

Reproductive toxicity of carbon nanomaterials: a review

I Vasyukova¹, A Gusev^{1,2} and A Tkachev³

¹G.R. Derzhavin Tambov State University, 33, Internatcionalnaya st., Tambov, 392000, Russia

²National Research Technological University "MISIS", 4, Leninsky ave., Moscow, 119991, Russia

³Tambov State Technical University, 106, Sovetskaya st, Tambov, 392000, Russia

E-mail: vasyukovaia@gmail.ru

Abstract. In the current review, we assembled the experimental evidences of an association between carbon nanomaterials including carbon black, graphite nanoplatelets, graphene, single- and multi-walled carbon nanotubes, and fullerene exposure and adverse reproductive and developmental effects, *in vitro* and *in vivo* studies. It is shown that carbon nanomaterials reveal toxic effect on reproductive system and offspring development of the animals of various system groups to a certain degree depending on carbon crystal structure. Although this paper provides initial information about the potential male and female reproductive toxicity of carbon nanomaterials, further studies, using characterized nanoparticles, relevant routes of administration, and doses closely reflecting all the expected levels of exposure are needed.

1. Introduction

Carbon nanomaterials become more and more attractive and they gain wider popularity in the field of power accumulation, biomedicine, catalysis etc. It happens because of their ability to improve material properties significantly even being introduced in a small dosage due to their truly unique physical-chemical characteristics like high chemical stability, large specific surface, high flexibility and electric conductivity [1-4]. With their high biocompatibility, carbon nanomaterials show a great potential in the area of biomedicine as containers for addressed drug delivery as well as for bioimaging and tissue engineering purposes [5-11].

Potential toxicity of the carbon nanomaterials has recently attracted great attention because of their intensive industrial and medical utilization that brings not only obvious advantages but also probable human health hazard. At the same time, wide application of these materials will inevitably result in uncontrolled increase of their content in the environment that can't help causing alarm about safety of industrial waste, working places and surrounding objects because of their biological effect being insufficiently studied [12-15]. The results of investigation of carbon nanomaterials effect on reproductive functions of various systematic groups are currently contradicting and not systematized. In the present paper we present the experimental results of assessment of toxic effects on reproductive system of the animals displayed by carbon nanoparticles with various structures.



2. Reproductive toxicity of carbon nanomaterials

2.1. Carbon Black

The results of the assessment of nanosized carbon black (CB) effect on the mice breed [16-18] show the absence of statistically significant difference in offspring quality from exposed females. However, in [19] malfunctions in gene expression of the offspring from mouse females exposed in pregnancy period were reported. The offspring of exposed mouse males have several indices of reproductive system functioning reduced, including an amount of sperm generated [20]. Results of the observations [21] of the influence on male reproductive system in experiments with mice of ICR line reveal negative effect of CB. Being administered intratracheally, the material at 0.1 mg/mouse dosage is shown to stimulate significant increase of testosterone level and force partial vacuolization of testicular tubules.

2.2. Graphite nanoplatelets, (GNPs)

Zanni et al. do not reveal any toxic effect of GNPs on reproductive functions of *Caenorhabditis elegans* [22].

2.3. Graphene

Toxicity assessment of graphene oxides (GO) at 0.5–100 mg/l dosage on model organisms *Caenorhabditis elegans* didn't show any toxic effect on reproductive system and offspring development [23-24].

In [25] the influence of nanostructured GO on reproductive system of mouse males of ICR line was in focus. GO were introduced through the tail vein in 6.25, 12.5 and 25 mg/kg dosage. According to the experimental results, secretion of gonadal hormones, testes and epididymis functioning as well as reproductive activity remains normal after the exposure. Among the offspring from unexposed females there was no significant difference revealed in the number, sex, weight and viability compared to the control group. Besides, authors give evidence to the absence of the toxic effect when GO are introduced intraperitoneally in higher dosage, namely up to 300 mg/kg. This fact lets them declare there is no or very small toxicity of GO for male reproductive system.

However, on exposing females, the GO negative effects reveal itself significantly both for female's organism and the offspring. In [26] the effect of GO on the females before and after breeding as well as on the offspring development was studied at GO dose of 6.25, 12.5, and 25 mg/kg. The dependence of toxic effect on fetuses upon pregnancy stage was detected. So, if females are exposed to GO before breeding or at the early pregnancy stages the offspring will be healthy with high probability. However, females' exposure in 6.25, 12.5 and 25 mg/kg dosage at the later stages of pregnancy leads to miscarriages and anomalies of fetuses, while the exposure at the maximal concentration led to death of 85% females. The results obtained give evidence to extremely high toxic effect of GO on females at late pregnancy stages. In [27] was shown that oral GO exposure of females in lactation period at 0.5 and 0.05 mg/ml dosage leads to the offspring defects consisting in blood biochemical indices altering, microstructure of gastrointestinal tract abnormalities, and general development inhibition. These results confirm the ability of GO to cause directly or indirectly offspring development abnormalities.

