-containing graphite looks soft and malleable, but when this carbon turn into nanosized, then it becomes a nanocarbon tube, which is tougher than steel. Catalyst based on nanoparticles is more effective, sometimes a hundred times compared to micro or macro materials. This extraordinary reactivity of nanomaterials is also responsible for higher toxicity as compared to other large-sized particles. Nanoparticles play an amazing role in toxicity, which is important for toxicologists, especially in respiratory diseases. Their size is a significant factor in the occurrence of disease. Studies on dissimilar sizes of carbon and titanium oxide have been carried out by scientists, and they have shown that decreasing nanoparticle size increases its toxicity for human organs. Respiratory toxicity and inflammation through nanoparticles were reported by Zhang et al. in 1998. In this study, they have highlighted the role of nanoparticle's size, surface chemistry, and oxidative stress in nanotoxicity. Other features like crystallinity, coating, and the longevity of particles have also been considered as important parameters for nanotoxicity (Schlesinger 1995). We can go ahead with these nanoparticles in a wise manner by reducing their harmful effects and thus allowing them to be used in the curing of diseases (Linkov et al. 2008; Zhang et al. 1998; Schlesinger 1995; Hyuk et al. 2009; Oberdorster et al. 1994; Holsapple et al. 2005). Although scientists have reported different impacts of nanoparticles on organisms' significance of particle size, size-specific tissue distribution and their size-specific toxicity for silver nanoparticles (AgNPs). Accumulation of AgNPs in aquatic species especially in their specific part, i.e., gill and liver, was reported by Scown et al. in 2010. Acute and chronic toxicities of nanomaterials and their comparison with bulk materials were done by Gaiser et al. in 2011; for Ag and CeO_2. They reported toxicity for nanosized Ag and micro-sized Ag for 0.1 mg/L concentration and found 57% and 13%, respectively. It means nanoparticles are more toxic than macro-sized particles. Global production of other popular nanoparticles, i.e., carbon nanotubes (CNT) and their composites, is also increasing (De Volder et al. 2013). This nanoparticle can be classified into two categories based on their structure, i.e., single-walled carbon nanotube (SCNT) or multiwalled carbon nanotube (MCNT). These nanoparticles have broad applications in different areas like in textiles, cosmetics, biomedicine, polymer chemistry, motor vehicles and sports equipment, and integrated circuits (IC) for electronic modules. Possibly human contact to CNTs is primarily through inhalation and dermal contact, especially during the manufacturing and

mishandling of nanoparticles (Schulte et al. 2014). CNT entry through the lungs in the human body may cause different problems like pulmonary damage, inflammation, oxidative stress, fibrosis, and cancer (Shvedova et al. 2009 and Kagan et al. 2010). The chronic situation can occur due to heavy exposure of carbon nanotube and results in DNA damage and mutation as mentioned by Singh et al. 2009 and Lindberg et al. in 2009 for mouse embryonic cells and human epithelial cells.

As growing demands and use of nanomaterials for different applications like in chemical and material industry, consumer products industry, electronic industry, food packaging industry, bioengineering fields, civil industry, and medical industry are increasing, simultaneously human and environmental problems are also increasing (United States Congress Joint Economic Committee Study 2007). It means nanomaterials have wide applications in different areas, but knowledge about their environmental and human toxicity and their dispersal routes are still in its infancy stage (Schulte et al. 2014; Kagan et al. 2010 and Oberdörster et al. 2009). In this alarming situation, the production and characterization of nanoparticles for the ecotoxicity evaluation is mandatory. In this review, we have focused on comprehensive toxicology and risk assessment of nanomaterials for the environment as well as for human beings.

2 Important Aspects of Nanoparticles

Size The lower size of nanomaterials increases surface area for attachment of chemical molecules which enhance its reactivity and its result into strong toxic effects (Linkov et al. 2008 and Hyuk et al. 2009). It means less size gives more surface area which provides higher reactivity for chemicals and generates more toxicity.

Surface Area of Particle In vitro studies have shown that nanoparticles have more pathological and destructive power rather than the same particles of macro size due to their higher surface area (Oberdorster et al. 1994; Holsapple et al. 2005; Linkov et al. 2008; Singh et al. 2007; Oberdo 2010).

Surface Chemistry Knowing surface chemistry for nanoparticles is very important because it links directly to their reactivity and toxicity. This is because of the presence of certain reactive groups on the surface, and their modification can show different biological effects like surface modification of the silica/quartz, which affects its cytotoxicity, inflammogenicity, and fibrogenicity. The degree of hydrophobicity and hydrophilicity of a surface is the major feature used to estimate the toxicity. The absorption of nanoparticles produced by hydrophobic polymers is greater than that of nanoparticles produced by hydrophilic polymers (Singh et al. 2007 and Oberdo 2010).

Chemical Nature Chemical components present on the surface have an important role for nanoparticles as they can react with metals. The surface modification of nanoparticles can decrease toxicity. Some authors have been reported that coating with pullulan can reduce the toxicity of superparamagnetic iron oxide nanoparticles (Singh et al. 2007 and Oberdo 2010).

Concentration According to Singh (2007) and Oberdo (2010), concentration of nanoparticle is very important because a lower concentration has useful applications, but a higher concentration shows toxicity (Singh et al. 2007 and Oberdo 2010).

Free Radical Production Almost all pathogenic particles generate free radicals in the free cell system and activate the redox cycle which in turn generates oxidative stress, inflammation cell destruction, and genotoxicity (Singh et al. 2007 and Hussain et al. 2009).

3 Nanomaterial's Characterization

As we already know, ecological or biological responses are depending on the physical and chemical nature of nanoparticles. To understand this toxicity and behavior of nanoparticles, we must characterize them for shape, size, phase, electronic structure, crystallinity, surface area, electronic structure, chemical composition, and functionality. Environmental factors like salinity, organic matter, pH, ionic strength, and temperature affect nanoparticles' behavior and toxicity. To determine the size, Brownian movement analysis of particle is done by dynamic light scattering (DLS). Characterization of nanoparticle dispersion through DLS was investigated by some researchers (Murdock et al. 2008). X-ray powder diffraction (XRD) based on Scherrer method is used to characterize the size of nanoparticles in a dry powder state. Scanning electron microscopy (SEM) or transmission electron microscopy (TEM) is generally used for morphological characterization. Elemental analysis of nanomaterial is done by energy-dispersive X-ray (EDX) spectrometry which is coupled with SEM. The surface charge of particles is determined through zeta potential measurement in the aqueous phase. Atomic-scale measurement of particles can be done through atomic force microscopy (AFM) and scanning tunneling microscopy (STM). UV-vis spectrophotometer is used to analyze the sample in the UV-visible range. This is a very important instrument to find out the absorption or transmission behaviors of nanoparticles. Fourier transform infrared spectroscopy (FTIR) is used to characterize functional groups present on nanoparticles (Perez et al. 2009 and Kishore et al. 2008). Chemical characterization of nanoparticles can be done through inductively coupled plasma mass spectrometry (ICP-MS) and coupled plasma optical emission spectroscopy (ICP-OES).

4 The Fate of Nanoparticles in the Aquatic Environment

The rapid growth of nanotechnology is responsible for the higher amount of production of nanoparticles for various applications. After the application, what will be the fate of those nanoparticles is still a remarkable question in the current scenario. Today we can find nanoparticles in the environment (soil, water, air), which were added through various routes. Aquatic ecosystems are more vulnerable to environmental contamination since they are at the receiving end of contaminants, mainly from runoff sources. Uptake of this nanoparticle by algae in the aquatic system has been already seen, which is further transferred to filter feeders and higher trophic levels. The nanoparticles which are not consumed by water organism are going to aggregate and settle down into sediment, and thereby they generate a threat to benthic organisms (Klaine et al. 2008). Nanomaterials can cross biological barriers due to their small size. In aquatic organisms, the main routes of entry are via assimilation or direct passage across the gill and other exterior surface epithelia. For example, nickel nanoparticles may be internalized via the cellular immune system, gut epithelium, and hepatopancreas routes in *Oreochromis mossambicus* (Jayaseelan et al. 2014). In eukaryotic cells, if the particle size is less than 100 nm, then internalize by endocytosis process and for >100 nm and up to 100,000-nm-sized particles absorb by phagocytosis process. Auffan et al. have reported the internalization of iron oxide nanoparticles via endocytosis process (Auffan et al. 2006). Aquatic pH, temperature, and presence of organic matter can affect the release of nanoparticles in the environment. The impact of pH on nanoparticles surface charge is well known and is further responsible for aggregation of nanoparticles (Gilbert et al. 2007). The effect of temperature on the toxicity of nanoparticles is also reported by some authors, and according to them, a higher temperature generates small aggregates and is responsible for more toxicity (Walters et al. 2013).

5 Toxicity of Nanoparticles

Toxicity assessment of nanoparticles is required to manage the applications of nanoparticles in an effective and eco-friendly manner. As aforesaid, toxicity through oxidative stress and inflammation in biological systems has been already reported. Toxicity depends on the size, type of material, coating, and chemical nature of nanoparticles. Due to different applications, titanium oxide-based nanoparticles are now available in plenty of amounts in the environment. Work on its toxicity and killing features of TiO2 nanoparticles is less reported in literature, and according to Lee et al. in 2005, it can be enhanced in the presence of UV radiations on endospore bacteria. The antimicrobial study of silver-coated surfaces has been already reported in 16 types of bacteria. The application of silver nanoparticles is mainly in medical design and dentistry. The killing of *Escherichia coli* organism through nanosilver has been already reported by Kim (2007). Zinc oxide (ZnO) nanoparticles'

application in sun creams and textile industry is already in trend. As shown in some studies, ZnO nanoparticle can easily penetrate the skin of rats and rabbits. Fullerene toxicity due to inhibition lipid oxidation and radicalization was also reported by Wang in 1999. According to Willems et al. (2000), a higher concentration of carbon nanotubes is toxic for organisms; accordingly, scientists have defined them as dangerous and suggested manipulation of the nanoparticles. As mentioned by Azizian et al. (2010), the toxicity of these nanoparticles can be reduced through coating because coating changes the functionality of the nanoparticle. Other nanoparticles like iron oxide particles have been reported for toxicity in human skin fibroblasts and superparamagnetic nanoparticles reported for diarrhea and ultimately death in rats (Kim 2001; Rishikesh 2009; Mahmoudi et al. 2009). As we have already seen the toxicity of different nanoparticles, now it requires to know the toxicity mechanism which is prescribed below for the comprehensive assessment of toxicity.

6 Mechanisms of Toxicity

The toxicity of nanoparticles depends on different factors like surface coatings, shape, size, solution chemistry, and aggregation behavior. The toxicity of various nanoparticles such as silver nanoparticles (Lankvel et al. 2010; Scown et al. 2010; Gaiser et al. 2011; Garcia-Alonso et al. 2011; Miao et al. 2010), copper oxide nanoparticles (Perez et al. 2009 and Heinlaan et al. 2011), titanium oxide nanoparticles (Kim et al. 2010), and nickel nanoparticles (Jayaseelan et al. 2014) have been studied in various aquatic species, such as *Daphnia magna* (Park and Choi 2010; Heinlaan et al. 2011), fish (Scown et al. 2010), algae (Oukarroum et al. 2012), and marine (Pan and Zhang 2006) and freshwater crabs (Walters et al. 2016a, b). In vitro as well as in vivo toxicity of nanoparticles has been already mentioned in literature, but the mechanism of toxicity is a little bit reported. According to Walters et al. (2016a, b), the reason for this toxicity is the generation of reactive oxygen species (ROS) which further produces oxidative stress in a cell that fails in the cellular antioxidant defense system and finally turns into cell death. Overproduction of reactive oxygen species (ROS) in a cellular system causes toxicity, and that results into modify protein radicals and nucleic acid (Stadtman and Berlett 1997 and Evans et al. 2004). Due to the production of ROS, lipid peroxidation (Butterfield and Kanski 2011), modulation of gene expression (Shi et al. 2004), and damage to DNA strand (Xia et al. 2012) events are generated, and all responsible for cell genotoxicity.

7 Environmental Toxicity

General toxicity of nanoparticles has been already reported by different authors, but in this article, basically, we have focused on ecotoxicity. After the production of nanomaterials and their application, what will be the fate of those nanoparticles?

Still, a matter of discussion. These nanoparticles after application find their route for water and start to accumulate in aquatic organism. This process is responsible for the generation of ecotoxicity (Oberdorster et al. 2006 and Moore 2006). The poisonous effect of nanoparticles on different organisms like bacteria, algae, fish, and mammals depends on their concentration. A basic understanding of this toxicity is still in the early stage. Nowadays *Daphnia magna* is used as an experimental model to evaluate the ecotoxicity of nanomaterials (Farre et al. 2009). Ecotoxicity for silver nanomaterials is reported in *Daphnia magna* by Park and Choi in 2010. In this case, an enhanced mortality rate has been observed by authors. Toxicity of CuO nanomaterials was evaluated by Heinlaan et al. (2011), and due to the toxicity, ultrastructural changes in the midgut of *Daphnia magna* were observed. In another study, Wiench et al. (2009) reported the toxicity of coated TiO2 nanoparticles on *Daphnia* reproduction. Accumulation and aggregation of nanoparticles in the exoskeleton of the aquatic organism were reported by Baun et al. in 2008. Uptake of AgNps by gills due to their capability to cross the semipermeable membrane is reported for *Danio rerio* by authors Scown et al. in 2010. As reported by Wu et al. (2010) for *Oryzias latipes*, this accumulation of silver nanoparticles may generate various effects in organisms like edema, spinal and fin folding abnormalities, heart malformations, and eye dysfunction. Toxicity of nanomaterials for freshwater algae, i.e., *Ochromonas danica*, was reported by authors Miao et al. in 2010. The effect of nanomaterial on genetic material is known as genotoxicity. It means ecotoxicity results may be turned into genotoxicity. Genotoxicity of nanoparticles is due to their large surface area and highly reactive site. Nanoparticles can easily cross the biological membrane and show different effects like chromosomal fragmentation, breakage of DNA strand, alterations in gene expression, point mutation, and carcinogenesis. Landsiedel et al. have reported the internalization of nanoparticles and their interaction with nucleic acid in the year 2009. The effect of various nanoparticles (ferric oxide, α- and β-alumina, calcined silica, etc.) on DNA strand breakage was studied by Oberholster et al. in 2011. According to Gurr et al. (2005), the effect of the small size of titanium oxide particles on chromosomal damage was more for then large-sized nanoparticles. The coating of nanoparticles can also affect ecotoxicity and genotoxicity differently. In one study coating of iron oxide with a positive charge enhances breakage of DNA strands (Hong et al. 2011), and in another study, coating of polyethylene glycol (PEG) shows mutagenicity while no effect of solid electrolyte interphase coating on genotoxicity (Lui et al. 2014). According to Ahamed et al. (2008), the toxicity of silver nanoparticles can be enhanced through the coating. The toxicity of silver nanoparticles for coating or non-coating was studied by AshaRani et al. (2008) in zebrafish embryos (*Danio rerio*). The moral of the story is that nanoparticles' toxicity can be in the form of ecotoxicity or genotoxicity; it depends on their size, shape, and concentrations.

8 Conclusions

As applications of nanomaterials in different consumer products are increasing, their environmental and human health hazard is also increasing. Due to this, their chance of appearing in the aquatic ecosystem through various routes as mentioned by different authors. The nanotechnology field is divided into various subfields and explained very well in literature, but nanotoxicity knowledge is still in its infancy stage. In this chapter, we have tried to cover in vivo as well as in vivo toxicity of nanoparticles. Description of aquatic toxicity of nanoparticles is very little in the literature. Escalating the production of nanoparticles and their toxicity has forced us to think in detail about ecotoxicity for their effective and green management for the environment. For this purpose, it requires systematic research in all fields like government, industry, academia, and different stakeholders like scientists, technocrats, etc. This research can explain gaps between current knowledge and future applications of nanomaterials and their toxicity, but this all cannot be possible without financial aid or support and strategies. Addressing these issues will require more intensive and panoramic research for the comprehensive development of the nanotoxicology field for environmental toxicity management. This chapter provides a review of nanotoxicology in a broader and in-depth way and especially for aquatic ecotoxicity. Nanomaterials toxicity depends on various parameters like size, shape, elemental nature, and functional groups as we have already discussed in this article. Based on their potential, they can generate oxidative stress in the organism which will turn into genotoxicity. Symptoms of genotoxicity are mutation, chromosomal damage, DNA damage, and cell death. Ecotoxicity not only depends on size and shape but also on chemical nature, exposure time, and their concentrations. Along with this, mechanism of toxicity is also very important for the effective management of ecotoxicity which we have already discussed in this article. The future application of nanomaterials requires deep research into new analytical and instrumental methods for addressing the toxicity of nanomaterials for the betterment of human society and the environment.

References

Ahamed M, Karns M, Goodson M, Rowe J, Hussain SM, Schlager JJ, Hong Y. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicology and Applied Pharmacology. 2008;233(3):404–410. https://doi.org/10.1016/j.taap.2008.09.015.

AshaRani, PV, Wu YL, Gong Z, Valiyaveettil S. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology. 2008;19(25):279–290. https://doi.org/10.1088/0957-4484/19/255102.

Auffan M, Decome L, Rose J, Orsiere T, DeMeo M, Briois V, Chaneac C, Olivi L, BergeLefranc JL, Botta A, Wiesner MR, Bottero JY. In vitro interactions between DMSA-coated maghemite nanoparticles and human fibroblasts: a physiological and cyto-genotoxical study. Environmental Science and Technology. 2006;40(14):4367–4373. https://doi.org/10.1021/es060691k.

Azizian J, Tahermansouri H, Biazar E, Heidari S, Chobfrosh Khoei D. Functionalization of carboxylated multiwall nanotubes with imidazole derivatives and their toxicity investigations. Inter J Nanomed. 2010;5: 907–914.

Baun A, Sorensen SN, Rasmussen RF, Hartmann NB, Koch CB. Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates. Aquatic Toxicology. 2008;86(13):379–387. https://doi.org/10.1016/j.aquatox.2007.11.019.

Borm PJA. Particle toxicology: from coal mining to nanotechnology. Inhal Toxicol. 2002;14:311–324.

Butterfield DA, Kanski J. Brain protein oxidation in agerelated neurodegenerative disorders that are associated with aggregate proteins. Mechanisms of Ageing and Development. 2011;122(9):945–962. https://doi.org/10.1016/S0047-6374(01)00249-4.

Colvin VL. The potential environmental impact of engineered nanomaterials. Nat Biotechnol. 2003;21:1166–1170. 2.

De Volder MFL, Tawfick SH, Ray H, Baughman RH, Hart AJ. Carbon nanotubes: present and future commercial applications. Science. 2013; 339(6119):535–539. [PubMed: 23372006]

Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. Mutation Research. 2004;567(1):1–61. https://doi.org/10.1016/j.mrrev.2003.11.001.

Farre M, Gajda-Schrantz K, Kantiani L, Barcelo D. Ecotoxicology and analysis of nanomaterials in the aquatic. Analytical and Bioanalytical Chemistry. 2009;393(1):81– 95. https://doi.org/10.1007/s00216-008-2458-1.

