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Environmental Toxicity Monitoring of Nanomaterials using *Vicia Faba* GENE-TOX Assay

Anita K. Patlolla*

Molecular Toxicology Research Laboratory, NIH-RCMI Center for Environmental Health, CSET, Jackson State University, Jackson, MS, USA

This is a short summary of environmental toxicity monitoring of nanomaterials (NM) by *Vicia faba* GENE-TOX assay. With the rapid growth in nanotechnology, it is important to understand the safety of engineered NM and their associated hazards. NMs have unique physical and chemical properties, due to which they have been used for a variety of purposes such as to create new consumer products, applications for life sciences and biotechnology. Yet, the potential adverse health effects remain poorly characterized for many NMs. The environmental fate and behavior of NMs is a rapidly expanding area of research. Several reports on the toxicity of NMs have been published however; its impact on plants is not yet thoroughly evaluated [1]. The toxicity of NMs is due to their unique small size and large surface area which allow them to translocate when inhaled [2]. When released into the environment through industrial or domestic waste, it might cause disruption in the microflora of soil, water and eventually alter the food chain hence disrupting the productivity of plant such as nitrogen assimilation and metabolism. Recent studies of NMs such as single and multi-walled nanotubes, nanofibers, fullerene derivatives, quantum dots, and metal oxide nanoparticles have gained much importance due to their toxicity on human cell line, bacteria, and rodents [3-8]. The adverse effects of engineered NMs are intensively being investigated due to the increasing interest in its potential toxicity. The studies on higher plants with both positive and negative effects of NM are very few. Nanoscale SiO_2 and TiO_2 enhanced nitrate reductase activity in soybean which hastened its germination and growth [9]. It was also reported by several authors [10-13] that Nano-TiO_2 enhanced photosynthesis, nitrogen metabolism and improved growth of spinach. Previous studies [14-17] with root tip meristematic cells of Allium cepa and Zea mays observed chromosomal aberrations, micronuclei and DNA damage exposed to silver nanoparticles, zinc oxide nanoparticles and coated magnetic nanoparticles of ferrofluid, demonstrating that NMs could penetrate plant system and may interfere with intracellular components causing damage to cell division.

In studies, to assess the toxicity of environmental polluting agent, higher plant species such as *Allium cepa, Zea mays, Tradescantia, Nicotiana tabacum, Crepis capillaris* and *Hordeum vulgare* and *Vicia faba* (*V. faba*) have been widely used. Higher plants are preferred over animal based bioassays due to their sensitivity to detect mutagens as well as other toxic agents, and also offer evaluation on the basis of a number of genetic endpoints, ranging from point mutations to DNA damage [18,19]. Higher plants are eukaryotes, having similar chromosomal morphology, cell division process and mutation mechanism to other living organisms. Furthermore, plant based bioassays can be quick, easy to culture, inexpensive, short term and can be conducted under a wide range of environmental conditions, pH and temperature. The higher plant genetic bioassays can also be performed to assess the toxicity of single or complex mixtures of chemicals.

Among plant based bioassays, the *V. faba* is regarded as favourable to evaluate the environmental quality, DNA damages and abnormalities in cell division as a result of exposure to different tested agent. *V. faba* offers many advantages and is ideal for use by scientists in the field of environmental mutagenesis for screening and monitoring of genotoxicity, cytotoxicity and mutagens according to the standard protocols and genetic makeup is similar to other living organisms [18,19]. *V. faba* chromosome aberration assay has been ongoing for decades. The USEPA Gene-Tox Program has allowed chromosome aberration frequencies in *V. faba* root tips to become a vital tool for assessing genotoxicity. As a result, these mitotic root meristems have been the leading cytogenetic material used to measure the potential genotoxicity of environmental pollutants. Plant assays are quick, simple, reliable, and inexpensive making them ideal for risk assessment of potential environmental mutagens. The International Programme on Chemical Safety (IPCS, WHO) and the United Nations Environmental Programme (UNEP) have validated the *V. faba* root tip chromosomal aberration assay as the standard test for chemical screening and in situ genotoxicity monitoring of environmental substances due to its efficiency [20-24].

An important aspect of hazard identification includes the potential for a novel agent to induce genotoxicity, as damage to the genetic material may result in the induction or promotion of carcinogenesis. When it is directly related to the exposure of the substance, genotoxicity is referred to as primary. However, secondary genotoxicity is the result of the substance interacting with cells or tissues and releasing factors, which cause the adverse effects, such as inflammation and oxidative stress. Cytogenetic biomarkers represent the intermediate steps in the pathway from exposure to disease. This is important because it allows estimating the risk of cancer in a population by analyzing the correlation between cytogenetic changes induced by carcinogens and the development of malignant changes. While the presence of these markers themselves is not sufficient to predict negative health outcomes, cells exposed to carcinogens are often found to have high levels of the cytogenetic markers. Such improper levels of these markers can lead to mutations that affect genes responsive for cell activity or growth and resulting in carcinogenesis [7,25].

There is a pressing requirement to define hazard identification and risk management strategy for NMs due to rapid growth in nanotechnology industry. In the near future with some estimates indicating a 19% predicted growth of the global nanotechnology industry between 2011 and 2013 [26]. When regulating the usage of nanotechnology, it is crucial to maintain an appropriate balance. If

***Corresponding author:** Dr. Anita K. Patlolla, Molecular Toxicology Research Laboratory, NIH-RCMI Center for Environmental Health, CSET, Jackson State University, Jackson, MS, USA, Tel: 601-979-0210; Fax: 601-979-5853; E-mail: anita.k.patlolla@jsums.edu

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regulation is too stringent, it can restrict innovation and hold back development; whereas, regulations that are relaxed can put consumers and the environment at risk. A delicate balance is required to prevent stifling innovation while ensuring that public and environmental safety is protected. Currently, the validity of the *in vitro* and *in vivo* genotoxicity assessment framework for NMs is still lacking, largely due to limited understanding of their pharmacokinetics including absorption, distribution, metabolism, excretion following the wide range of NM exposure routes. Additionally, current *in vitro* and *in vivo* genotoxicity assays do not take into consideration the consequences of chronic exposure which might be more detrimental than those resulting from acute exposure [27]. A strategic approach is clearly required with regards to the genotoxic testing strategy for the safety assessment and regulation of NM. The use of *Vicia faba* GENE-TOX assay would be appropriate to test the genotoxicity of NM, as it is fast, sensitive and could detect toxicants below the permissible limit and enables the evaluation of action mechanisms of the tested agents. It is a reliable bioassay and provides an important method for screening environmental contamination and results can be used as a warning for other biological and ecological systems.

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