2.4. Single-walled carbon nanotubes

Studies of single-walled carbon nanotubes (SWCNT) biological effect on embryonic development of model species of *Danio rerio* (zebrafish) reveal significant embryonic development inhibition induced by more than 120 mg/l exposure. DWCNT exposure also leads to embryonic development inhibition, but at greater concentrations (more than 240 mg/l). The data obtained point to probable negative effects of SWCNT on flora and fauna when penetrated into the water environment [28].

SWCNT exposure of *Drosophila melanogaster* females to 0, 0.005%, 0.01%, 0.05%, 0.1%, 0.5% mass / volume concentrations over 14 days has no effect on reproductive system of females as well as on offspring's quantity and quality [29].

Investigation results of CNT embryotoxicity on mammals [30] prove the negative effect of non-functionalized and oxidized SWCNT from 100 ng/mouse to 30 µg/mouse dosage on mouse embryos development. The injurious effect on test objects of oxidized SWCNT is stronger than of non-functionalized ones, since the part of miscarriages and fetuses malfunctions within the former group was higher than that within the latter one. Similar results were obtained by authors on higher dosage investigation [31]. They introduced oxidized SWCNT at 3.3-1000 µg/kg dosage in pregnant females intravenously. When nanomaterial was administered at high concentrations the number of miscarriage was dramatically increased. Groups exposed to small dosage had apparent defects of embryonic development. There was an evidence of oxidative stress in placenta and embryo, but it didn't succeed in registering SWCNT there. These data are also confirmed by [29]. It was shown that oral SWCNT administration at 10 mg/kg dosage in pregnant females of CD-1 line during organogenesis significantly increased the number of fetuses resorption as well as led to morphological and skeletal anomalies development. In similar studies devoted to reproductive toxicity assessment when SWCNTs were introduced intraperitoneally at 1 and 10 mg/kg dosage in pregnant females the absence of toxic effect on maternal organism was observed. However, decrease in the number of offspring was detected in the group taking SWCNT at 10 mg/kg dosage. Such indices as lactation period, offspring viability and sex distribution didn't show any deviation. [32].

In [33] the study of ability to penetrate breast milk and distribution in offspring's tissue of tritium labeled shorted SWCNT-COOH was carried out on the model of lactating outbreeding rats. The material was introduced at 0.8 mg/kg dosage through one-time intravenous injection. As a result, authors establish SWCNTs penetration into rats breast milk which estimated peak concentration was approximately 0.12 µg/l. Quite uniform distribution of SWCNT in tissue and organs of pups including brain, kidneys, skeletal muscles etc. was detected. Maximal concentration was observed in blood, cardiac muscle and spleen, while the minimal one was in lungs.

As for toxic effect of SWCNT on male reproductive system, the result of mammalian spermatogonial chromosome aberration test also revealed the ability of SWCNT to increase the rate of teratosperm [34]. This fact indicates the toxicity of SWCNT for reproduction and development.

2.5. Multi-walled carbon nanotubes

Fluorescently labeled multi-walled carbon nanotubes (MWCNT) spread to the whole blastoderm after one-time injection in *Danio rerio* embryos. But in 96 hours after MWCNT introduction embryonic cells were completely cleaned from foreign substance by immune response generation. Their further development didn't have any anomalies, however, the next generation of *Danio rerio* from MWCNT exposed ones had less viability indices. It may be evidence to delayed negative MWCNT effect to the animals [35].

In order to assess the effect on female reproductive system of the mammals MWCNT were administered in rats through stomach pump at 40, 200, and 1000 mg/(kg·day). All animals had survived to the end of experiment. The results show that repeated MWCNT gavage at the studied dosage cause minimal toxic effect on female reproductive system. Thymus weight reduction was only detected in the group with high MWCNT dosage [36].

Assessment of MWCNT influence on mammal's embryonic development hasn't yielded unambiguous results yet. In [37, 38] MWCNT influence on impregnation successfulness was investigated, mouse females of the line C57Bl/6J were exposed. Nanomaterial was administrated intratracheally at the dosage of 67 µg per animal. As a result, inflammatory reactions in lung and liver of exposed females were detected, and insufficient delay of pregnancy time took place, but there was no effect on the offspring's quality. On the 125th day of the life of male offspring the analysis of their sperm genesis per day was performed. According to it, there was no deviation from control values. As authors think, some pregnancy period delay might be related to inflammatory processes in the

female's lung. Similar results are also obtained by intraperitoneal MWCNT introduction in mouse females of NMRI line at the dosage of 1 and 10 mg per mouse. Negative effect of MWCNT on female organism and pregnancy process was not detected, but there was certain rise in anxiety state among the offspring [39].