Gaiser BK, Biswas A, Rosenkranz P, Jepson MA, Lead JR, Stone V, Tyler CR, Fernandes TF. Effects of silver and cerium dioxide micro- and nano-sized particles on Daphnia magna. Journal of Environmental Monitoring. 2011;13:1227–1235. https://doi.org/10.1039/c1em10060b.

Garcia-Alonso J, Khan FR, Misra SK, Turmaine M, Smith BD, Rainbow PS, Luoma SN, Valsami-Jones E. Cellular internalization of silver nanoparticles in gut epithelia of the estuarine polychaete Nereis diversicolor. Environmental Science and Technology. 2011;45:4630–4636. https://doi.org/10.1021/es2005122.

Gilbert B, Lu G, Kim CS. Stable cluster formation in aqueous suspensions of iron oxyhydroxide nanoparticles. Journal of Colloid and Interface Science. 2007;313(1):152– 159. https://doi.org/10.1016/j.jcis.2007.04.038.

Gurr JR, Wang AS, Chen CH, Jan KY. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology. 2005;213:66–73.

Heinlaan M, Kahru A, Kasemets K, Arbeille B, Prensier G, Dubourguier H-C. Changes in the Daphnia magna midgut upon ingestion of copper oxide nanoparticles: a transmission electron microscopy study. Water Research. 2011;45:179–190. https://doi.org/10.1016/j.watres.2010.08.026.

Holsapple MP, Farland WH, Landry TD, et al. Research strategies for safety evaluation of nanomaterials, part II: Toxicological and safety evaluation of nanomaterials, current challenges and data needs. Toxicol Sci. 2005;88:12–17.

Hong SC, Lee JH, Lee J, Kim HY, Park JY, Cho J, Han DW. Subtle cytotoxicity and genotoxicity differences in the superparamagnetic iron oxide nanoparticles coated with various functional groups. International Journal of Nanomedicine. 2011;6:3219–3231. https://doi.org/10.2147/IJN.S26355.

Hussain S, Boland S, Baeza-Squiban A, et al. Oxidative stress and proinflammatory effects of carbon black and titanium dioxide nanoparticles: role of particle surface area and internalized amount. Toxicol. 2009;260:142–149.

Hyuk Suh W, Suslick SK, Stucky Galen D, SuhYoo-Hun. Nanotechnology, nanotoxicology, and neuroscience. Pro Neurobiol. 2009; 87(3):133–170.

Jayaseelan C, Rahuman AA, Ramkuma R, Perumal P, Rajakumar G, Kirthi G, Santhoshkumar AV, Marimuthu S. Effects of sub-acute exposure to nickel nanoparticles on oxidative stress and

histopathological changes in Mozambique tilapia Oreochromis mossambicus. Ecotoxicology and Environmental Safety. 2014;107:220–228. https://doi.org/10.1016/j.ecoenv.2014.06.012.

Kagan VE, Shi J, Feng W, Shvedova AA, Fadeel B. Fantastic voyage and opportunities of engineered nanomaterials: what are the potential risks of occupational exposures? J Occup Environ Med. 2010; 52(9):943–946. [PubMed: 20829674]

Kim Do-Kyung. Superparamagnetic Iron Oxide Nanoparticles for Biomedical Applications. Stockholm 2001 Licentiate Thesis. Royal Institute of Technology Department of Material Science and Engineering

Kim JS. Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Bio Med. 2007; 3:95–101.

Kim KT, Klaine SJ, Cho J, Kim S-H and Kim SD. Oxidative stress responses of Daphnia magna exposed to TiO2 nanoparticles according to size fraction. Science of the Total Environment. 2010;408(10):2268–2272. https://doi.org/10.1016/j.scitotenv.2010.01.041.

Kishore PS, Viswananthan B, Varadarajan TK. Synthesis and characterization of metal nanoparticles embedded conducting polymer-polyoxometalate. Nanoscale Research Letters. 2008;3:14–20. https://doi.org/10.1007/s11671-007-9107-z.

Klaine SJ, Alvarez PJJ, Batley GE, Gernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR. Nanomaterials in the environment: behaviour, fate, bioavailability, and effects. Environmental Toxicology and Chemistry. 2008;27(9):1825–1851. https://doi.org/10.1897/08-090.1.

Landsiedel R, Kapp MD, Schulz, M, Wiench K, Oesch F. Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations – many questions, some answers. Mutation Research. 2009;681(2–3):241–258. https://doi.org/10.1016/j.mrrev.2008.10.002.

Lankvel DPK, Oomen AG, Krystek P, Neigh A, Troost-de Jong A, Noorlander JCH, Geertsma RE, De Jong WH. The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomaterials. 2010;31:8350–8361. https://doi.org/10.1016/j.biomaterials.2010.07.045.

Lee SH, Pumprueg S, Moudgil B, Sigmund W. Inactivation of bacterial endospores by photocatalytic nanocomposites. Colloids and Surfaces B: Biointerfaces, (2005);40(2):93–8.

Lindberg HK, Falck GS-M, Suhonen S, et al. Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells in vitro. Toxicol Lett. 2009; 186(3):166–173. [PubMed: 19114091]

Linkov I, Satterstrom FK, Corey LM. Nanotoxicology and nanomedicine: making hard decisions. Nanomedicine: Nanotech Bio and Med. 2008;4:167–171.

Lui Y, Xia Q, Liu Y, Zhang S, Cheng F, Zhong Z, Wang L, Li H, Xiao K. Genotoxicity assessment of magnetic iron oxide nanoparticles with different particle sizes and surface coatings. Nanotechnology. 2014;25(42):425101. https://doi.org/10.1088/0957-4484/25/42/425101.

Mahmoudi M, Simchi A, Milani AS, Stroeve P. Cell toxicity of superparamagnetic iron oxide nanoparticles. J Colloid Inter Sci. 2009; 336(2):510–518

Miao A-J, Luo Z, Chen C-S, Chin W-S, Santschi PH, Quigg A. Intracellular uptake: a possible mechanisms for silver engineered nanoparticle toxicity to a freshwater alga Ochromonas danica. PLoS One. 2010;5(12):e15196. https://doi.org/10.1371/journal.pone.0015196.

Moore MN. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environment International. 2006;32(8):967–976. https://doi.org/10.1016/j.envint.2006.06.014.

Murdock RC, Braydich-Stolle L, Schrand AM, Schlager JJ, Hussain SM. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicological Sciences. 2008;101(2):239–253. https://doi.org/10.1093/toxsci/kfm240.

Oberdo G. Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. J Inte Med. 2010;267:89–105.

Oberdorster E, Zhu SQ, Blickley TM, Clellan-Green P, Haasch ML. Ecotoxicology of carbon-based engineered nanoparticles: effects of fullerene (C-60) on aquatic organisms. Carbon. 2006;44(6):1112–1120. https://doi.org/10.1016/j.carbon.2005.11.008.

Oberdorster G, Ferin J, Lehnert BE. Correlation between particle-size, in-vivo particle persistence, and lung injury. Environ Health Persp. 1994;102:173–179.

Oberdörster G, Stone V, Donaldson K. Toxicology of nanoparticles: a historical perspective. Nanotoxicology. 2009; 1(1):2–25.

Oberholster PJ, Musee N, Noth A-M, Chelule PK, Focke WW, Ashton PJ. Assessment of the effect of nanomaterials on sediment-dwelling invertebrate Chironomus tentans larvae. Ecotoxicology and Environmental Safety. 2011;74:416–423. https://doi.org/10.1016/j.ecoenv.2010.12.012.

Oukarroum A, Bras S, Perreault F, Popovic R. Inhibitory effects of silver nanoparticles in two green algae, Chlorella vulgaris and Dunaliella tertiolecta. Ecotoxicology and Environmental Safety. 2012;78(1):80–85. https://doi.org/10.1016/j.ecoenv.2011.11.012.

Pan L, Zhang H. Metallothionein, antioxidant enzymes and DNA strand breaks as biomarkers of Cd exposure in a marine crab, Charybdis japonica. Comparative Biochemistry and Physiology, Part C. 2006;144(1):67–75. https://doi.org/10.1016/j.cbpc.2006.06.001

Park S-Y and Choi J. Geno- and ecotoxicity evaluation of silver nanoparticles in freshwater crustacean Daphnia magna. Environmental Engineering Research. 2010;15(1):23–27. https://doi.org/10.4491/eer.2010.15.1.428.

Perez S, Farre M, Barcelo D. Analysis, behavior and ecotoxicity of carbon-based nanomaterials in the aquatic environment. Trends in Analytical Chemistry. 2009;28:820–832. https://doi.org/10.1016/j.trac.2009.04.001.

Rishikesh MS. Nanosized cancer cell-targeted polymeric immunomicelles loaded with super paramagnetic iron oxide nanoparticles. Nanopart Res. 2009;6:1–9.

Schulte PA, Geraci CL, Murashov V, et al. Occupational safety and health criteria for responsible development of nanotechnology. J Nanopart Res. 2014; 16(1):2153. [PubMed: 24482607]

Scown TM, Santaos EM, Johnston BD, GAiser B, Baalousha M, Mitov S, Lead JR, Stone V, Fernandes TF, Jepson, M, van Aerle R, Tyler CR. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. Toxicological Sciences. 2010;115(2): 521–534. https://doi.org/10.1093/toxsci/kfq076.

Shi H, Hudson LG, Liu KJ. Oxidative stress and apoptosis in metal ion-induced carcinogenesis. Free Radical Biology and Medicine. 2004;37(5):582–593. https://doi.org/10.1016/j.freeradbiomed.2004.03.012.

Shvedova AA, Kisin ER, Porter D, et al. Mechanisms of pulmonary toxicity and medical applications of carbon nanotubes: two faces of Janus? Pharmacol Ther. 2009; 121(2):192–204. [PubMed: 19103221]

Singh N, Manshian B, Jenkins JS, et al. Nano-Genotoxicology: The DNA damaging potential of engineered nanomaterials. Biomaterials. 2009; 30(23–24):3891–3914. [PubMed: 19427031]

Singh S, Shi T, Duffin R, et al. Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine TiO2: role of the specific surface area and of surface methylation of the particles. Toxico Appl Pharmacol. 2007;222(2): 141–151.

Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. Chemical Research and Toxicology 1997;10(5):485–494. https://doi.org/10.1021/tx960133r.

Toensmeier PA. Nanotechnology faces scrutiny over environment and toxicity. Plastics Engineering. 2004;60(11):14–17.

United States Congress Joint Economic Committee Study. Nanotechnology: The Future is Coming Sooner than you Think. Washington, DC: JEC; 2007

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochelle Jr MF, Rejeski D, Hull MS. Nanotechnology in the real world: redeveloping the nanomaterials consumer products inventory. Journal of Nanotechnology. 2015;6:1769–1780. https://doi.org/10.3762/bjnano.6.181.

Walters C, Pool E, Somerset V. Aggregation and dissolution of silver nanoparticles in a laboratory-based freshwater microcosm under simulated environmental conditions. Toxicology and Environmental Chemistry. 2013;95(10):1690–1701. https://doi.org/10.1080/02772248.2014.904141.

Walters C, Pool E, Somerset V. Nanotoxicity in aquatic invertebrates. In: Larramendy ML, Soloneski S, editors. Invertebrates—Experimental Models in Toxicity Screening. 1st ed. Croatia: InTech; 2016a. p. 13–34. https://doi.org/10.5772/61715. Available from: http://www.intechopen.com/books/invertebrates-experimental-models-in-toxicity-screening/nanotoxicity-in-aquatic-invertebrates

Walters CR, Cheng P, Pool E, Somerset V. Effect of temperature on oxidative stress parameters and enzyme activity in the tissues of cape river crab (Potamonautes perlatus) following exposure to silver nanoparticles (AgNP). Journal of Toxicology and Environmental Health, Part A. 2016b;79(2):1–11. https://doi.org/10.1080/15287394.2015.1106357.

Wang C. C60 and Water-soluble fullerene derivatives as antioxidants against radical-initiated lipid peroxidation. J Med Chem. 1999;42: 4614–4620.

Wiench K, Wohlleben W, Hisgen V, Radke K, Salinas E, Zok S, Landsiedel R. Acute and chronic effects of nano- and non nano-scale TiO2 and ZnO particles on mobility and reproduction of the freshwater invertebrate Daphnia magna. Chemosphere. 2009;76(10):1356–1365. https://doi.org/10.1016/j.chemosphere.2009.06.025.

Willems I, Konya Z, Colomer J-F, et al. Control of the outer diameter of thin carbon nanotubes synthesized by catalytic decomposition of hydro-carbons. Chem Phys Lett. 2000;317:71–76

Wu Y, Zhou Q, Li H, Liu W, Wang T, Jiang G. Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (Oryzias latipes) using the partial-life test. Aquatic Toxicology. 2010;100(2):160–167. https://doi.org/10.1016/j.aquatox.2009.11.014.

Xia Q, Chiang H-M, Zhou Y-T, Yin J-J, Liu F, Wang C, Guo L, Fu PP. Phototoxicity of kava—formation of reactive oxygen species leading to lipid peroxidation and DNA damage. The American Journal of Chinese Medicine. 2012;40(6):1271–1288. https://doi.org/10.1142/S0192415X12500942.

Zhang QW, Kusaka Y, Sato K, Nakakuki K, Kohyama N, Donaldson K. Differences in the extent of inflammation caused by intratracheal exposure to three ultrafine metals: role of free radicals. J Toxicol Env Heal. 1998;53:423–438.

Schlesinger RB. Toxicological evidence for health effects from inhaled particulate pollution: does it support the human experience? Inhal Toxicol. 1995;7:99–109

Insecticidal Activities of Different Extracts of *Calotropis procera*

Devendra Kumar, Anuj Ranjan, Abhishek Chauhan, Dhan Prakash, and Tanu Jindal

Abstract *Calotropis procera* has an important biological role in controlling pests and disease-spreading vectors. A search for biopesticide is a novel approach for reducing the risk and persistence of chemical and synthetic pesticides. Different parts of plants such as leaves, bark, roots, flowers, seeds, etc. are extensively studied by numerous authors for insecticidal properties; however, the outcomes are not available in compiled form. Efforts are done to compile the research done on its insecticidal properties in the past 20 years. The available data has been classified based on target insect taxonomical orders, plant part with details of different fractions of extract, their activity, insecticidal activity, dosage, and extent of lethality. Preliminary data on larvicidal, insecticidal, adulticidal, and LC_{50} were retrieved along with plant parts, solvent/solvent fraction detail, and insect details (taxonomical order, larva/adult, and stage of larva). Later the data was classified for each plant part extracted in each solvent/fraction with taxonomical order of insect, the dosage used, the lethality of the dosage, and author details.

Keywords *Calotropis procera* · Chemical constituents · Insecticidal properties · Bio-efficacy · Vectors · Biopesticide

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_7

1 Introduction

Calotropis is a genus of the Asclepiadaceae family having six species of shrubs. They are generally distributed throughout the tropics of Asia, South America, Africa, and the Middle East (Rahman and Wilcock 1991). The plant is a hardy, pubescent, evergreen, erect, compact shrub up to 4.5 m high, covered with cottony tomentum (Mohamed et al. 2015). It grows abundantly in arid and semiarid regions with warm climate in dry, sandy, and alkaline soils and does not need any irrigation, fertilizers, pesticides, or other agronomic practices (Rahman and Wilcock 1992: Tezara et al. 2010; Quazi et al. 2013a, b).

In Ayurveda, *Calotropis procera* Linn. is a valuable medicinal plant commonly known as "Sodom apple" and locally known as aak in India (Sharma et al. 2011; Mueen et al. 2005). Three varieties of *C. procera* mentioned in the literature of Dhanvantari Nigantu as Suklarkah, Rajarkah, and Sveta mandarah are well known for its high medicinal properties (Singh et al. 2015). It has been widely used for the treatment of various diseases such as leprosy, ulcers, piles, and diseases of the spleen, liver, and abdomen in Indian traditional system medicine as well as Unani, Arabic, and Sudanese (Oloumi 2014; Meena et al. 2015; Sweidan and Abu Zarga 2015).

1.1 Morphology

Leaf is 10–15 cm long and 4.5 to 6.5 cm broad, simple, opposite, sub-sessile, slightly thick, fleshy, coriacious, broadly cuneate, obovate or obovate oblong, slightly cordate, and auricled at base with tuff of short simple hairs on the upper side near the place of the attachment to the petiole. Flowers consist of five small triangular dirty white sepals; five thick ovate petals, (1 cm x 1 cm) which are white at the base and purple at the tips; and five purple-tipped stamens, which surround a white fived-lobed stigma. Fruits of *C. procera* are green, spongy ovoid fruits (follicles), up to 15 cm long by 10 cm wide. They split open to release plumed, papery light brown seeds with a pappus of white filaments up to 6 cm long on one side. The root occurs in the entire condition. The bark is separated from the wood 0.5–2.0 cm in diameter bearing rootlets with a diameter varying from 0.2 to 0.5 cm, externally whitish-grey, wrinkled in the fresh condition, plenty of whitish latex exudes from cuts or wounds in the bark.

The plant is used by various tribes of the world as a curative agent for ailments such as skin disease, elephantiasis, toothache, asthma, leprosy, and rheumatism as it possesses acaricidal, schizonticidal, antimicrobial, anthelmintic, insecticidal, anti-inflammatory, antidiarrheal, anti-cancerous, and larvicidal activities with other beneficial properties (Parihar and Balekar 2016).

1.2 Phytochemistry of C. procera

The plant has many reported phytochemicals which make it medicinally important plant. The whole plant contains alpha- and beta-amyrin, taraxasterol, gigantin, giganteol, isogiganteol, beta-sitosterol, and wax (Upadhyay 2014). It also has triterpenoids, calotropursenyl acetate and calopfriedelenyl, norditerpenyl ester, calotropternyl ester oleanene triterpenes like calotropoleanyl ester, procerleanol A and B and cardiac glycosides calotropogenin, calotropin, uscharin, calotoxin and calactin, uscharidin and calotropagenin, trypsin, α-calotropeol, β-calotropeol and β-amyrin, and 7 saturated and 11 unsaturated fatty acids (Perwez and Mohammad 2009; Quazi et al. 2013a, b).

C. procera is well known for producing latex, a milky liquid consisting of several biologically active compounds, including proteins, amino acids, carbohydrates, lipids, vitamins, alkaloids, carbonates, resins, tannins, and terpenes (Khanzada et al. 2008). The main cardenolides in the various parts of the plant are uscharin and calotropagenin in the latex; calotropin and calotropagenin in the leaves; uscharidin, calotropin, proceroside, and calactin in the stem; calotoxin and calactin in the root bark; and coroglaucigenin and uzarigenin in the fruit pericarp. The seeds contain 0.23–0.47% cardenolides, mainly coroglaucigenin or frugoside (Shobowale et al. 2013). Hesse and Ludwing (1960) reported the cardiac glycosides calotropin, calactin, uscharidin, uscharin calotoxin, and voruscharin from the latex of *C. procera* (Saber et al. 1969). Mudarine is the principal active constituent in the leaves along with toxic glycosides, namely, calotropin, uscharin, and calotoxin (Hesse and Ludwing 1960), The main constituents of latex are cardenolides, triterpenoids, anthocyanins, alkaloids, resins, and proteolytic enzymes (Cheung et al. 1983; Chauhan et al. 2018).

The leaves and stalks bear calotropin and calotropagenin (Khan and Malik 1989). Phytochemical investigation of the roots of *C. procera* yields two new phytoconstituents, procerursenyl acetate and proceranol along with N-dotriacont-6-ene, glyceryl mono-oleolyl-2-phosphate, methyl myristate, methyl behenate, and glyceryl-1,2-dicapriate- 3-phosphate (Hanna et al. 2002). Its bark has shown the presence of lupeol, β-sitosterol, oleanolic acid in chloroform fraction, phenolics benzoyllineolone, benzoyl isolineolone, madaralban, and madar fluavil[9]. Flower of *C. procera* are reported to have quercetin-3-rutinoside, anthocyanin, cyanidin-3-rhamnoglucoside, calotropenyl acetate, and multiflavenol (Alam and Ali 2009) (Chatterjee and Pakarashi 1995).

In some other studies on *C. procera* proteolytic enzymes, cardenolides, alkaloids, tannins, triterpenes, and flavonoids with diverse biological activities such as cytotoxic, anti-cancerous, anti-inflammatory, analgesic, anti-nociceptive, and hepatoprotective activities have also been observed (Qureshi et al. 2007; Arya and Kumar 2005; Sehgal et al. 2006).

2 Insecticides Activity

Chemical and synthetic pesticides are persistent for a long time, and they pose risk to the environment, and on most occasions, they are toxic to nontarget organisms. Therefore, the search for a natural alternative to these pesticides is always a suitable alternative for conventional chemical control and integrated pest management. The search for natural products to control destructive insects and vectors of diseases is desirable due to the prevalent occurrence of vector resistance to synthetic insecticides (Choedon et al. 2006). An alternative to conventional chemical control is the utilization of natural products from plants and essential oils (Cheng et al. 2009; Chauhan et al. 2016).

C. procera has several alkaloids, flavonoids, terpenes, terpenoids, enzymes, and other inorganic elements (Quazi et al. 2013a, b). These compounds also exhibit insecticidal properties apart from other bioactivities. Numerous reports are claiming the insecticidal properties of plant extract and essential oils of *C. procera* (De Morais et al. 2007; Begum et al. 2013; Esmaeily et al. 2012). The present proliferation of these vectors is due to the increasing resistance of mosquitoes to current insecticides (Irannejad et al. 2012). In many parts of the world, plant-derived products have been used to repel or kill mosquitoes and other domestic insect pests (Knio et al. 2008; Pavela 2015).

The latex of *C. procera* has shown larvicidal efficacy against all three important vector species, viz. *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinqefasciatus*, vectors of dengue, malaria, and *lymphatic filariasis*, respectively (Conti et al. 2010; Shahi et al. 2010). Latex of this plant has complex chemical constituents such as N-acetyl-β-d-glucosaminase (Ramos et al. 2006). Hence, due to the presence of diverse chemical substances, plant latex serves as a defence material and prevents herbivorous insects from feeding (Giordani et al. 1992). The effect of several commercial and locally extracted biorational pesticides including *C. procera* showed activity against the Egyptian alfalfa weevil (EAW) and *Hypera brunneipennis* (Konno 2011).

AChE inhibitory activity has been reported in the methanolic extract of *C. procera*. Matthew and Adaramoye (2014) studied the same against rat brain homogenate using catechin as standard AChE inhibitor; the study found better inhibition than catechin (Al-Doghairi and El Hag 2003). Calotoxin from the *C. procera* is a great inhibitor of AChE studied by molecular docking with human AChE. It has a very strong hydrogen bond formation with aromatic amino acids present in the binding cavity (Matthew and Adaramoy 2014). Acetylcholinesterase is a key enzyme which hydrolyses the neurotransmitter, acetylcholine, in the nervous system (Rohit et al. 2016; Grundy and Still 1985), and it is primarily responsible for the termination of cholinergic neurotransmission at synapses in both humans and insects (Wang et al.

2004; Fournier and Mutero 1994; Ranjan et al. 2018). Therefore, AChE is known to be the target of many organophosphate- and carbamate-based insecticides (Carlier et al. 2008).

Although the insecticidal activities of *C. procera* have been extensively studied on various pests, mosquitoes, larvae, stored grain pests, etc., several authors have studied various parts such as latex, flowers, stem, roots, and barks for insecticidal properties. Given the vast usage and importance of the plant as insecticidal activities, it is highly required to bring the majority of the available data on one platform.

3 Conclusion

The present book chapter has compiled the maximum scholarly articles published on insecticidal properties of *C. procera*. The main focus was based on the hypothesis to bring all the published data on insecticidal properties of *C. procera* on one platform. We have compiled insecticidal activities of crude extracts of plant parts such as leaves, aerial/floral parts, seeds, roots, whole plant, flowers, stem, and their fractions of different solvents like hexane, chloroform, methanol, ethanol, acetone, dichloromethane, ethyl acetate, and aqueous extract as summarized in Tables 1, 2, 3, 4, 5, 6, 7, and 8. Insects studies were also classified based on the taxonomical order. The effects of the treatment which have been included in the tables are in terms of LC_{50}, LC_{90}, and percentage mortality. Data mining was done using Google Scholar, PubMed Central, and ScienceDirect. Most of the publications were retrieved by Google Scholar. The following keywords were used in different combinations to retrieve the research articles: *C. procera*, larvicidal, insecticide, mortality, LC_{50}, adulticides, biopesticides, crude extract, solvent fraction, etc. Search results were filtered based on the publication years to select papers published after the year 2000.

Preliminary data on larvicidal, insecticidal, adulticidal, and LC_{50} were retrieved along with plant parts, solvent/solvent fraction detail, and insect details (taxonomical order, larva/adult, and stage of larva). Later the data was classified for each plant part extracted in each solvent/fraction with taxonomical order of insect, the dosage used, and the lethality of the dosage.

Table 1 Insecticidal activities (LC50 and LC90) of methanol extract from different plant parts of *C. procera* against various insects

Parts of plants	Insect	Dosage	% of insecticides	References
Leaves (methanol)	*An. stephensi* (Diptera)	109.71 mg/l	LC50	Shahi et al. (2010)
		234.61 mg/l	LC90	
		65.0(mg/l)	LC50 (48 h)	Bansal et al. (2012)
	An. stephensi; 1st instar larva (Diptera)	0.38%	LC50 (72 h)	Singh et al. (2005)
		0.81%	LC90 (72 h)	
	An. stephensi 1st and 2nd instar larva (Diptera)	0.02%	LC50 (72 h)	
		0.06%	LC90 (72 h)	
	Cx. quinquefasciatus (Diptera)	387.93 mg/l	LC50	Shahi et al. (2010)
		630.66 mg/l	LC90	
	Cx. quinquefasciatus 1st and 2nd instar larva (Diptera)	0.05%	LC50 (72 h)	Singh et al. (2005)
		0.18%	LC90 (72 h)	
		0.85%	LC50 (72 h)	
		2.22%	LC90 (72 h)	
	Cx. quinquefasciatus	259.3 (mg/l)	LC50 (48 h)	Bansal et al. (2012)
	Aedes aegypti 1st and 2nd instar larva (Diptera)	0.03%	LC50 (72 h)	Singh et al. (2005)
		0.20%	LC90 (72 h)	
	Aedes aegypti 1st instar larva (Diptera)	0.74%	LC50 (72 h)	
		2.06%	LC90 (72 h)	
	Ae. aegypti (Diptera)	118.7(mg/l)	LC50 (48 h)	Bansal et al. (2012)
	S. litura 3rd instar larva (Lepidoptera)	28.45%/w	LC50	Bakavathippan et al. (2012)
		2019.37%/w	LC90	
	Clostera cupreata (Lepidoptera)	1%	50 (72 h)	Singh and Arya (2011)
Seeds (methanol)	*An. stephensi* (Diptera)	89.8 (mg/l)	LC50 (48 h)	Bansal et al. (2012)
	Ae. aegypti (Diptera)	115.4 (48 h)	LC50 (48 h)	
	Cx. quinquefasciatus (Diptera)	141.5 (48 h)	LC50 (48 h)	
Flower (methanol)	*An. stephensi*	68.7 (mg/l)	LC50 (48 h)	
	Ae. aegypti (Diptera)	361.7 (mg/l)	LC50 (48 h)	
	Cx. quinquefasciatus (Diptera)	616.2 (mg/l)	LC50 (48 h)	

Table 2 Insecticidal activities (LC50 and LC90) of aqueous extract from different plant parts of *C. procera* against various insects

Parts of plants (aqueous leaves)	Insect	Dosage	% of insecticides	References
	An. arabiensis 2nd instar larva (Diptera)	273.53 (ppm)	LC50	Abdalla et al. (2009)
	An. arabiensis 3rd instar larva (Diptera)	366.44 (ppm)		
	An. arabiensis 4th Instar larva (Diptera)	454.99 (ppm)		
	An. arabiensis 2nd instar larva (Diptera)	783.43 (ppm)	LC90	
	An. arabiensis 3rd instar larva (Diptera)	1018.59 (ppm)		
	An. arabiensis 4th instar larva (Diptera)	1224.62 (ppm)		
	*Cx. quinquefasciatus*2nd instar larva (Diptera)	187.93 (ppm)	LC50	
	Cx. quinquefasciatus 3rd instar larva (Diptera)	218.27 (ppm)	LC50	
	Cx. quinquefasciatus 4th instar larva (Diptera)	264.85 (ppm)	LC50	
	Cx. quinquefasciatus 2nd instar larva (Diptera)	433.51 (ppm)	LC90	
	Cx. quinquefasciatus 3rd instar larva (Diptera)	538.27 (ppm)	LC90	
	Cx. quinquefasciatus 4th instar larva (Diptera)	769.13 (ppm)	LC90	
	Musca domestica (Diptera)	21.70%	LC50	Kamalam et al. (2013)
		21,1%		Kamalam et al. (2013)
	Musca domestica 2nd instar larva (Diptera)	6306.44 (ppm)	LC50	Islam and Akhtar 2013
Whole plants (aqueous)	*Musca domestica* 2nd instar larva (Diptera)	557.894 (micro litre	LC50	Islam and Akhtar (2013)
Seeds (aqueous)	*An. stephensi* (Diptera)	16.8 (mg/l)	LC50 (48 h)	Bansal et al. (2012)
	Ae. aegypti (Diptera)	16.3 (mg/l)		
	Cx. quinquefasciatus (Diptera)	22.1(mg/l)		
Stem (aqueous)	*Musca domestica* 2nd instar larva (Diptera)	7428.63 (ppm)	LC50	Islam and Akhtar (2013)
Root (aqueous)		5399.93 (ppm)		

Table 3 Insecticidal activities (LC50 and LC90) of Acetone extract from different plant parts of *C. procera* against various insects

Parts of plants	Insect	Dosage	% of insecticides	Reference
Seeds (acetone)	*An. stephensi* (Diptera)	119.9 (mg/l)	LC50 (48 h)	Bansal et al. (2012)
	Ae. aegypti (Diptera)	146.9 (mg/l)		
	Cx. quinquefasciatus (Diptera)	126.4 (mg/l)		

Table 4 Insecticidal activities (LC50 and LC90) of chloroform extract from different plant parts of *C. procera* against *S. litura* and *N. depunctalis*

Parts of plants	Insect	Dosage	% of insecticides	Reference
	N. depunctalis (Lepidoptera)	2.854%/w	LC50	
Whole plant (chloroform)	*N. depunctalis* (Lepidoptera)	3.55%	LC50	Gogoi and Bora (2012)
		1.29%	LC50	

Table 5 Insecticidal activities (LC50 and LC90) of ethyl acetate extract from different plant parts of *C. procera* against *S. litura* insect larvae

Parts of plants	Insect	Dosage	% of Insecticides	Author
Leaves (ethyl acetate)	*S. litura* 3rd instar larva (Lepidoptera)	87.48%/w	LC50	Bakavathippan et al. (2012)

Table 6 Insecticidal activities (LC50 and LC90) of ethanol extract from different plant parts of *C. procera* against various insect

Parts of plants	Insect	Dosage	% of insecticides	References
Leaves	*S. litura* 3rd instar larva (Lepidoptera)	10.76%/w	LC50	Bakavathippan et al. (2012)
Flower	*Culex* sp. (Diptera)	2.50%	LC50	Azmathullah (2011)

Table 7 Insecticidal activities (LC50 and LC90) of petroleum ether extract from different plant parts of *C. procera* against various insects

Parts of plants	Insect	Dosage	% of insecticides	Author
Seeds (petroleum ether)	*An. stephensi* (Diptera)	181.2 (mg/l)	LC50 (48 h)	Bansal et al. (2012)
	Ae. aegypti (Diptera)	27.2 (mg/l)		
	Cx. quinquefasciatus (Diptera)	25.3 (mg/l)		

Table 8 Insecticidal activities (LC50 and LC90) of hexane extract from different plant parts of *C. procera* against on third instar larva of *S. litura*

Parts of plant	Insect	Dosage	% of insecticides	Reference
Leaves (hexane)	*S. litura* 3rd instar larva (Lepidoptera)	18.04%w	LC50	Bakavathippan et al. 2012

References

Abdalla, M., Elimam, K. H., Elmalik, F. S. Ali 2009. Efficacy of leaves extract of *Calotropis procera* Ait.(Asclepiadaceae) in controlling Anopheles arabiensis and Culex quinquefasciatus mosquitoes. *Saudi J. Bio. Sci 2*(16), 95-100.

Alam, P., Ali, M. 2009. Phytochemical investigation of Calotropis procera Ait roots. Indian Journal of chemistry Vol 48 B, pp. 443–446

Al-Doghairi, M. A., El Hag, E. 2003. Effect of several biopesticides on alfalfa weevil larvae, Hypera brunneipennis (Boheman). Pak J Biol Sci, 6(8), 777-781.

Arya, S., Kumar, V. L. 2005. Antiinflammatory efficacy of extracts of latex of Calotropis procera against different mediators of inflammation. Mediators of inflammation 2005 (4), 228-232.

Azmathullah, N. M. (2011). Phytochemical screening of Calotropis procera flower extracts and their bio-control potential on Culex sp. mosquito larvae and pupae. International Journal of Pharmaceutical & Biological Archive 2(6).

Bakavathippan, G. A., Baskaran, S., Parvej, M., Jeyaparvathi, S. (2012). Effect of *Calotropis procera* leaf extract on *Spodoptera litura* (Fab.). J. Biopesticides 5:135.

Bansal, S. K., Singh, K. V., Sharma, S., Sherwani, M. R. K. 2012. Laboratory observations on the larvicidal efficacy of three plant species against mosquito vectors of malaria, dengue/dengue hemorrhagic fever (DF/DHF) and lymphatic filariasis in the semi-arid desert. Journal of environmental biology 33(3), 617.

Begum, N., Sharma, B., Pandey, R. S. 2013. Calotropis procera and Annona squamosa: potential alternatives to chemical pesticides. British Journal of Applied Science & Technology 3(2), 254.

Carlier, P. R., Anderson, T. D., Wong, D. M., Hsu, D. C., Hartsel, J., Ma, M., Bloomquist, J. R. 2008. Towards a species-selective acetylcholinesterase inhibitor to control the mosquito vector of malaria, Anopheles gambiae. Chemico-biological interactions 175(1–3), 368–375.

Chatterjee A., Pakarashi S. C. (1995). The treatise of Indian medicinal plants. (Publications and Information Directorate, CSIR, New Delhi) Vol. 4 130

Chauhan **A.**, Ranjan A. and Jindal, T (2016) Insecticidal Activity of Methanolic Extract of *Calotropis procera* against *Callosobruchus maculatus* using Moong Seeds (Vigna radiata) Journal of Biomedical and Pharmaceutical Research 5(6) 75–82.

Chauhan A., Ranjan A., Jindal T. (2018) Biological Control Agents for Sustainable Agriculture, Safe Water and Soil Health. In: Jindal T. (eds) Paradigms in Pollution Prevention. Springer Briefs in Environmental Science. Springer, Cham https://doi.org/10.1007/978-3-319-58415-7_6

Cheng, S. S., Liu, J. Y., Huang, C. G., Hsui, Y. R., Chen, W. J., Chang, S. T. 2009. Insecticidal activities of leaf essential oils from Cinnamomum osmophloeum against three mosquito species. Bioresource Technology 100(1), 457–464.

Cheung, H. A., Chiu, F. C., Watson, T. R., Wells, R. J. 1983. Cardenolide glycosides of the Asclepiadaceae. New glycosides from Asclepias fruticosa and the stereochemistry of uscharin, voruscharin and calotoxin. Journal of the Chemical Society, Perkin Transactions 1 2827–2835.

Choedon, T., Mathan, G., Arya, S., Kumar, V. L., Kumar, V. 2006. Anticancer and cytotoxic properties of the latex of Calotropis procera in a transgenic mouse model of hepatocellular carcinoma. World Journal of Gastroenterology: WJG 12(16), 2517.

Conti, B., Canale, A., Bertoli, A., Gozzini, F., Pistelli, L. 2010. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito Aedes albopictus (Diptera: Culicidae). Parasitology research 107(6), 1455–1461.

De Morais, S. M., Facundo, V. A., Bertini, L. M., Cavalcanti, E. S. B., dos Anjos Júnior, J. F., Ferreira, S. A., … & de Souza Neto, M. A. 2007. Chemical composition and larvicidal activity of essential oils from Piper species. Biochemical Systematics and Ecology 35(10), 670–675.

Esmaeily, S., Samih, M. A., Zarabi, M., Jafarbeigi, F. 2012. Comparative Study of Insecticides and Calotropis procera Extract on Biological Parameters of Bemisia tabaci (Genn.). Annals of Plant Protection Sciences 20(1), 14–18.

Fournier, D., Mutero, A. 1994. Modification of acetylcholinesterase as a mechanism of resistance to insecticides. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 108(1), 19–31

Giordani, R., Benyahia, S., Teissère, M., Noat, G. 1992. Purification and properties of N-acetyl-β--glucosaminidase from Hevea brasiliensis latex. Plant Science 84(1), 25–34.

Gogoi, H., Bora, D. 2012. Bio-efficacy Potential of Some Ethnically Important Plants Against *Nymphula depunctalis* Guenée (Lepidoptera: Pyralidae) Larvae with Special Reference to *Calotropis procera* and *Zanthoxylum nitidum*. National Academy Science Letters 35(3), 169–175.

Grundy, D. L., Still, C. C. 1985. Inhibition of acetylcholinesterases by pulegone-1, 2-epoxide. Pesticide biochemistry and physiology 23(3), 383–388.

Hanna AG., Shalaby NMM., Morsy NAM., Simon A., Tóth G., Malik S., Duddeck H. 2002; Structure of a calotropagenin-derived artifact from Calotropis procera. Magnetic Resonance in Chemistry; 40:599–602

Hesse and Ludwing, G. 1960. African arrow poisons XIV. Voruscharine, a second sulfur-containing heart poison from Calotropis procera L. Justus Lieb. Ann Chem 632, 158–171.