In order to assess the effect on the rat's embryos MWCNT were administered into stomach at the dosage of 40, 200, and 1000 mg/kg per day over 13 days, beginning with 6th day of pregnancy. No anomalies of embryonic development were detected. On the base of the results obtained authors made up decision that even high-dosage oral administration of MWCNT can be considered safe for embryo development [36].

However, the results [40] prove the ability of MWCNT to cross placental barrier and alter embryonic development being introduced at various pregnancy stages. Oxidized MWCNT with radioactive labels on the surface were introduced by intravenous injections in pregnant females of laboratory mice at 20 mg/kg dosage. The probability of spontaneous expulsion of a fetus and offspring development anomalies were assessed. In that work, MWCNT placenta crossing was established by means of transmission electron microscopy and paper chromatography. Significant increase of resorption number and external anomalies of embryos development was also mentioned. Similar results were also obtained by authors [41]. In their investigations, they introduced MWCNT intraperitoneally and intratracheally in pregnant mouse females of ICR line one time on the 9th day of pregnancy at 2, 3, 4, and 5 mg/kg. All fetuses were detached from womb on 18th pregnancy day to assess external and skeletal development anomalies. When MWCNT were introduced intraperitoneally sufficient development defects were observed at all concentrations. Intratracheal administration resulted in embryonic development anomalies at 4 and 5 mg/kg dosages only. At the same time, the higher is MWCNT dosage, the greater is the number of breed with fetuses having skeletal anomalies. It leaves MWCNT embryotoxicity question open-ended.

As for toxic effect of MWCNT on male reproductive system, there is little information about it now. Scientific team from China [42] studied MWCNT influence on male reproductive function. They have shown that repeated intravenous injections of water-soluble MWCNT in mice males can result in reversible testis damage without affecting fertility. According to obtained experimental data, MWCNT accumulated in testes of exposed laboratory mice of BALB/c line leading to oxidative stress and reducing gonadal epithelium thickness on 15th day, but all defects were regenerated on 60th and 90th day. Quantity, quality, and consistence of the sperm, as well as levels of three basic gonadal hormones have no significant damage over all the experiment. Fertility of the animals under investigation remained within the normal limits, pregnancy period and successfulness of the birth process in control and experimental groups had no difference. This work confirms the probability of crossing hemato-testicular barrier by MWCNT, that can't help causing concerns about offspring quality.

In [43] dose-dependent decrease in testosterone level and fertilizing capacity was revealed for orally administered MWCNT at 0.3, 3, and 30 mg/kg. At the same time, no anomalies of development of the offspring from exposed males were detected.

In vitro assessment of the influence of MWCNT at 10-200 µg/ml concentrations on sperm didn't reveal any cytotoxic effect on spermatozoa. Moreover, when nanotube concentration was not so high (10-40 µg/ml) spermatozoa activation was detected, the number of active cells during their viability period increased in some cases by more than 20% [44].

2.6. Fullerene

In [45-47] an assessment of fullerene influence on embryonic development of zebrafish was performed. It is shown that fullerenes induce embryonic cell necrosis and lead to the increase in embryonic mortality. Fullerene exposure also effect negatively on embryonic development of mammals that is confirmed by *in vitro* and *in vivo* investigations on laboratory mice [48].

In [49] the ability of fullerenes to ease the consequences of diabetes treatment due to their antioxidant properties has been reported. It is shown that daily oral fullerene administration in diabetic male rats of Vistar line over 5 weeks results in reduction of diabetic-induced oxidative stress and

diabetic treatment consequences such as testicular dysfunction and spermatogenesis disruption. The authors also noted the absence of toxic effect on male reproductive system of healthy animals.

3. Conclusion

So, carbon nanomaterials reveal toxic effect on reproductive system and offspring development of the animals of various system groups to a certain degree depending on carbon crystal structure. However, the reported results are insufficient to establish reliable nanomaterial's reproductive toxicity gradation based upon its carbon allotropic modification.

Currently, one can consider CNT and CB to be studied most carefully from the point of view of reproductive toxicity. There are experimental evidence to their ability to cross the hemato-testicular [42] and placental [19, 40] barriers, as well as breast milk penetration [33].

Graphene was first synthesized quite recently; therefore, their biological effect is studied to the least degree among modifications presented. Nevertheless, there are experimental evidences of graphene toxic effect on female reproductive system and offspring development.

In regard to fullerene and graphite nanoplatelets, the data obtained to the present time doesn't allow their reproductive and embryonic toxicity assessment.

Main toxicity mechanism of carbon nanomaterials as well as of many other types of nanoparticles is considered to be the oxidative stress [50-52]. Their effect, for example, in liver can be reduced to the minimum. However, testes don't have such protection mechanisms [53]. This is the cause of male reproductive system being more sensitive to external adverse impacts [54, 55] than female's one. Consequently, it is affected by anthropogenic environmental pollution much more often [56], as confirmed by most of carbon nanomaterial toxicity research. It should be noted that negative consequences for the offspring reveal itself if parent animals have been exposed to any allotropic modification of the carbon.

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