Irannejad, M. K., Samih, M. A., Jahromi, K. T., Alizadeh, A. 2012. The side Effects of Several Pesticides and Plant Extracts on Biological Parameters of Green Lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) After Treating Eggs and 3rd Instar Under Laboratory Conditions. Plant Protection (Scientific Journal of Agriculture) 35, 3.

Islam, M. S., Aktar, M. J. 2013. Larvicidal efficacies of some plant extracts and their synergistic effects with cypermethrin on the life-history traits of *Musca domestica* L. *Int. J. Innov. Biosci 3,* 92–103.

K.K. Mueen Ahmed, A.C. Rana, V.K. Dixit (2005). Calotropis species (Asclepediaceae) a comprehensive review Pharmacognosy Magazine, 1 (2), pp. 48–52

Kamalam, J. N., Anburaj, J., Sundaravadivelan, C., Kumar, P., Kuberan, T., Dhasarathan, P. 2013. Effect of pyrotechnic chemicals in digestive tract microflora and proximate composition in fish Oreochromis mossambicus. Adv. Appl. Sci. Res 4(1), 188–194.

Khan, A. Q., Malik, A. 1989. A steroid from Calotropis procera. Phytochemistry, 28(10), 2859–2861

Khanzada, S. K., Shaikh, W., Kazi, T. G., Sofia, S., Kabir, A., Usmanghani, K., Kandhro, A. A. 2008. Analysis of fatty acid, elemental and total protein of *Calotropis procera* medicinal plant from Sindh, Pakistan. Pak J Bot 40(5), 1913–1921.

Knio, K. M., Usta, J., Dagher, S., Zournajian, H., Kreydiyyeh, S. 2008. Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, Ochlerotatus caspius. Bioresource technology 99(4), 763–768.

Konno, K. 2011. Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein. Phytochemistry 72(13), 1510–1530

Matthew O. A. Adaramoy O.A. 2014. Anti-Acetylcholinesterase Activity of Methanol extract of Calotropis Procera Leaves. International Journal of Science and Research 3(11), 724–727.

Meena, A. K., Yadav, A. K., Niranjan, U. S., Singh, B., Nagariya, A. K., Sharma, K., Rao, M. M. 2015. A review on *Calotropis procera* Linn and its ethnobotany, phytochemical, pharmacological profile. Drug Invention Today 185–190.

Mohamed, N. H., Liu, M., Abdel-Mageed, W. M., Alwahibi, L. H., Dai, H., Ismail, M. A., Shoreit, A. A. 2015. Cytotoxic cardenolides from the latex of *Calotropis procera*. Bioorganic & medicinal chemistry letters, 25(20), 4615–4620.

Oloumi, H. 2014. Phytochemistry and ethno-pharmaceutics of Calotropis procera. Ethno-Pharmaceutical Products 1(2), 1–8.

Parihar, G., Balekar, N. 2016. *Calotropis procera*: A phytochemical and pharmacological review. Thai Journal of Pharmaceutical Sciences 40(3).

Pavela, R. 2015. Essential oils for the development of eco-friendly mosquito larvicides: a review. Industrial Crops and Products 76, 174–187.

Perwez, A., Mohammad, A. 2009. Phytochemical investigation of *Calotropis procera* roots. Indian Journal of chemistry B 48, 443–446.

Quazi, S., Mathur, K., Arora, S., Wing, P. 2013a. *Calotropis procera*: An overview of its phytochemistry and pharmacology. Indian J. Drugs 1(2), 63–69.

Quazi, S., Mathur, K., Arora, S., Wing, P. 2013b. *Calotropis procera:* An overview of its phytochemistry and pharmacology. Indian J. Drugs 1(2), 63–69.

Qureshi, A. A., Patil, T., Swamy, A. H. M., Gouda, V. A., Setty, R. S. 2007. Hepatoprotective and antioxidant activities of flowers of Calotropis procera (Ait) R. Br. in CCl4 induced hepatic damage. Indian journal of experimental biology 45, 304–310.

Rahman, M. A., Wilcock, C. C. 1991. A taxonomic revision of *Calotropis* (Asclepiadaceae). Nordic Journal of Botany 11(3), 301–308.

Rahman, M. A., Wilcock, C. C. 1992.Diversity of life-form and distribution of the Asclepiadaceae in south-west Asia and the Indian subcontinent. Journal of biogeography 51–58.

Ramos, M. V., Bandeira, G. D. P., Freitas, C. D. T. D., Nogueira, N. A. P., Alencar, N. M. N., Sousa, P. A. S. D., Carvalho, A. F. U. 2006. Latex constituents from Calotropis procera (R. Br.) display toxicity upon egg hatching and larvae of Aedes aegypti (Linn.). Memórias do Instituto Oswaldo Cruz 101(5), 503–510.

Ranjan A., Chauhan, A., & Jindal, T. (2018). In-silico and in-vitro evaluation of human acetylcholinesterase inhibition by organophosphates. Environmental toxicology and pharmacology, 57, 131–140.

Rohit M., Taranalli Ashok., Rasal Vijaykumar., Kamlesh Kashniyal, 2016. Molecular Docking Study of Cassia tora, Brassica campestris and Calotropis procera as Acetylcholinesterase Inhibitor 50(1) 116–122.

Saber, A. H., Maharan, G. H., Rizkallah, M. M., Ste Saber, A. H. 1969. Sterols and pentacyclic triterpenes of Calotropis procera. Bull Fac Pharm Cairo Univ 7(1).

Sehgal, R., Roy, S., Kumar, V. L. 2006. Evaluation of cytotoxic potential of latex of Calotropis procera and Podophyllotoxin in Allum cepa root model. Biocell 30(1), 9–13.

Shahi, N., Hanati-Bojo, A., Iranshahi, M., Vatandoost, H., Mansour, A. 2010. Larvicidal efficacy of latex and extract of *Calotropis procera* against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). Vector Borne Diseases 47, 185–188.

Sharma AK, Kharb R, Kaur R (2011) Pharmacognostical aspects of Calotropis procera (Ait.) R. Br. Int J Pharm Bio Sci 2(3):480–488

Shobowale, O. O., Ogbulie, N. J., Itoandon, E. E., Oresegun, M. O., Olatope, S. O. A. 2013. Phytochemical and antimicrobial evaluation of aqueous and organic extracts of Calotropis procera ait leaf and latex. Nigerian Food Journal 31(1), 77–82.

Singh, A. P., Pathak, D., Verma, N. K., Panda, P. 2015. Journal of Chemical and Pharmaceutical Research 2015, 7 (5): 1366–1369.

Singh, K. P., Arya, S. 2011. Phytochemical profile and evaluation of insecticidal efficacy of *Calotropis procera* against defoliators. Journal of Medicinal Plants Research 5(31), 6738–6743.

Singh, R. K., Mittal, P. K., Dhiman, R. C. 2005. Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family-Asclepiadaceae) against mosquito larvae. Journal of Communicable Diseases 37(2), 109.

Sweidan, N. I., Abu Zarga, M. H. 2015. Two novel cardenolides from Calotropis procera. Journal of Asian natural products research 17(9), 900–907.

Tezara, W., Colombo, R., Coronel, I., Marín, O. 2010. Water relations and photosynthetic capacity of two species of *Calotropis* in a tropical semi-arid ecosystem. Annals of botany 107(3), 397–405.

Upadhyay, R. 2014. Ethnomedicinal, pharmaceutical and pesticidal uses of *Calotropis procera* (Aiton)(Family: Asclepiadaceae). International Journal of Green Pharmacy 8(3), 135.

Wang, J. C., Hinrichs, A. L., Stock, H., Budde, J., Allen, R., Bertelsen, S., Jones, K. 2004. Evidence of common and specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. Human molecular genetics 13(17), 1903–1911.

Toxicology of Organophosphate and Recent Trends in Prophylactic Approaches

Anuj Ranjan, Abhishek Chauhan, and Tanu Jindal

Abstract Organophosphate (OP) pesticides are potent nerve agents. Improper use and poor monitoring lead to intentional and unintentional exposures. Most of the suicidal cases are due to intentional consumption of OP. Organophosphate is a vast family of chemicals that inhibit Acetylcholinesterase (AChE) in synaptic gap of the neurons thereby causing neurotoxicity. Oximes and atropines are conventional therapy given to poisoning patients; however, many OP inhibit AChE irreversibly and do not respond to oximes. There are several developments in the area of OP poisoning treatment. This review has covered the usage, consumption, pathophysiology of OP exposure, and recent advances in the prophylactic approach to combat human poisoning. The review has also discussed the limitation and major drawbacks in the treatment of OP poisoned cases.

Keywords Organophosphate pesticides · Acetylcholinesterase · Organophosphates poisoning · Prophylactic approaches

1 Introduction

Pesticides are a group of chemicals that become environmental pollutants if used in an intensive and poorly managed manner for protection against diseases and pests. Organophosphates (OPs) are a group of chemicals with cholinesterase/acetylcholinesterase (AChE) inhibitory properties being used for crop protection for the last 60 years. These are esters of phosphoric acid or thiophosphoric acids. Metabolites of OPs are prevalent across diverse populations (Aprea et al. 2000; Curl et al. 2003; Barr et al. 2004). This group of compounds is also referred to as nerve agents, and their derivatives are sarin, soman tabun, VX, etc. OPs are also responsible for

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_8

countless human poisoning cases that are associated with OP exposure usually following absorption either through the skin, by inhalation, or by ingestion (Iorizzo et al. 1996).

Organophosphates were first synthesized in the nineteenth century, but in the early 1920s, the German chemist Gerhard Schrader could identify the lethality of these chemicals against insects. He later began research into their development as insecticides. Gerhard Schrader synthesized several commercial OPs among which parathion is still in practice (Costa 2006). Lethality of OPs was later recognized as a nerve agent, and at the start of the second world war, 1939, tabun was made available to the German armies. With time, the study of OPs was concentrated on the improvement of less toxic OPs (Gupta 2011). Nevertheless, usage of OPs rapidly increased in the 1970s, when the use of many organochlorines was prescribed since it was environmentally persistent.

The general chemical structure of an Organophosphates contains a central phosphorus atom (P) and the distinctive phosphoric (P=O) or thiophosphoric (P=S) bond (Fig. 1).

where X is the leaving group and is generally substituted by nucleophilic substitution by the oxygen of serine in the active site of AChE. The degree of AChE inhibition is determined by the nature of the leaving group. A higher tendency of leaving the group provides a greater affinity of the inhibitor to the enzyme. OPs with easily releasing X group can be potent cholinesterase inhibitors (Ecobichon and Joy 1993). This efficiency shows the ability of OPs to phosphorylate the AChE enzyme in the nervous system.

In the case of warfare agents, the leaving group has fluorine (F), that bears a high affinity for hydrolysis makeing OP an extremely potent AChE inhibitor. The active configuration of OPs is an oxono structure; it binds to the AChE active site with the central phosphorus attached to the oxygen atom. The majority of novel OPs have the thion or P=S linkage. In this circumstance, metabolic activation with CYP enzymes (cytochrome P450) first metabolize the thiono to an oxono group – only then can the OPs inhibit the AChE (Gupta 2011). Compounds bearing the P=S group are thiophosphoryl, and they are considerably less toxic than associated phosphoryl derivatives. Characteristically they are poor inhibitors of AChE in both mammals and insects. Metabolism in mammals is likely to remove lipophilic side groups from the phosphorus atom, whereas in the case of insects, it tends to oxidize the compound thus eliminating the terminal sulfur and substituting it with terminal oxygen, which permits the compound to more capably act as an AChE inhibitor (Gupta 2011).

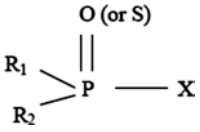

Fig. 1 A typical structure of OP

2 Organophosphate Exposure and Poisoning in Humans

The major toxicity of OP compounds is due to the covalent binding of phosphate radicals to the active sites of the cholinesterase that transform it into an enzymatically inert protein (Namba et al. 1971; Haddad et al. 1998). Organophosphates, therefore, act as irreversible cholinesterase inhibitors since the phosphorylated AChE is not spontaneously reversible until intervenes pharmacologically. Exposure to OPs consequently impedes the synaptic transmission peripherally at muscarinic neuroeffector junctions and nicotinic receptors within sympathetic ganglia and also at skeletal myoneural junctions. This is accomplished by overstimulation of acetylcholine receptor sites that lead to a variety of physiological and metabolical disorders. Interruption of transmission also occurs at the acetylcholine receptor sites within the central nervous system (Namba et al. 1971). Table 1 includes the clinical manifestation of OP positioning.

OPs are the utmost extensively used pesticides worldwide, and their metabolites are prevalent across different populations. The key routes of exposure are shown in Fig. 2. Humans are prone to OP exposure via food, drink, and also breathing polluted air (Vermeire et al. 2003). The workers who are exposed to closed work areas and agricultural fields or people living near farms are also very prone to OP exposure (Gupta 2011). Though the frequency of severe acute organophosphorus pesticide poisoning is comparatively lesser among developed countries, some patients have been reported with an acute low dose of unintentional or occupational exposures (Roberts et al. 2005).

Despite the apparent benefits of these uses, OPs are harmful to humans, particularly children. Children's behaviors such as the nature of feeding behavior, crawling on the floor, putting random objects in their mouth, etc. also put them at high risk (Silvers et al. 1994).

Table 1 Clinical manifestation of OP poisoning according to receptor type

Muscarinic	Nicotinic	Central
Blurred vision	Muscle paralysis	Unconsciousness
Nausea	Paralysis	Confusion
Vomiting	Pallor	Toxic psychosis
Diarrhea	Muscle weakness	Seizure
Salivation	Hypertension	Fatigue
Lacrimation	Tachycardia	Respiratory depression
Bradycardia	Mydriasis (rare)	Dysarthria
Abdominal pain		Ataxia
Diaphoresis		Anxiety
Wheezing		
Urinary and fecal incontinence		

Source: Kumar et al. (2010)

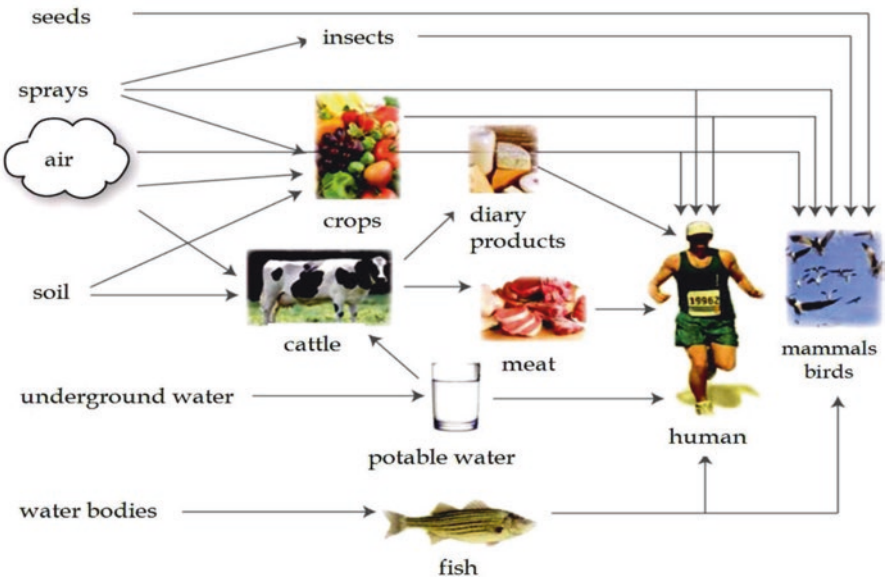

Fig. 2 Various routes of Organophosphates exposure to human (WHO, 2001)

3 Global Status of Human Poisoning by Organophosphate

The extensive use of OPs has caused increased numbers of human poisonings. Most of these poisoning was noticed in developing nations, where protections are usually inadequate (Jeyaratnam 1990; Eddleston 2000). The US Environmental Protection Agency (1979) in 1970 reported that in the United States, 3000 hospitalizations per year were recorded with a fatality rate of 50% in the pediatric age group and 10% in adults (EPA, 1979). The American Association of Poison Control Centers specified in 1983 that the frequency of insecticide exposures involved 33,000 cases of OPs out of 77,000 (Veltri and Litovitz 1984). The WHO in 1990 published first global estimates of the level of insecticide poisoning according to which 3 million cases of pesticide poisonings happened worldwide yearly which included 220,000 deaths; the majority of death was intentional (WHO, 1990). According to the WHO estimates in the year 2001, 849,000 people die worldwide from suicide annually (WHO, 2002). Though the poisoning is the commonest form of suicide in rural Asia, it accounts for over 60% of all deaths (Somasundaram and Rajadurai 1995; Phillips et al. 2002; Joseph et al. 2003). An investigation in Bangladesh indicated that 14% of altogether deaths (3971 of 28,998) of women between 10 and 50 years of age were due to self-poisoning and the majority with pesticides (Yusuf 2002). The problem is particularly severe in Sri Lanka (Berger 1988) where pesticide poisoning was a very common cause of intentional death in six rural districts during 1995 (MoH, Srilanka, 1995). In several countries, the prevalence of highly toxic pesticides used in agriculture has made a selection of pesticides as the agents of choice for

self-harm well known to both healthcare workers and public health authorities (Nalin 1973; Kasilo et al. 1991; Daisley and Hutchinson 1998).

3.1 National Status

The significance of insecticides/pesticides in India can be assumed from the point that agriculture is a major segment of the Indian economy. It adds 17.2% of India's GDP and is the source of revenue for nearly 70% of the country's workforce. Worldwide, due to merging in the agrochemical sector, only the top few multinational firms govern nearly 60% of the marketplace. But in India, the industry is much segmented; there are 30 to 40 big manufacturers and around 400 formulators. The pattern of usage is slanted to insecticides, which estimates 67% of the total pesticide consumption in 2006. As per the National Crime Records Bureau (NCRB), in the year 2014, 26% of the total cases, i.e., 34,254, and in the year 2015, 27.9% of the total cases, i.e., 37,232 were confirmed due to poisoning (Chatterjee et al. 2020).

Indian states (including union territories) have consumed nearly 57,000 MT of chemical pesticide during 2010–2017, and India's highly imported chemical pesticide is profenophos (192.5 MT) followed by phenothoate (36.75 MT) during 203–2017. A graph appanded below shows the usage of chemical pesticides (in metric tonnes) by each state and union territory of India from the year 2010 to 2017. Uttar Pradesh is the top user of chemical pesticides followed by Maharashtra and Punjab during 2010–2017 (Table 2). Among indigenous OP pesticides, chlorpyrifos is highly used OPs followed by phorate and malathion. However, for the year 2017, data are provisional (Figs.3-4 and Table 3) (PPQS, Govt. of India, Feb. 2017).

4 Organophosphates and the Target Enzyme Acetylcholinesterase

Neurotoxicity due to OPs refers to the inhibition of AChE which terminates cholinergic transmission by hydrolyzing the neurotransmitter acetylcholine, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation (Fig. 5).

The toxicological effects of OPs are mainly exerted through nonreversible phosphorylation of AChE in the central nervous system. AChE inhibition causes subsequent accumulation of ACh at the synapses causing overstimulation of nicotinic and muscarinic ACh receptors and obstructed neurotransmission. This results in cholinergic hyperstimulation and neurotoxicity, followed by loss of metabolic balance, and if any effective treatment is delayed, it may lead to death (Worek et al. 2007).

OPs are substrate analogs to ACh so it goes to an active site like its natural substrate and binds covalently to the –OH group of serine. During acetylation, OP is

Table 2 Top consumer states of chemical pesticides in India during 2010-2017

S. No.	States/UTs	2010–2011	2011–2012	2012–2013	2013–2014	2014–2015	2015–2016	2016–2017	Total
1	Uttar Pradesh	8460	8839	9057	10164	9736	10457	10142	66855
2	Maharashtra	8317	6723	6618	10969	8663	11665	13496	66451
3	Punjab	5730	5625	5730	5723	5689	5743	5843	40083
4	Andhra Pradesh	8869	9289	2803	4253	4050	2713	1884	33861
5	Haryana	4060	4050	4050	4080	4070		4050	24360

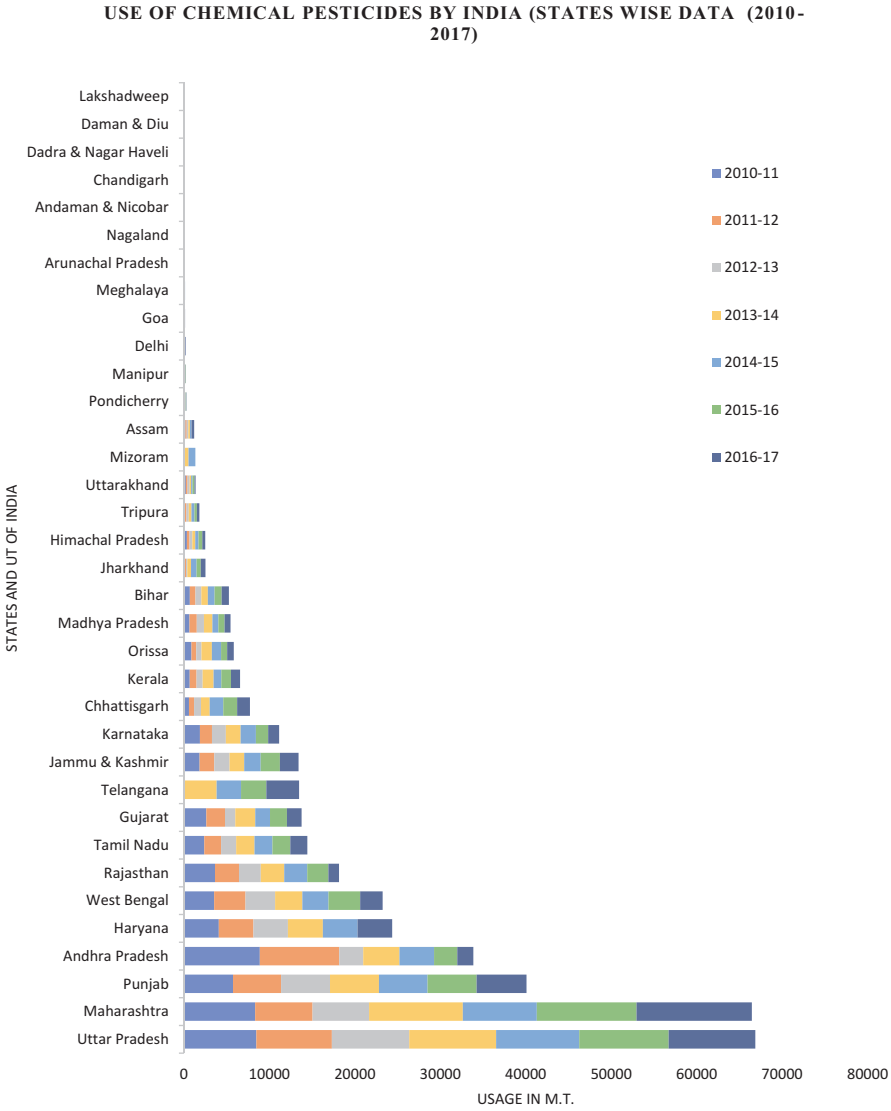

Fig. 3 Usage of chemical pesticide by different Indian states and union territories (Data for 2017 is provisional)

split, and the enzyme is phosphorylated (Fig. 6). While the acyl-enzyme is rapidly hydrolyzed to release the free enzyme, dephosphorylation of the OP-AChE is very slow (order of days), and the phosphorylated enzymes cannot hydrolyze the neurotransmitter (Boublik et al., 2002). OPs binding to the crucial active site residues convert the complex into OP-AChE abducts; this process is called aging of AChE (Fig. 7).

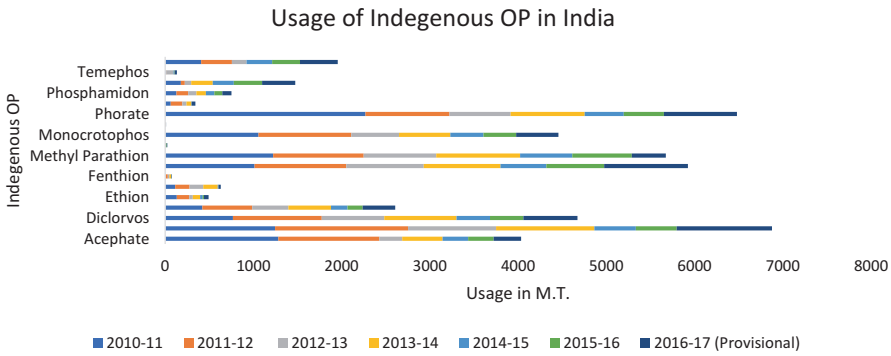

Fig. 4 Usage pattern of indigenous OPs in India during 2010–2017

4.1 Inhibition and Aging of Acetylcholinesterase

A study of the crystal structure of *Tc*AChE also revealed the catalytic site which shows serine residue lies near the base of a narrow gorge penetrating towards the bottom of the enzyme's center. This narrow gorge feature of the active site of AChE is nearly 20 Å from its surface (Schumacher et al. 1986; Sussman et al. 1991). The active site comprises a catalytic triad of glutamate, histidine, and serine and works for catalytic hydrolysis of ACh as shown in Fig. 8.

Acetylation of the serine residue at the active site completes the first mechanistic level to generate the acyl-enzyme, which is then hydrolyzed by a conserved water molecule leading to substrate turnover and deacylation to restore the enzyme to its unbound state (Kryger et al. 2000; Ofek and Soreq 2013).

The crystal structure of human AChE's (*h*AChE) showed similar structural composition and conservation of amino acid residues lining the catalytic gorge. Structural analysis of AChE reveals sub-binding sites near the mouth of the catalytic gorge and in the proximity of the enzyme's active site (Fig. 9). Anionic site-bearing residues tryptophan, phenylalanine, and tyrosine interact with the quaternary ammonium group of ACh (Dvir et al. 2010)).

On either side of the catalytic triad lie an acyl binding pocket and an oxyanion hole. Both the acyl binding pocket and oxyanion hole work to position ACh which helps to expedite nucleophilic attack of its carbonyl carbon by the active site serine hydroxyl group. The discovery of the amino acid residues that comprise the catalytic triad and sub-binding sites was determined through various mutagenesis studies. To explicate the residues of *h*AChE's active site (Ser203, His447, Glu334), alanine was used to displace each of the integral residues yielding a fully inactivated enzyme (Shafferman et al. 1992).

A supplementary allosteric binding site near the entrance of the AChE catalytic gorge is known as the peripheral anionic site (PAS) lying 14 Å from the enzyme's active site. Among several subsidiary binding sites, PAS is believed to play a critical role in initiating the enzyme's catalytic reaction (Dougherty and Stauffer 1990). The

Table 3 Consumption of indigenously made OPs in India during 2010–2017

S. No.	Pesticides	2010–2011	2011–2012	2012–2013	2013–2014	2014–2015	2015–2016	2016–2017 (Provisional)	Total
1	Chlorpyriphos	1245.85	1506.752	997.164	1113.876	470.6884	461.456	1080.858	6876.645
2	Phorate	2270.23	951.652	692.641	840.044	443.006	455.094	826.52	6479.187
3	Malathion	1012.23	1040.352	877.135	870.602	519.627	655.619	949.019	5924.584
4	Methyl parathion	1224.17	1024.929	823.14	950.436	591.226	674.237	385.026	5673.164
5	Dichlorvos	767.12	1007.425	709.0098	817.8136	379.8386	378.039	613.112	4672.358

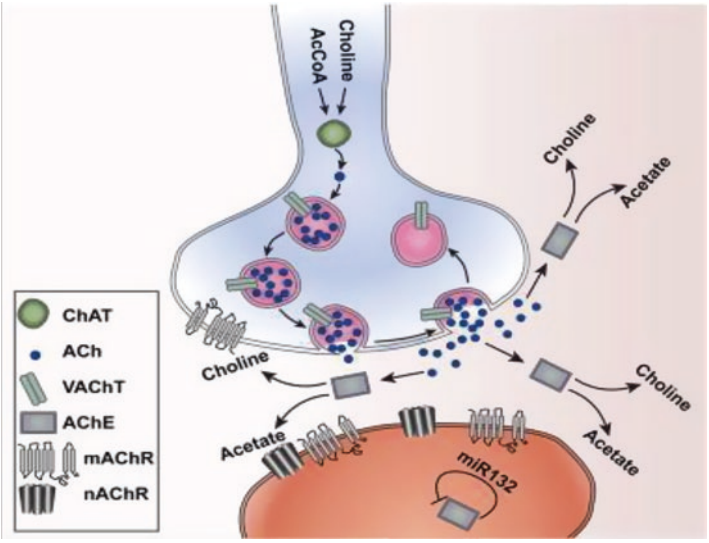

Fig. 5 Pathway of ACh signal nerve-nerve transmission in the central nervous system. (Source: Ofek and Soreq 2013)

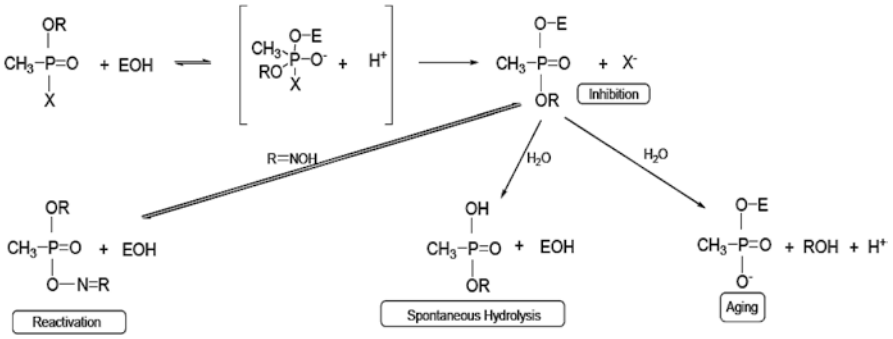

Fig. 6 Reaction mechanism at the active site of AChE showing the aging of AChE. (Source: Mirjana et al. 2013)

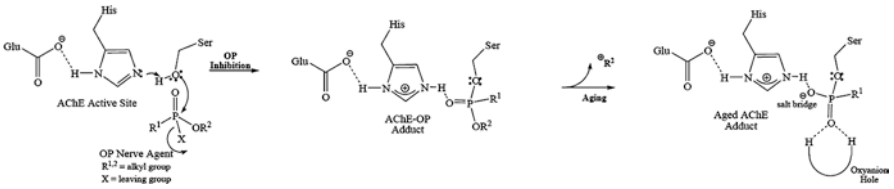

Fig. 7 AChE inhibition mechanism, inhibition induced by OPs; reactivation, spontaneous hydrolysis, and aging of the phosphorylated enzyme. (Source: Mirjana et al. 2013)

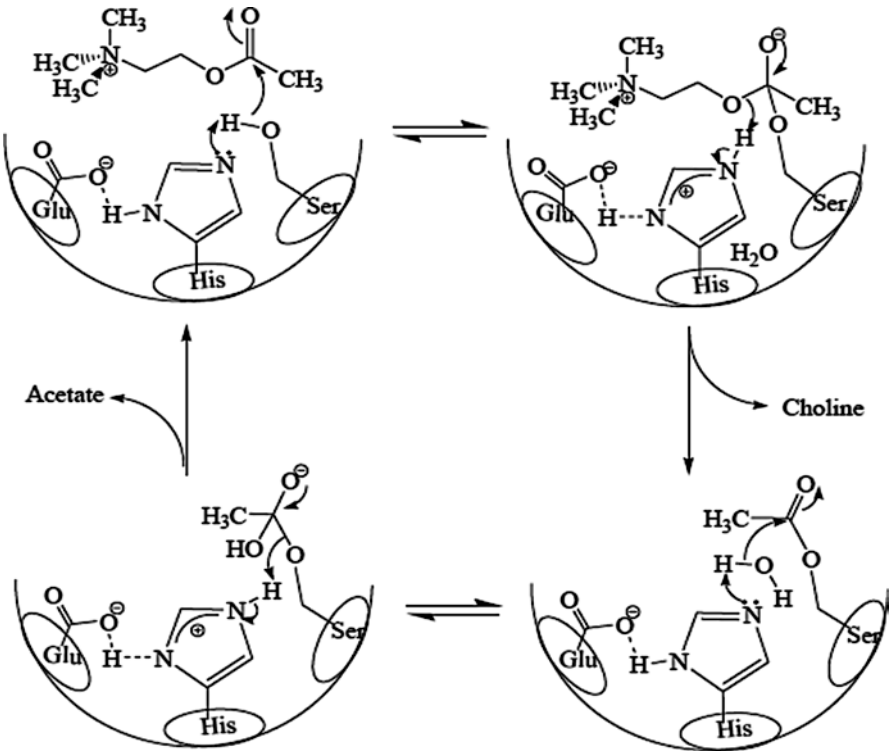

Fig. 8 Mechanism of Ach hydrolysis by catalytic reaction of AChE. (Source: Alexander, 2013)

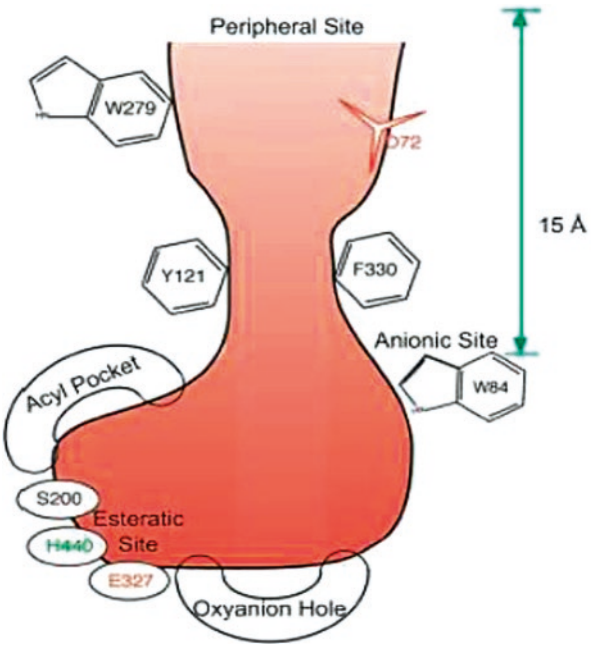

Fig. 9 Representative diagram of active-site gorge of *Tc*AChE emphasizing subsidiary binding sites and catalytic active site

PAS consists of two tyrosines, tryptophan, and 3 aspartate residues, and it functions as an additional binding site for the quaternary ammonium moiety of ACh and has been linked to the substrate inhibition comportment experienced at higher substrate concentrations (Bourne et al. 2003). Delineation of the residues lining the catalytic gorge involved numerous AChE mutagens, particularly those substituting amino acid residues of the PAS. Such investigations accounted for effects on kinetic activity and binding affinity and along with molecular modeling provided topographic insights of the PAS (Barak et al. 1994). Site-specific inhibitors, such as propidium, offer the use of fluorescence detection in mutagenesis studies to further identify amino acid residues comprising the PAS and confirm structural features that contribute to AChE catalytic efficiency. AChE's PAS plays a critical role in substrate hydrolysis due to allosteric variations that can either aid in substrate access to the active site or inhibit enzyme activity due to PAS binding (Changeux 1966; Radić et al. 1991; Shafferman et al. 1992; Barak et al. 1994).

4.2 Role of Aromatic Amino Acids

The active site of AChE is different from other esterases using its structure and nature of conservation. The active site is nearly 20 Å deep from the surface and has two pockets connected through a narrow gorge. AChE's active site is lined with 14 conserved aromatic amino acids which makes it special. Catalytic triad which in which includes Glu-Ser-His at the bottom of the catalytic gorge. Asp 74 and Trp 86 are located at the neck of the gorge, and the cavity wall is lined with two Phe and Trp amino acids (Fig. 10) Importance of the residue Trp86 has been extensively studied as it plays a crucial role in stablizing the OP's interaction in the binding gorge (Ranjan et. al. 2018).

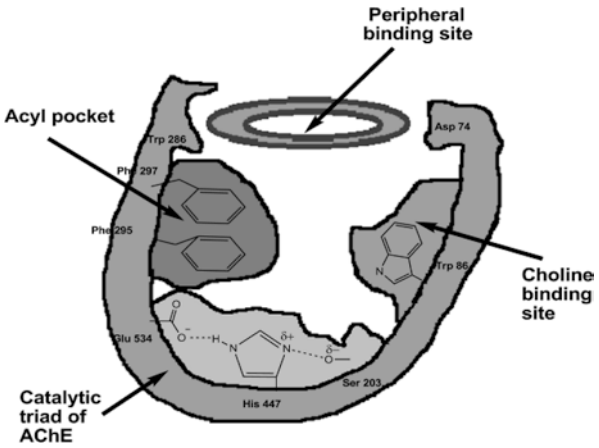

Fig. 10 Schematic representation of AChE binding sites

Studies on MoAChE using site-specific mutants of the associated amino acids for the differences in the reactivity of E2020 towards AChE suggested that residues at the peripheral anionic site such as Asp74 (72), Tyr72(70), Tyr124(121), and Trp286(279) in mammalian AChE may be important in the binding of E2020 to AChE (Saxena et al. 2003).

Molecular modeling studies suggested that E2020 interacts with the active site and the peripheral anionic site in AChE; as the gorge is larger, E2020 cannot simultaneously interact at both sites.

4.3 Reactivation of Aged Acetylcholinesterase and Prophylactic Approaches

OPs inhibit AChE irreversibly and hence neurotoxicity is caused. Reactivation of AChE is the key to reinforce the cholinergic transmission. In this section, we have discussed the emerging approaches for the clinical management of OP-poisoned patients.

4.3.1 Usage of Oximes

Oximes are widely used for the reactivation of AChE inhibited by OPs (Eyer 2003). Wilson and colleagues discovered pralidoxime in the mid-1950s, and it was introduced into clinical practice shortly and effectively (Namba and Hiraki 1958). Followed by pralidoxime many other variants such as obidoxime and trimedoxime also were developed, but pralidoxime remains the most widely used. Pralidoxime is available in four salts: chloride, iodide, methylsulfate, and mesylate. Among these chloride and iodine salts are mostly used (Bismuth et al. 1992). Apart from that chloride salts of pralidoxime have advantages over iodide with respect to its smaller molecular weight (173 vs 264) which increases the active compound per gram by 50% than does iodide. Another drawback of using iodine salt is that it puts patients at risk of thyroid toxicity (Eyer and Buckley 2006). Regardless of the importance of pralidoxime with parathion poisoning, its effectiveness has been debated among Asian clinicians who are unconvinced of its benefit (De Silva et al. 1992; Singh et al. 1995; Peter and Cherian 2000). It was observed that low-dose infusions of pralidoxime might cause harm (Johnson et al. 1996); however, the absence of clinical benefit could relate to study design (suboptimum dose or bias in allocation). Alternatively, some study also suggests that pralidoxime is ineffective on the patients perhaps because of many factors such as specific pesticide ingested, the amount ingested, or the patients' long delay before pralidoxime was monitored (Eddleston et al. 2002; Peter and Moran 2004).

The efficiency of pralidoxime is also being affected firstly by pesticide ingested and secondly by the degree of baseline AChE inhibition and subsequent reversal

(Eyer and Buckley 2006). Above all major studies suggests that higher doses of pralidoxime can be beneficial if patients are treated early with good supportive care. Comparative observations suggest that the reactivation ability of pralidoxime and obidoxime varies with the pesticide ingested (Thiermann et al. 1999; Eyer 2003; Eddleston et al. 2005). AChE inhibited with OP having diethyl group, e.g., parathion and quinalphos, is effectively reactivated by oximes; however OP having dimethyl, e.g., monocrotophos or oxydemeton-methyl, poorly responds to oximes and AChE inhibited by S-alkyl-linked OP, for example, profenofos is not reactivated by oximes at all (Eddleston et al. 2002).

This difference is probably partly because of variation in the speed of AChE aging induced by these different pesticides. Another study about discussing the dosage of pralidoxime has not found any benefit of higher dose pralidoxime in a patient with moderate dimethyl or diethyl OP poisoning (Pawar et al. 2006). Further studies are needed to establish and interpret the clinical evidence regarding oxime usage according to the OP consumed by the patient (Eddleston et al. 2002), although the WHO recommends that oximes should be given to those who develops symptoms who need atropine (Johnson et al. 2000).

4.3.2 Usage of Bioscavenger

In case of OP poisoning in humans, OP penetrates through the skin, lungs, and eyes and then diffuses towards central cholinergic synapses, ganglia, neuromuscular junctions, non-cholinergic targets, etc. The idea of using bioscavenger is to neutralize the OPs using enzymes so that the effect that a certain amount of OP could lead on the body would be diluted. In another word, bioscavengers engage the OP, so that they would be less available to the cholinergic systems and neuromuscular junctions. Usually, bioscavengers are injected intravenously or intramuscularly which helps in neutralizing OP present in the bloodstream (Masson 2015). Bioscavengers present in our body (endogenous bioscavenger) help partially neutralize the OP in the skin, lungs, liver, blood, blood capillaries, etc. However, some of them are converted into thiono-OP activated into oxon- or triortho-cresyl phosphate (TOCP) activated into cresyl saligenyl phosphate (CSP) which are potential cholinesterase inhibitors (Masson 2015).

The idea of using exogenous bioscavengers to protect against nerve agents and poisoned patients was brought into the picture some 30 years ago (Wolfe et al. 1987), with the existing knowledge of endogenous enzymes that are the target of OPs in the body and defense against OP poisoning (Adie 1956). Generally, three types of bioscavengers are discussed, namely, stoichiometric, pseudocatalytic, and catalytic bioscavengers. Stoichiometric bioscavenger neutralize OP in a mole-to-mole reaction, and when these bioscavenger is continuously restored after phosphorylation, they are said to be pseudocatalytic bioscavengers. The third type is catalytic bioscavengers whose substrates are OP (Wolfe et al. 1987; Broomfield 1992).

Stoichiometric Bioscavengers Cholinesterases such as fetal bovine serum AChE, human butyrylcholinesterase (BChE), and human AChE are the first-generation bioscavengers. Human BChE is known to be used for the past 20 years as a standby of pharmacological drugs for pretreatment of OP poisoning as well as postexposure management (Saxena et al. 2006; Mumford et al. 2013). They are irreversibly inhibited by phosphorylation of catalytic serine residue. Therefore, to neutralize the OP using stoichiometric bioscavengers, a huge dose of bioscavenger is required which leads to a higher cost per dose. The FDA-approved human BChE to use as bioscavenger could cost up to $20,000 (Brazzolotto et al. 2012). The main limitation of stoichiometric bioscavengers is the cost/dose of the single-use enzyme.

Catalytic Bioscavengers By nature, these bioscavengers have a higher bimolecular rate constant, and they are multiple-use enzymes. Their bimolecular rate constant (k-OP/Kd) ranges between 10^4 and 10^6 M^{-1} min^{-1} for pesticide (Main 1979) and between 10^6 and 10^8 M^{-1} min^{-1} for nerve agents (Delfino et al. 2009). A study reported that catalytic bioscavenger with a molecular weight of 40 kDa having kcat/Km 10^7 M^{-1} min^{-1} must form a dose of 55 mg to drop blood OP concentration from 3 mM to 0.03 mM in 1 min and 5.5 mg if kcat/Km = 10^8 M^{-1} min^{-1} (Rochu et al. 2007). Another in vivo study reported the extreme effectiveness of catalytic bioscavengers against nerve agents (Worek et al. 2014).

A good bioscavenger must have the following criteria: (i) its bimolecular rate constant must be higher, (ii) its activity must be broad-spectrum to act upon varieties of OP, and (iii) it must act upon toxic stereoisomers of OP as well. There are biological and safety constraints which can be dealt with: (i) long biological life, i.e., the mean residence time in the bloodstream for injected bioscavengers, (ii) it must be immuno-tolerant, (iii) it must have no iatrogenic effects, and (iv) it should not have any biological contaminants such as viruses, coagulation factors, etc. There are many other criteria that limits the bioscavengers application in combating OP poisoned pateints at a large scale. It is also having constraints in manufacturing at an industrial scale. An ideal bioscavenger should be (i) easily produced preferably from expression system or natural sources under good manufacturing practice (GMP) facilities; (ii) thermally stable in liquid or lyophilized formulations in either storage or field conditions; (iii) able to deliver through injection, aerosol, or any other means; and (iv) available at an economically affordable cost.

4.3.3 Nanotechnology Approach

In a study, biomimetic nanoparticles having poly(lactic-co-glycolic acid) (PLGA) polymeric core surrounded by erythrocyte membranes assist as an anti-organophosphate agent by exploiting the AChE present on the surface of the RBC for OP scavenging (Fig. 11). It helps in retaining the activity of AChE bound on the membrane of RBCs and is able to bind to an OP dichlorvos, prohibiting the binding with endogenous AChE in the synapse. The RBC surface modified with NPs has

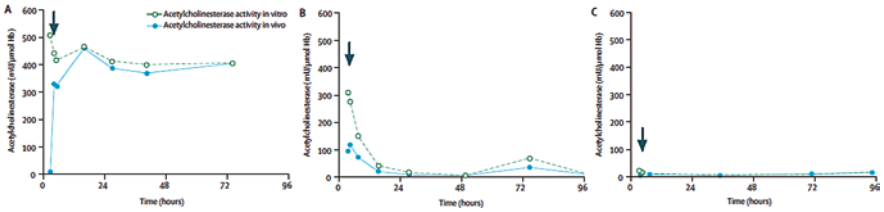

Fig. 11 Reactivation of inhibited AChE (**a**) parathion and quinalphos, (**b**) monocrotophos or oxydemeton-methyl, and (**c**) profenofos using oximes inhibited by oximes

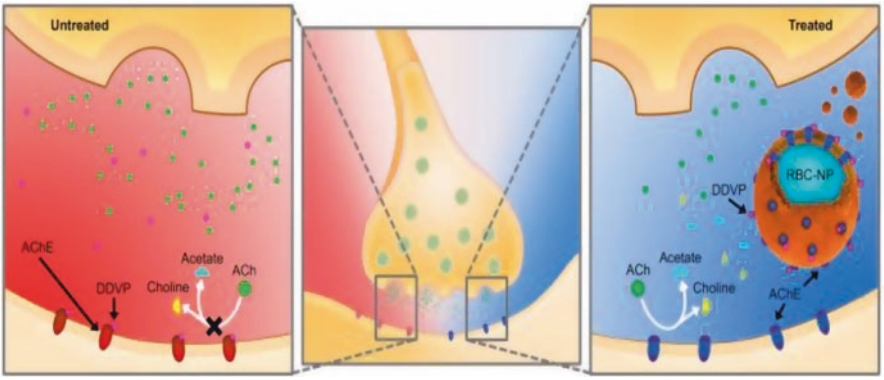

Fig. 12 Schematic representation of nanoparticle-based bioscavenger of OP. Without treatment (left): dichlorvos (DDVP) irreversibly binds to AChE to prevent the breakdown of acetylcholine (ACh) into choline and acetate. When RBC-NPs are introduced (right), they help scavenge the DDVP molecules by exploiting the erythrocyte AChE thereby preserving the binding of OP with endogenous AChE (Pang et al., 2015)

shown to improve the erythrocytic AChE activity and also significantly improved the chance of survival of dichlorvos poisoned mouse (Pang et al. 2015) (Fig. 12).

Another nano-based drug delivery system utilized mixed cationic liposomes based on L-α-phosphatidylcholine and dihexadecylmethylhydroxyethylammonium bromide (DHDHAB) for delivering the 2-PAM into the body. This liposome was designed to overcome the blood-brain barrier crossing by using the intranasal route. Liposomes carrying 2-PAM shows excellent encapsulation capability (~ 90%) and were effective against paraoxon-induced AChE inhibition in the brain. This approach was able to reactivate 12 ± 1% of brain AChE. The uniqueness of this approach was its implementation which is a noninvasive approach, via the "nose-brain" pathway ((Pashirova et al. 2018b). Paraoxon-poisoned rats (0.8 × LD50) were treated with 3-HPA-loaded solid lipid nanoparticles (SLN) and 2-PAM+3-HPA-loaded SLNs (dose 5 mg/kg each). The study shows solid lipid nanoparticles (SLNs) loaded with 2-PAM displayed longer circulation time in the bloodstream concerning free 3-HPA and free 2-PAM. Brain AChE reactivation was observed up to 30% (slowly achieved in 5 h after administration of 3-HPA-SLNs). However, the combination of two oximes could increase reactivation up to 35% (Pashirova et al. 2018a).

5 Discussion

Management of OP poisoning remains a challenge for many developing and underdeveloped countries as it leads to numerous morbidities and mortalities. Severe OP poisoning causes deaths within a few minutes of exposure mainly caused by the failure of the respiratory system due to paralysis of respiratory muscles. It causes disruption of nicotinic ACh receptors which is a key consequence of OP-induced lethality. Hyperstimulation of peripheral muscarinic receptors also leads to choking due to extreme bronchorrhea and bronchoconstriction. OPs are small lipophilic molecules that readily cross the blood-brain barrier and affect the central nervous system (CNS) and brain. Treatment of OP-poisoned patients remains challenging and limited up to the usage of oxime-based reactivators as they remove OP from inhibited AChE to reactivate its activity (Worek and Thiermann 2013). However, several nerve agents like tabun and soman inhibit AChE irreversibly despite the application of clinically used oxime (Lee 2003). Atropine, pyridinium oximes, and benzodiazepines are mostly used in medical emergencies as primary anti-OP treatment (Jokanović 2009). Atropine is universally accepted for the treatment of OP-induced toxicity symptoms like bronchospasm, vomiting, diarrhea, increased sweating, miosis, urinary incontinency, etc., but there is no unanimous guideline for its administration and dosage. Underdosing and overdosing both could be fatal in the case of atropine administration. Underdosing of atropine might delay the optimal reactivation of inhibited cholinesterase followed by central respiratory failure, followed by hypoxia and hypotension, whereas overdosing may lead to excessive anticholinergic toxicity that is also lethal (Wang et al. 2014). Regardless of decades of research, there is no single broad-spectrum oxime that has been developed which can act against all OP compounds (Worek et al. 2016).

Bioscavenger treatment has emerged as a recent medical innovation used to detoxify OP poisoning. Stoichiometric or catalytic. PON-1 is a known catalytic bioscavenger under development (Yeung et al. 2008; Ekinci and Beydemir 2009). PON-1 is a calcium-dependent enzyme responsible for hydrolyzing many OPs very efficiently (Ferretti et al. 2001). Administration of purified PON-1 intravenously is significantly able to help survive and protect guinea pigs poisoned with sarin and soman (Valiyaveettil et al. 2011). However, the large-scale production of PON-1 is questionable as a therapeutic candidate. Another issue with the PON-1 obtained using recombinant microbial expression system is its lowered hydrolytic activity and storage stability (Lushchekina et al. 2018). It has been reported that 200 mg of anti-OP bioscavenger can protect a human against two times the LD50 of highly toxic OP as soman (Saxena et al. 2006). Apart from PON-1, human BChE is another potential bioscavenger candidate in consideration (Stevens et al. 2008). In guinea pig model, the administration of human BuChE has been able to provide defense against soman at 5.5 times higher the dose of LD50 and VX at 8 times higher the dose of VX (Allon et al. 1998). Human BChE isolated from human blood could not be feasible; moreover the production of the same in transgenic animals has posed immunogenic fears. Among anti-OP bioscavengers, AChE is believed to be a promising candidate than BuChE with sophisticated stereoselectivity and ability to scavenge agent VX efficiently (Saxena et al. 2006; Yeung et al. 2008). However, the

search for an affordable source of large-scale production capability of AChE is still a major hurdle. Due to such major hurdles and translational challenges, the AChE development as an anti-OP bioscavenger has been discontinued hoping that an alternative strategy for OP bioscavenging could be really of more therapeutic importance.

NPs has been playing a key role in medicine and drug development, and it has grown rapidly in the past few years (De Jong and Borm 2008). In recent years, NPs have been used for the removal of toxins and chemicals from the body or detoxification. In this regard, very little work has been done to use NPs for the detoxification of OP. The study discussed in this review has discussed biomimetic surfaces prepared using engineered NPs that could intercept the binding of OP and endogenous AChE. Thereby it is useful in reducing the severity of OP. The polymeric NPs when coated on cells were able to functionalize the membrane-bound proteins into enzymatic active forms ((Pang et al. 2015; Pashirova et al. 2018a, b).

Acknowledgments The authors acknowledge the support of Dr. Ashok K. Chauhan, Founder President, Amity Universe, for his constant support and encouragement. The authors also thank Dr. Atul Chauhan, Chancellor, Amity University, Uttar Pradesh, for providing all the required facilities.

References

Adie PA (1956) The effect of the sarinase levels of liver on the survival of rabbits injected with sarin. Can J Biochem Physiol 34:654–659

Allon N, Raveh L, Gilat E, et al (1998) Prophylaxis against soman inhalation toxicity in guinea pigs by pretreatment alone with human serum butyrylcholinesterase. Toxicol Sci 43:121–128

Aprea C, Strambi M, Novelli MT, et al (2000) Biologic monitoring of exposure to organophosphorus pesticides in 195 Italian children. Environ Health Perspect 108:521

Barak D, Kronman C, Ordentlich A, et al (1994) Acetylcholinesterase peripheral anionic site degeneracy conferred by amino acid arrays sharing a common core. J Biol Chem 269:6296–6305

Barr DB, Bravo R, Weerasekera G, et al (2004) Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the US population. Environ Health Perspect 112:186

Berger LR (1988) Suicides and pesticides in Sri Lanka. Am J Public Health 78:826–828

Bismuth C, Inns RH, Marrs TC (1992) Efficacy, toxicity and clinical use of oximes in anticholinesterase poisoning. BUTTERWORTH-HEINEMANN, STONEHAM, MA(USA) 1992

Bourne Y, Taylor P, Radić Z, Marchot P (2003) Structural insights into ligand interactions at the acetylcholinesterase peripheral anionic site. EMBO J 22:1–12

Brazzolotto X, Wandhammer M, Ronco C, et al (2012) Human butyrylcholinesterase produced in insect cells: huprine-based affinity purification and crystal structure. FEBS J 279:2905–2916

Broomfield CA (1992) A purified recombinant organophosphorus acid anhydrase protects mice against soman. Pharmacol Toxicol 70:65–66

Boublik Y, Saint-Aguet P, Lougarre A, et al (2002) Acetylcholinesterase engineering for detection of insecticide residues. Protein engineering 15(1):43–50

Changeux J-P (1966) Responses of acetylcholinesterase from Torpedo marmorata to salts and curarizing drugs. Mol Pharmacol 2:369–392

Chatterjee S, Verma VK, Hazra A, Pal J (2020) An observational study on acute poisoning in a tertiary care hospital in West Bengal, India. Perspect Clin Res 11:75

Costa LG (2006) Current issues in organophosphate toxicology. Clin Chim acta 366:1–13

Curl CL, Fenske RA, Elgethun K (2003) Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. Environ Health Perspect 111:377

Colovic, M.B., Krstic, D.Z., Lazarevic-Pasti, T.D., Bondzic, A.M. and Vasic, V.M., 2013. Acetylcholinesterase inhibitors: pharmacology and toxicology. Current neuropharmacology, 11(3):315–335

Daisley H, Hutchinson G (1998) Paraquat poisoning. Lancet 352:1393–1394

De Jong WH, Borm PJA (2008) Drug delivery and nanoparticles: applications and hazards. Int J Nanomedicine 3:133

De Silva HJ, Wijewickrema R, Senanayake N (1992) Does pralidoxime affect outcome of management in acute organophosphorus poisoning? Lancet 339:1136–1138

Delfino RT, Ribeiro TS, Figueroa-Villar JD (2009) Organophosphorus compounds as chemical warfare agents: a review. J Braz Chem Soc 20:407–428

Dougherty DA, Stauffer DA (1990) Acetylcholine binding by a synthetic receptor: implications for biological recognition. Science (80-) 250:1558–1560

Dvir H, Silman I, Harel M, et al (2010) Acetylcholinesterase: from 3D structure to function. Chem Biol Interact 187:10–22

Ecobichon DJ, Joy RM (1993) Pesticides and neurological diseases. CRC Press

Eddleston M (2000) Patterns and problems of deliberate self-poisoning in the developing world. Qjm 93:715–731

Eddleston M, Eyer P, Worek F, et al (2005) Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. Lancet 366:1452–1459

Eddleston M, Szinicz L, Eyer P, Buckley N (2002) Oximes in acute organophosphorus pesticide poisoning: a systematic review of clinical trials. Qjm 95:275–283

Ekinci D, Beydemir Ş (2009) Evaluation of the impacts of antibiotic drugs on PON 1; a major bioscavenger against cardiovascular diseases. Eur J Pharmacol 617:84–89

Eyer P (2003) The role of oximes in the management of organophosphorus pesticide poisoning. Toxicol Rev 22:165–190

Eyer P, Buckley N (2006) Pralidoxime for organophosphate poisoning. Lancet 368:2110

Environmental Protection Agency (1979) National study of hospital admitted pesticide poisonings for 1974 to 1976. Washington DC, US Government Printing Office, 1979

Ferretti G, Bacchetti T, Marchionni C, et al (2001) Effect of glycation of high density lipoproteins on their physicochemical properties and on paraoxonase activity. Acta Diabetol 38:163–169

Gupta RC (2011) Toxicology of organophosphate and carbamate compounds. Academic Press

Haddad LM, Shannon MW, Winchester J (1998) Clinical Management of Poisoning and Drug Overdose. 3. Aufl, Saunders, Philadelphia

Iorizzo L, Bianchi A, Gamberini G, et al (1996) Assessment of human exposure to pesticides in greenhouses and effectiveness of personal protective devices. Arh Hig Rada Toksikol 47:25–32

Jeyaratnam J (1990) Acute pesticide poisoning: a major global health problem

Johnson MK, Jacobsen D, Meredith TJ, et al (2000) Evaluation of antidotes for poisoning by organophosphorus pesticides. Emerg Med 12:22–37

Johnson S, Peter J V, Thomas K, et al (1996) Evaluation of two treatment regimens of pralidoxime (1 gm single bolus dose vs 12 gm infusion) in the management of organophosphorus poisoning. J Assoc Physicians India 44:529–531

Jokanović M (2009) Medical treatment of acute poisoning with organophosphorus and carbamate pesticides. Toxicol Lett 190:107–115

Joseph A, Abraham S, Muliyil JP, et al (2003) Evaluation of suicide rates in rural India using verbal autopsies, 1994-9. Bmj 326:1121–1122

Kasilo OJ, Hobane T, Nhachi CFB (1991) Organophosphate poisoning in urban Zimbabwe. J Appl Toxicol 11:269–272

Kryger G, Harel M, Giles K, et al (2000) Structures of recombinant native and E202Q mutant human acetylcholinesterase complexed with the snake-venom toxin fasciculin-II. Acta Crystallogr Sect D Biol Crystallogr 56:1385–1394

Kumar SV, Fareedullah MD, Sudhakar Y, et al. (2010) Current review on organophosphorus poisoning. Archives of Applied Science Research 2(4):199–215

Lee EC (2003) Clinical manifestations of sarin nerve gas exposure. Jama 290:659–662

Lushchekina S V, Schopfer LM, Grigorenko BL, et al (2018) Optimization of cholinesterase-based catalytic bioscavengers against organophosphorus agents. Front Pharmacol 9:211
Lodge AM (2013) Kinetic evaluation of the inhibition and reactivation of human acetylcholinesterase. The University of Iowa, 2013
Main AR (1979) Mode of action of anticholinesterases. Pharmacol Ther 6:579–628
Masson P (2015) Catalytic bioscavengers: the new generation of bioscavenger-based medical countermeasures. In: Handbook of Toxicology of Chemical Warfare Agents (Second Edition). Elsevier, pp 1107–1123
Mumford H, Docx CJ, Price ME, et al (2013) Human plasma-derived BuChE as a stoichiometric bioscavenger for treatment of nerve agent poisoning. Chem Biol Interact 203:160–166
Nalin DR (1973) Epidemic of suicide by malathion poisoning in Guyana. Trop Geogr Med 25:8–14
Namba T, Hiraki K (1958) PAM (pyridine-2-aldoxime methiodide) therapy for alkylphosphate poisoning. J Am Med Assoc 166:1834–1839
Namba T, Nolte CT, Jackrel J, Grob D (1971) Poisoning due to organophosphate insecticides: acute and chronic manifestations. Am J Med 50:475–492
Ofek K, Soreq H (2013) Cholinergic involvement and manipulation approaches in multiple system disorders. Chem Biol Interact 203:113–119
Pang Z, Hu C-MJ, Fang RH, et al (2015) Detoxification of Organophosphate Poisoning Using Nanoparticle Bioscavengers. ACS Nano 9:6450–6458. https://doi.org/10.1021/acsnano.5b02132
Pashirova TN, Braïki A, Zueva I V, et al (2018a) Combination delivery of two oxime-loaded lipid nanoparticles: Time-dependent additive action for prolonged rat brain protection. J Control Release
Pashirova TN, Zueva I V, Petrov KA, et al (2018b) Mixed cationic liposomes for brain delivery of drugs by the intranasal route: the acetylcholinesterase reactivator 2-PAM as encapsulated drug model. Colloids Surfaces B Biointerfaces 171:358–367
Pawar KS, Bhoite RR, Pillay CP, et al (2006) Continuous pralidoxime infusion versus repeated bolus injection to treat organophosphorus pesticide poisoning: a randomised controlled trial. Lancet 368:2136–2141
Peter J V, Moran JL (2004) Role of oximes in human organophosphate poisoning—a critical look at the evidence. Crit Care Update 153–163
Peter P V, Cherian AM (2000) Organic insecticides. Anaesth Intensive Care 28:11
Phillips MR, Li X, Zhang Y (2002) Suicide rates in China, 1995–99. Lancet 359:835–840
Radić Z, Reiner E, Taylor P (1991) Role of the peripheral anionic site on acetylcholinesterase: inhibition by substrates and coumarin derivatives. Mol Pharmacol 39:98–104
Roberts DM, Fraser JF, Buckley NA, Venkatesh B (2005) Experiences of anticholinesterase pesticide poisonings in an Australian tertiary hospital. Anaesth Intensive Care 33:469
Rochu D, Chabriere E, Masson P (2007) Human paraoxonase: a promising approach for pretreatment and therapy of organophosphorus poisoning. Toxicology 233:47–59
Saxena A, Fedorko JM, Vinayaka CR, et al (2003) Aromatic amino-acid residues at the active and peripheral anionic sites control the binding of E2020 (Aricept®) to cholinesterases. Eur J Biochem 270:4447–4458
Saxena A, Sun W, Luo C, et al (2006) Bioscavenger for protection from toxicity of organosphosphorus compounds. J Mol Neurosci 30:145–147
Schumacher M, Camp S, Maulet Y, et al (1986) Primary structure of Torpedo californica acetylcholinesterase deduced from its cDNA sequence. Nature 319:407
Shafferman A, Kronman C, Flashner Y, et al (1992) Mutagenesis of human acetylcholinesterase. Identification of residues involved in catalytic activity and in polypeptide folding. J Biol Chem 267:17640–17648
Silvers A, Florence BT, Rourke DL, Lorimor RJ (1994) How children spend their time: a sample survey for use in exposure and risk assessments. Risk Anal 14:931–944

Singh S, Batra YK, Singh SM, et al (1995) Is atropine alone sufficient in acute severe organophosphorus poisoning?: experience of a North West Indian Hospital. Int J Clin Pharmacol Ther 33:628–630

Somasundaram DJ, Rajadurai S (1995) War and suicide in northern Sri Lanka. Acta Psychiatr Scand 91:1–4

Stevens RC, Suzuki SM, Cole TB, et al (2008) Engineered recombinant human paraoxonase 1 (rHuPON1) purified from Escherichia coli protects against organophosphate poisoning. Proc Natl Acad Sci 105:12780–12784

Sussman JL, Harel M, Frolow F, et al (1991) Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. Science (80-) 253:872–879

Thiermann H, Szinicz L, Eyer F, et al (1999) Modern strategies in therapy of organophosphate poisoning. Toxicol Lett 107:233–239

Valiyaveettil M, Alamneh Y, Rezk P, et al (2011) Protective efficacy of catalytic bioscavenger, paraoxonase 1 against sarin and soman exposure in guinea pigs. Biochem Pharmacol 81:800–809

Veltri JC, Litovitz TL (1984) 1983 annual report of the American Association of Poison Control Centers national data collection system. Am J Emerg Med 2:420–443

Vermeire T, MacPhail R, Waters M (2003) Integrated human and ecological risk assessment: A case study of organophosphorous pesticides in the environment. Hum Ecol Risk Assess 9:343–357

Wang W, Chen Q-F, Li Q-B, et al (2014) Efficiency of anisodamine for organophosphorus-poisoned patients when atropinization cannot be achieved with high doses of atropine. Environ Toxicol Pharmacol 37:477–481

WHO (2001) Organophosphorous pesticides in the environment- Integrated Risk Assessment, Geneva: WHO

WHO (1990) In collaboration with UNEP. Public Health Impact of Pesticides used in Agriculture. Updated June 2007, WHO, Geneva

WHO (2002) The World Health report 2002. Reducing risks, promoting healthy life. WHO, Geneva

Wolfe AD, Rush RS, Doctor BP, et al (1987) Acetylcholinesterase prophylaxis against organophosphate toxicity. Fundam Appl Toxicol 9:266–270

Worek F, Aurbek N, Thiermann H (2007) Reactivation of organophosphate-inhibited human AChE by combinations of obidoxime and HI 6 in vitro. J Appl Toxicol An Int J 27:582–588

Worek F, Seeger T, Reiter G, et al (2014) Post-exposure treatment of VX poisoned guinea pigs with the engineered phosphotriesterase mutant C23: a proof-of-concept study. Toxicol Lett 231:45–54

Worek F, Thiermann H (2013) The value of novel oximes for treatment of poisoning by organophosphorus compounds. Pharmacol Ther 139:249–259

Worek F, Thiermann H, Wille T (2016) Oximes in organophosphate poisoning: 60 years of hope and despair. Chem Biol Interact 259:93–98

Yeung DT, Lenz DE, Cerasoli DM (2008) Human paraoxonase I: A potential bioscavenger of organophosphorus nerve agents. In: The Paraoxonases: Their Role in Disease Development and Xenobiotic Metabolism. Springer, pp 151–170

Yusuf S (2002) Clinical research and trials in developing countries. Stat Med 21:2859–2867

Pesticide Usage and Impact on Health of Women in Agriculture

Shalini Thakur, Khushbu Gulati, and Tanu Jindal

Abstract In India, around 84% of the total percentage of women mainly rely on agricultural sector for meeting their livelihood needs. Women's participation rate in farming is about 47% in tea plantations, 46.84% in cotton cultivation, 45.43% growing oil seeds, and 39.13% in the production of vegetables. The indiscriminate usage of pesticides within the agricultural sector poses a serious threat to our environment as well as creates health issues for people. It has been found that currently in India, < 76% of women farmers are either using "moderately" or "highly hazardous" pesticides like organochlorine, organophosphorus, and carbamates, while it was found that around 88% of them never used any protective equipment like masks, gloves, etc. while handling pesticides. About 50% of sprayers mixed different brands of pesticides, many of which were substitutable to each other. The bosom malignancy is considered as the second most normal disease after the uterine cervix disease among the provincial ladies. Out of the 107 instances of malignancy at Talwandi Sabo in Punjab state, it was discovered that around 80 affirmed disease cases were ladies. Similarly, at Chamkaur Sahib, out of 71 cancer cases, around 46 were found to be females. Another study conducted in Himachal Pradesh revealed that 27.45% of the sample of mother's milk had pesticide contamination of p,p′-DDE, p,p′-DDT, and chlorpyrifos. There is an earnest requirement for making more mindfulness among the ladies ranchers and experts in authorizing and guaranteeing the utilization of defensive stuff while taking care of pesticides. The talk focuses on relationship between the extent of pesticide use and signs and symptoms of illnesses due to exposure among women farmers of India.

Keywords Pesticides · Environmental health · Exposure · Risk · Pesticide exposure · Personal protective equipment · Agriculture · Health impact

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2_9

1 Introduction

In India, the cases of breast cancer have increased drastically and are required to be the essential wellspring of malignant growth in ladies in the coming occasions (Donepudi et al. 2014). High rate of breast cancer incidences in farm women occur because of following abnormal conditions, i.e., lifestyle, advancing age, early menarche, late menopause, positive first relative, late age of first birth, diet, socioeconomic status, exposure to pesticides, etc. (Jaga and Dharmani 2005; Zumbado et al. 2005; Steingraber 2009). To further substantiate, the International Agency for Research on Cancer has ordered pesticides as cancer-causing agents and the cultivating-based network to be at a serious danger for extreme and constant wellbeing impacts related with pesticides (Ejaz et al. 2004). It's not only necessary that the cancer patterns will differ only throughout the world but can also vary within a population of the same country. The greater part of the new malignant growth cases that are distinguished each year have a place with the nonindustrial nations. A point-by-point study would demonstrate very accommodating to decide the modifiable reasons for malignancy influencing in the agricultural nations (Thakur et al. 2008). It's conceivable to decrease the impacts of this sickness by distinguishing its causal specialists present in the climate (Engel et al. 2017). People can be presented to pesticides through their word related action and additionally climate, and to additionally prove this, perceptions have exhibited the evil impacts of the drawn-out presentation to pesticides and bug spray (Devakumar 2002). Organochlorine bug sprays (OCs) are a class of chlorinated hydrocarbon insect poisons generally utilized worldwide in horticulture. The cancer-causing properties of 15 OCs have been inspected by the International Agency for Research on Cancer.

The Agrarian Health Study led investigations of authorized pesticide utensils and found that lindane and DDT were related with NHL, while the utilization of chlordane and heptachlor was related with more instances of leukemia. Confirmations of hepatocellular carcinoma have been found by utilization of DDT, dichlorodiphenyldichloroethylene (ρ, ρ'-DDE), and β-HCH, while pre-indicative serum DDE and chlordane metabolites are discovered to be connected with testicular germ cell tumors. Prostate, skin, lung, rectum, and pancreatic malignant growths have a serious relationship with huge introduction of OCs (Jaga and Dharmani 2005). In this survey study, we characterized the effect of pesticide use on ladies' wellbeing working in horticulture area and their separate relationship to bosom malignancy.

2 Definition of Pesticide

According to the Food and Agriculture Organization (FAO), pesticide is a mixture of chemicals that are used for averting, terminating, or regulating pests, including anthropoid vectors or animal virus and undesirable plant and animal species, causing damage during or interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood, and wood products.

3 Agrochemicals Linked with Breast Cancer

Triazine Triazine is a gathering of mixes which are utilized as herbicides everywhere on the world. This gathering is comprised of atrazine, simazine, propazine, and cyanazine. Among all mixes, atrazine is a regularly utilized herbicide; around 76 million pounds of it is applied every year as it is practical. Atrazine is profoundly responsible for setting off bosom malignant growth in ladies and other natural unsettling influences. In the recent years, atrazine is routinely utilized as the prime herbicide and thus defiled groundwater and soil with it. In summer and spring, significant levels of atrazine have been seen in water and soil, especially in developed regions like in the midwest where it is chiefly used to control weeds in cornfields.

Atrazine is a perceived endocrine disruptor. It influences pituitary-ovarian pivot diminishing prolactin and luteinizing hormone levels, the progressions which add to increment in mammary organ tumor (Cooper et al. 2000). There is recorded proof of atrazine making emotional harm to conceptive structures in frogs, fish, and other untamed life (Hayes et al. 2003); anyway correlational human investigations are inadequate. Atrazine additionally instigates expanded aromatase chemical action bringing about expanded degrees of estrogen which is straightforwardly connected with breast disease (Fan et al. 2007). Studies by Ueda et al. indicated critical speeding up in tumor cell multiplication when exploratory rodents with existing bosom disease were presented to atrazine mixes (Ueda et al. 2005). Studies by Raynor et al. presumed that Interco introduction of atrazine aggravates brings about deferred improvement of mammary organs, a realized danger factor for bosom neoplasm (Raynor et al. 2005). In USA, the drinking water guidelines for atrazine remains at 3 ppb, while there are several investigations which uphold the way that the herbicide is destructive even at levels of 0.1 parts per billion. Because of the industrious groundwater tainting brought about by the herbicide, its utilization was suspended quite a while back by Europe in 2005. In any case, it is still generally utilized in the USA as the Environmental Protection Agency (EPA) is hesitant to boycott atrazine as it considers atrazine's danger/advantage proportion for its utilization as a herbicide.

4 Dichlorodiphenyltrichloroethane (DDT/DDE)

DDT was a generally common bug spray on the planet, effective in destruction of jungle fever from the USA and different nations, however to a detriment of obliterating ecological issues and human wellbeing dangers. The connection between the degree of DDT and DDE in the blood and identification of bosom threat has been set up with the assistance of numerous case-control examines. Cohn. BA et al. directed a forthcoming, settled case-control study to help comprehend the connection between time of presentation to DDT and ensuing bosom disease event later in their life. In that review 129 cases created bosom malignant growth by the age of

50 years rather than 129 controls (p = 0.02). It inferred that ladies who are presented to DDT and its metabolites in early 50% of their youth (initial 14 years of ladies' life) have multiple times more odds of creating bosom malignancy in later phases of their life in contrast with ladies who had same measure of introduction after initial 14 years of their life (Cohn et al. 2007). Review case-control concentrates by Charlier et al. demonstrate that specific poisons, for example, DDT and its halfway items, are available in consequential higher fixation in ladies having bosom malignancy when contrasted with the benchmark group (Charlier et al. 2003). An investigation by Demers et al. discovered a positive connection between the high dosages of DDE and its metabolites with forceful tumors with lymph hub association showing that DDT/DDE may disturb threat of mammary organs, if not start it (Demers et al. 2000).

5 Dieldrin and Aldrin

Dieldrin and aldrin were regularly utilized bug sprays in corn fields till the late 1980s the point at which they were prohibited in USA in 1987 because of concerns presented to climate and human wellbeing. Dieldrin primarily goes about as a xenoestrogen and furthermore disturbs androgenic pathways. Dieldrin when added into MCF-7 human bosom disease cell in an in vitro test leads to cells' quickened development and multiplication (Andersen et al. 2002; Soto et al. 1994). Uncovering exploratory rodents prenatally and neonatally with earth applicable dosages of dieldrin brought about expanded frequency of bosom malignant growth among them; the likely system of it very well might be that dieldrin intervened with changes in cell articulation of BNDF and cell signal receptors Heroin bosom tissue (Cameron and Foster 2009). In 1998 a companion study led by Copenhagen Center for imminent investigations presumed that dieldrin is related with expanded rate, frequency of forceful tumor, and higher mortality in instances of bosom malignant growth. The analysis indicated a peril of bosom malignant growth which was identified with an expanded measure of dieldrin dose. This investigation likewise inferred that tumor reviewing and arranging is legitimately relative to blood dieldrin levels (Hoyer et al. 2000).

6 Heptachlor

Heptachlor is a notable cancer-causing bug spray which was broadly utilized before 1980. Its business was prohibited in 1988 aside from controlling the fire ants in underground structures like covered cushion, mounted electric force transformers, and in underground digital TV and phone link boxes. Heptachlor has long half-life in climate, and its buildups can be discovered 14 years after its planned use. Heptachlor epoxide, a metabolite of heptachlor, gets aggregated in fat tissues

including mammary organ. Heptachlor epoxide alters the hepatocytes in this way causing hepatocellular carcinoma. Cassidy et al. decided the degrees of HE, OC, and DDE in fat tissue for a bunch of 34 ladies assessed for bosom disfigurements and found that solitary HE demonstrated a positive impact for episodes of bosom malignant growth inside the biopsies (Cassidy et al. 2005). HE is a xenoestrogenic compound and when joined with HE's capacity to communicate with NO instigates a transformed U expansion in intracellular oxidants causing DNA harm and resulting in danger. Heptachlor likewise actuated kinase flagging pathways bringing about quickened multiplication of malignant growth cells (Cassidy et al. 2005; Cassidy 2010).

7 Persistent Organic Pollutants

Determined natural toxins (POP) are natural mixes which are impervious to ecological debasement and lipophilic, coming about in bio aggregation in human tissues causing endocrine, insusceptible, and conceptive framework dysfunctions and danger including bosom disease. This gathering incorporates polychlorinated biphenyls, chlorinated dioxins, furans, DDT, and so forth. Polychlorinated Biphenyls (PCB) Though PCBs were prohibited in the USA in 1977, as they are persistent natural toxins, they bio aggregate in human fat tissue throughout some undefined time frame and furthermore discharged in bosom milk. A pilot study completed to quantify and think about PCB levels in bosom fat tissue in ladies with threatening and generous neoplasm indicated huge more elevated levels of PCBs in ladies with dangerous neoplasm contrasted with considerate partner (Falck et al. 1992). Numerous investigations reasoned that hereditary polymorphism assumes a fundamental part in the relationship among PCBs and bosom disease hazard. They additionally reasoned that ladies with CYP1A1-m2 hereditary variation, likewise alluded to as the exon 7 variation (present in 10–15% of white ladies and higher level of African American ladies) are more inclined to PCB-initiated threatening changes in bosom tissues (Li et al. 2012a, b). The presence of PCB in huge numbers inside the bosom tissue is straightforwardly identified with the expanding occurrences of bosom disease (Muscat et al. 2003).

8 Polybrominated Diphenyl Ethers (PBDE)

These mixes are basically and practically like PCBs and were broadly utilized after PCBs were prohibited. An ongoing report done by Zhi-Hua Li et al. in China discovered that PBDEs, as PBDE-209 enlarge the creation of tumor cell lines such that's portion subordinate by modifying cell development cycle instigating S stage among G2 and M stage. PBDE-209 is likewise known to incompletely hinder the cell apoptosis in bosom malignant growth cells (MCF-7) and furthermore stifle

Gö6976- and PD98059-prompted apoptosis in all cell lines. It was inferred that PBDE actuates quick development of solid just as malignancy cell lines inside the bosom just as a ladies' regenerative framework, justifying further examinations to affirm the part of BDEs in bosom disease and other neoplasms of conceptive framework among ladies (Li et al. 2012a, b).

9 Other Pesticides

In a vault-based case-control investigation of bosom malignant growth in ranch worker's organization individuals in California, Mills PK, Yang R researched new analyzed 128 bosom disease patients and 640 malignant growth free controls which uncovered this affiliation (Mills and Yang 2005). Another enormous planned associate examination embraced by Lawrence et al. to assess the relationship among pesticides and event of bosom malignant growth among ladies whose spouses work in agrarian fields uncovered an ascent in disease cases among ladies presented to 2, 4, 5-triclorophenoxypropionic corrosive (Lawrence et al. 2005).

10 Relation of Pesticides with Estrogen

The majority of the pesticides have xenoestrogenic properties. An epidemiological investigation was completed to discover the estrogen receptor-positive bosom malignancies and their relationship with natural elements which presumed that ER+ bosom disease grows forcefully when presented to pesticides having xenoestrogenic impact than ER− bosom malignancy (Hilaire et al. 2011).

11 Discussion

Agriculture assumes a significant part in Indian economy with the lion's share of the populace living at towns. Pesticides locate its widespread use in the towns inferable from its part in controlling vector-borne illnesses and harm to crops. A developing number of very much planned epidemiological and subatomic examinations give generous proof that the pesticides utilized in agrarian, business, home, and gardens are connected with an extra risk of causing malignant growth (Alavanja et al. 2013).

Pesticides assume a significant function corresponding to risks connected with causing bosom malignant growth in view of their pervasiveness and due to the capacity of specific pesticides to incite mammary tumors in creature models identified with bosom disease etiology. A few pesticides are known to have evident endocrine-upsetting impacts which have caused certain fears on the grounds that many realized the danger factors for bosom malignancy are identified with

hormonal nature. Much exploration on pesticides and bosom malignant growth has zeroed in explicitly on organochlorine bug sprays in light, to some extent, of their endocrine-disturbing action; in any case, different classes of less-contemplated insect sprays and different pesticides likewise show such action (Giulivo et al. 2016; Smith 1999).

The general population is presented to bug sprays and different pesticides, for the most part at low levels, through the boundless utilization of these synthetic substances in farming and through their utilization in homes, yards, and spots that are open and available to individuals. Ladies who are occupied with farming work or who live in rural territories are probably going to encounter higher introductions to a more prominent scope of pesticides (Enge et al. 2005; Cabello et al. 2003). Such agrarian presentations can either influence straightforwardly subsequently from a lady's treatment of pesticides (i.e., blending, applying, or both), or they can be aberrant, coming about because of working in fields containing pesticide deposits. Other circuitous pesticide presentations may result from shower float, sullied drinking water, or treatment of things polluted in or close to territories of pesticide application (Engel et al. 2017). Bosom disease is the most pervasive malignancy among ladies and is the main source of disease demise among ladies inside the scope of 35–54 years (Reynolds et al. 2004; Abdalla et al. 2003).

The occurrence of bosom malignant growth is low in India, yet rising (Bobdey et al. 2015a, b). Bosom malignancy is the most common disease of metropolitan Indian ladies and the second in the country ladies after cervical disease. The pervasiveness of this sickness has been consistently expanding step by step, and it is assessed to have ascended by half between the year 1965 and 1985 (Murthy et al. 2007).

It has been seen that the quantity of disease cases are ascending by 0.5–2% per annum all over India and among all age bunches however more so inside the more youthful age gatherings of under 45 years (Murthy et al. 2007). Ladies between the age gathering of 41–60 years were being recognized with instances of bosom disease. Prior reports have even shown a concerning actuality that the instances of bosom malignant growth have been accounted for in patients from India around 10 years sooner when contrasted with the individuals from different nations. It was found in an investigation that individuals experiencing bosom malignancy in India were generally under 60 years, and such populace remained at a walloping over 80% of the populace considered from urban communities like Dibrugarh (44.2 years), Delhi (46.8 years), Jaipur (47 years), and Bangalore and Chennai (49.6 years).

As per a recent report by the National Cancer Registry Program, it was discovered that the normal age scope of patients answered to have bosom disease from everywhere India was accounted for to be between 50 and 53 years. In different examinations, it was discovered that the more youthful populace under 35 years old was, the more inclined to bosom malignant growth occurrences in India which involves concern.

The other significant finish of the investigation directed was that the bosom malignant growth episodes were discovered to be more common in metropolitan

spots when contrasted with the rustic settings. There are numerous investigations that have attempted to investigate the connection between malignancy cases and their pervasiveness in rustic zones. One such examination found that individuals recognized with malignant growth cases in the rustic zones commonly come to think about this illness at later stages, and that is the reason their endurance chances decline instead of their metropolitan partners (Doescher and Jackson 2009; Stamenic and Strnad 2011). Such occurrences have been primarily analyzed for disease instances of the colorectal adenocarcinomas of the breast and prostate (Celaya et al. 2010; Benuzillo et al. 2009; Stamatiou and Skolarikos 2009). It has been seen that wellbeing aberrations do exist on a wide reach when one looks at the wellbeing foundation of the towns and urban areas to that of the towns.

A few danger factors have been depicted as expected drivers of this epidemiological polarization. Admittance to medical care, including good ways from clinical offices, doctor to-populace proportion, accessibility of malignant growth recognition advances, and screening techniques comprise a portion of the critical parts of social hardship and rurality (Goodridge et al. 2010; Onega et al. 2008). The third most significant perception was that the bosom malignancy occurrences were discovered to be hardly more in individuals living in the towns where agribusiness remains as a transcendent occupation.

References

Donepudi MS, Kondapalli K, Amos SJ, Venkanteshan P. (2014). Breast cancer statistics and markers. *J Cancer Res Ther.*10(3):506–11.

Jaga K, Dharmani C. (2005). The epidemiology of pesticide exposure and cancer: A review. *Rev Environ Health.* 20(1):15–38.

Zumbado M, Goethals M, Alvarez-León EE, Luzardo OP, Cabrera F, Serra-Majem L, et al. (2005). Inadvertent exposure to organochlorine pesticides DDT and derivatives in people from the Canary Islands (Spain). *Sci Total Environ*. 339(1–3):49–62.

Ejaz S, Akram W, Lim CW, Lee JJ, Hussain I. (2004) Endocrine disrupting pesticides: a leading cause of cancer among rural people in Pakistan. *Exp Oncol*.26:98–105.

Steingraber S. (2009). What we know about pesticides and breast cancer. *Rev Environ Health*.24(4):345–55.

Thakur JS, Rao BT, Rajwanshi A, Parwana HK, Kumar R. (2008). Epidemiological study of high cancer among rural agricultural community of Punjab in Northern India. *Int J Environ Res Public Health*. 5(5):399–407.

Engel LS, Werder E, Satagopan J, Blair A, Hoppin JA, Koutros S, et al. (2017). Insecticide use and breast cancer risk among farmers' wives in the agricultural health study. *Environ Health Perspect.*125(9):097002.

Devakumar C. (2002). Endosulfan-Update on Regulations on Its Use. *Pesticide Research Journal.* 14: 355–61.

National Cancer Institute, "Surveillance Epidemiology and End Results." http://seer.cancer.gov/statfacts/html/breast.html

R. L. Cooper, T. E. Stoker, L. Tyrey, J. Goldman and W. McElroy (2000). Atrazine Disrupts the Hypothalamic Control of Pituitary-Ovarian Function. *Toxicology Science*, 53(2): 297–307.

T. Hayes, K. Haston, M. Tsui, A. Honag, C. Haeffele and A. Vonk (2003). Atrazine-Induced Hermaphroditism at 0.1 ppb in American Leopard Frogs (Ranapipiens): Laboratory and Field Evidence. *Environmental Health Perspectives*.111(4):568–575.

W. Q. Fan, T. Yanase, H. Morinaga, T. Yanase, H. Morinaga, S. Gondo, T. Okabe, M. Nomura, T. Komatsu, K.-I. Morohashi, T. Hayes, R. Takayanagi and H. Nawata (2007). Atrazine-Induced Aromatase Expression in SF-1 Dependent: Implications for Endocrine Disruption in Wildlife and Reproductive Cancers in Humans. *Environmental Health Perspectives*.115:720–727.

M. Ueda, T. Imai, T. Takizawa, H. Onodera, K. Mitsumori, T. Matsui and M. Hirose (2005). Possible Enhancing Effects of Atrazine on Growth of 7, 12-Dimethylbenz(a) Anthracene-Induced Mammary Tumors in Ovariectomized Sprague-Dawley Rats. *Cancer Science*. 96(1):19–25.

J. L. Raynor, R. R. Enoch and S. E. Fenton (2005). Adverse Effects of Prenatal Exposure to Atrazine during a Critical Period of Mammary Gland Growth. *Toxicological Sciences*. 87(1): 255–266.

B. A. Cohn, M. S. Wolff, P. M. Cirillo and R. I. Sholtz (2007). DDT and Breast Cancer in Young Women: New Data on the Significance of Age at Exposure. *Environmental Health Perspectives*. 115(10):406- 1414.

C. Charlier, A. Albert, P. Herman, E. Hamoir, U. Gaspard, M. Meurisse and G Plomteux, (2003). Breast Cancer and Serum Organochlorine Residues. *Occupational and Environmental Medicine*. 60(5):348–351.

A. Demers, P. Ayotte, J. Brisson, S. Dodin, J. Robert and E. Dewailly (2000). Risk and Aggressiveness of Breast Cancer in Relation to Plasma Organochlorine Concentrations. *Cancer Epidemiology Biomarkers & Prevention*. 9(2):161–166.

H. R. Andersen, A. M. Vinggaard, T. H. Rasmussen, et al. (2002) Effects of Currently Used Pesticides in Assays for Estrogenicity, Androgenicity, and Aromatase Activity in Vitro. *Toxicology Applied Pharmacology*. 179(1):1–12.

H. L. Cameron and W. G. Foster (2009). Developmental and Lactational Exposure to Dieldrin Alters Mammary Tumorigenesis in Her2/Neu Transgenic Mice. *PLoS One*, Vol. 4(1):e4303

A. P. Høyer, P. Grandjean, T. Jørgensen, J. W. Brock and H. B. Hartvig (2000). Dieldrin as a Risk Factor for Breast Cancer and for Increased Mortality Once Breast Cancer Is Detected. *Journal of Clinical Epidemiology*. 2002(53):323–330.

R. Cassidy (2010). Cancer and Chlordane-Treated Homes: A Pinch of Prevention Is Worth a Pound of Cure. *Leukemia & Lymphoma*. 51(7):1363–1364.

R. A. Cassidy, S. Natarajan and G. M. Vaughan (2005). The Link between the Insecticide Heptachlor Epoxide, Estradiol, and Breast Cancer. *Breast Cancer Research and Treatment*. 90(1):55–64.

F. Falck Jr., A. Ricci Jr., M. S. Wolff, J. Godbold and P. Deckers (1992). Pesticides and Polychlorinated Biphenyl Residues in Human Breast Lipids and Their Relation to Breast Cancer. *Archives of Environmental Health*. 47(2):143–146.

Z.-H. Li, X.-Y. Liu, N. Wang, J.-S. Chen, Y.-H. Chen, et al. (2012b). Effects of Decabrominated Diphenyl Ether (PBDE- 209) in Regulation of Growth and Apoptosis of Breast, Ovarian, and Cervical Cancer Cells. *Environmental Health Perspectives*. 120(4):541–546.

J. Muscat, J. Britton, M. Djordjevic, M. Citron, M. Kemeny, E. Devereaux, B. Pittman, S. Stellman (2003). Adipose Concentrations of Organochlorine Compounds and Breast Cancer Recurrence in Long Island, New York. *Cancer Epidemiol Biomarkers and Prevention*. 12:1474.

Z.-H. Li, X.-Y. Liu, N. Wang, J.-S. Chen, Y.-H. Chen, J.-T. Huang, C.-H. Su, F. K. Xie, B. Yu and D.-J. Chen (2012a). Effects of Decabrominated Diphenyl Ether (PBDE-209) in Regulation of Growth and Apoptosis of Breast, Ovarian, and Cervical Cancer Cells. *Environmental Health Perspectives*. 120:4.

P. K. Mills and R. Yang (2005). Breast Cancer Risk in Hispanic Agricultural Workers in California. *International Journal of Occupational and Environmental Health*. 11(2):123–131.

E. Lawrence, D. Hill, J. Hoppin, J. Lubin, C. Lynch, J. Pierce, C. Samanic, D. Sandler, A. Blair and M. Alavanja (2005). Pesticide Use and Breast Cancer Risk among Farmers' Wives in the Agricultural Health Study. *American Journal of Epidemiology*.161(2):121–135.

S. Hilaire, R. Mandal, A. Commendador, S. Mannel and D. Derryberry (2011). Estrogen Receptor Positive Breast Cancers and Their Association with Environmental Factors. *International Journal of Health Geographics*.11(1):32.

Michael C R Alavanja, Matthew K Ross, Matthew R Bonner (2013). Increased cancer burden among pesticide applicators and others due to pesticide exposure. *CA Cancer J Clin.* 63(2):120–42

Giulivo M, Lopez de Alda M, Capri E, Barceló D (2016). Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. *A review. Environ Res*. 151:251–64.

Smith D (1999). Worldwide trends in DDT levels in human breast milk. *Int J Epidemiol.* 28(2):179–88.

Enge LS, Hill DA, Hoppin JA, Lubin JH, Lynch CF, Pierce J, et al. (2005). Pesticide use and breast cancer risk among farmers' wives in the agricultural health. study. *Am J Epidemiol.*161(2):121–35.

Cabello G, Juarranz A, Botella LM, Calaf GM (2003). Organophosphorus pesticides in breast cancer progression. *J Submicrosc Cytol Pathol*.35(1):1–9.

Reynolds P, Hurley SE, Goldberg DE, Yerabati S, Gunier RB, Hertz A, et al. (2004). Residential proximity to agricultural pesticide use and incidence of breast cancer in the California Teachers Study cohort. *Environ Res*.96(2):206–18.

Abdalla MH, Gutierrez-Mohamed ML, Farah IO (2003). Association of pesticide exposure and risk of breast cancer mortality in Mississippi. *Biomed Sci Instrum*.39:397–401.

Bobdey S, Balasubramanium G, Kumar A, Jain A (2015a). Cancer screening: Should cancer screening be essential component of primary health care in developing countries? *Int J Prev Med*. 26:56.

Bobdey S, Balasubramanium G, Kumar A, Jain A. (2015b). Cancer screening: Should cancer screening be essential component of primary health care in developing countries*? Int J Prev Med*.6:56. 19.

Murthy NS, Agarwal UK, Chaudhry K, Saxena S. (2007). A study on time trends in incidence of breast cancer-Indian scenario. *Eur J Cancer Care*.16:185–6.

Doescher MP, Jackson JE. (2009). Trends in cervical and breast cancer screening practices among women in rural and urban areas of the United States. *J Public Health Manag Pract*.15(3):200–9.

Stamenic V, Strnad M. (2011). Urban-rural differences in a population-based breast cancer screening program in Croatia. *Croat Med J*.52(1):7686.

Celaya MO, Berke EM, Onega TL, Gui J, Riddle BL, Cherala SS, et al. (2010). Breast cancer stage at diagnosis and geographic access to mammography screening (New Hampshire, 1998–2004). *Rural Remote Health*.10(2):1361.

Benuzillo JG, Jacobs ET, Hoffman RM, Heigh RI, Lance P, Martínez ME. (2009). Rural-urban differences in colorectal cancer screening capacity in Arizona. *J Community Health.* 34(6):523–8.

Stamatiou K, Skolarikos A. (2009). Rural residence and prostate cancer screening with prostate-specific antigen. *Rural Remote Health*.9(2):1227.

Goodridge D, Lawson J, Rennie D, Marciniuk D. (2010). Rural/urban differences in health care utilization and place of death for persons with respiratory illness in the last year of life. *Rural Remote Health*. 10(2):1349.

AM Soto, Chung, KL, Sonnenschein C. (1994). "The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen sensitive cells." Environ. Health Perspect. 102:380–383.

Onega T, Duell EJ, Shi X, Wang D, Demidenko E, Goodman D. (2008). Geographic access to cancer care in the U.S. Cancer. 112(4):909–18.

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T. Jindal (ed.), *New Frontiers in Environmental Toxicology*,
https://doi.org/10.1007/978-3-030-72173-2