Risk analysis and solution of using graphene: Material, synthesis, and application (Mini review)

L Destiarti¹, I Kartini², Riyanto³, Roto² and Mudasir²*

¹Chemistry Department, Mathematics and Natural Sciences Faculty, Universitas Tanjungpura, Pontianak, Indonesia
²Chemistry Department, Mathematics and Natural Sciences Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia
³Chemistry Department, Mathematics and Natural Sciences Faculty, Universitas Islam Indonesia, Yogyakarta, Indonesia

*E-mail: mudasir@ugm.ac.id

Abstract. Graphene is a nanomaterial with unique physical and chemical properties. The two-dimensional hexagonal sp² structure in the honeycomb lattice has high thermal conductivity, high electricity, mechanical strength, and large surface area. The nano properties are significantly different from the bulk material. The review of the material, synthesis and application aspects of graphene gave rise to risk analysis in each field of study. Graphene material does not yet have adequate information regarding the risk of danger. Because graphene is nano-sized, this material can enter the human body through inhalation, ocular, cutaneous and oral. Graphene synthesis involves using chemicals that will produce hazardous products and reduce agents with high toxicity. The risk becomes more and more when the challenges of mass production of graphene are faced. Graphene can be applied as sensors, nanoelectronics, and biomedical applications. In this biomedical application, graphene has direct contact with humans and can increase reactive oxygen species in the body. The recommendation to overcome the risk is to use personal protective equipment and handle graphene material properly. The toxic materials involved in the synthesis step can be replaced with other environmentally friendly materials. Antidotes substances can reduce the toxicity of graphene materials so that the risks graphene in its application can be overcome.

1. Introduction
Nanomaterials are materials that have a size of 1-100 nm. Various types of nanomaterials are available with an extensive range of applications. This function is because the properties of nanomaterials are unique, including carbon-based nanomaterials. This material is highly preferred because it is easy to obtain and low cost to make composite materials. Carbon has allotropic forms, namely fullerene (0D), carbon nanotube (1D), graphene and its derivatives (2D), and graphite (3D). The use of nanomaterials is closely related to the development of science and technology.

One of the purposes of science and technology is to solve environmental problems and improve environmental quality. When the innovation is discovered, some other risks arise. The researchers have to understand the properties of materials themselves, find out environmental-friendly technology for synthesis, and make some health and environmental application innovations. Lately, graphene material
has been a fascinating material. Graphene is a 2D material having a hexagonal lattice structure with sp² hybridization where the carbon atoms are composed of a honeycomb lattice structure.

Graphene has high thermal conductivity (5000 W m⁻¹ K⁻¹), high electrical properties (6000 S/cm), Young's modulus of 1 TPa, the mechanical strength of 130 GPa, high optical transmittance (97.7%), and high theoretical area (2630 m² g⁻¹) [1]. The purchase of graphene material is equipped with a Material Safety Data Sheet (MSDS), which provides information on material properties, toxicity, hazards, and how to handle the waste. Since graphene is a carbon-based material, its level of threat is debatable. The information obtained from the MSDS is also still limited, depending on the supplier who provides the material.

There are two synthesis techniques of graphene, namely top-down and bottom-up. Top-down methods include mechanical exfoliation, arc discharge method, oxidative exfoliation and GO reduction (chemical reduction, heat reduction, electrochemical reduction, other reduction methods), liquid-phase exfoliation, and unzipping of CNT. Bottom-up processes include chemical vapour deposition, epitaxial growth, substrate-free gas-phase synthesis, template route, and total organic synthesis [2]. Based on the literature, the researchers tend to use the top-down technique. The Hummers-Marcano technique is popular, where this synthesis technique involves the use of large amounts of chemicals and highly toxic reducing agents.

Graphene can be applied to nanoelectronic devices, transparent conductive films, transistors, chemical and biochemical sensors, actuators, clean energy equipment, flexible optoelectronic devices, and data storage technologies [1]. In addition, Biological and Biomedical applications used graphene [3,4]. The potential risk factors will appear to the production process, and its application, the properties of nanotoxicity both in vitro and in vivo need to be studied.

Based on the material, synthesis, and application aspects described above, it is crucial to study the risk analysis of the use of these materials. The risks studied are from the perspective of endangering humans/living things and the environment. In addition, recommendations are given to overcome the dangers so that this material can be used safely.

2. Risk Analysis of Graphene

Graphene is a carbon-based nanomaterial. However, due to the nano-sized size, the properties change from the bulk form. The level of danger of this graphene material is still debated because carbon-based materials are often found in everyday life and are safe to use. The idea that graphene is not a risk is its low toxicity, production and use in small quantities, its similarity to harmless materials (carbon only). Several other researchers state that graphene is a risky material, namely under certain conditions or due to a lack of information related to risk [5].

Information obtained regarding the level of danger of graphene material through the MSDS is still not sufficient. The information in MSDS depends on the provider of the material itself [6,7]. Compared to the two documents, one has complete knowledge, and another is moderate. Because this graphene material is nano-sized, handling it also poses risks in terms of inhalation (inhalation), or exposure through the eyes (ocular) and skin (cutaneous), or even through the mouth (oral) [8].

Graphene is a stable and hydrophobic material. Awodele, et al. (2018) stated that graphene could cause environmental problems and health problems [9]. Graphene can bind to the cell surface and cause physical and chemical damage to the cell membrane. In addition, graphene can interact with proteins and nucleic acids, change their structure and function and produce ROS that can damage membranes, lipids, proteins, and nucleic acids.

3. Risk Analysis in Graphene Synthesis

In general, two graphene material synthesis techniques are available, namely top-down and bottom-up. More specifically, this paper discusses the top-down technique because this technique offers a variety of precursors and involves more straightforward equipment than the bottom-up technique. As mentioned in the introduction, the top-down method converts the precursor into graphene through exfoliation and
reduction techniques. Hummers technique, modification of Hummers and Marcano, and electrochemical exfoliation technique are often used to synthesize graphene.

The life cycle assessment methodology has been tested to determine the effect of the environment. In the GO and rGO synthesis techniques, the synthesis method chosen depends on its use as a bulk material or thin layer. The research results by Lujan, et al. (2019) show that there are no barriers to the industrial-scale production of graphene [10]. The cumulative energy requirements are cap values of 20.7 and 68.5 GJ/Kg, which are relatively high. However, this figure still shows a value that is relatively safe for the environment. The suggestion given by this publication is that the Hummers method is suitable for the production of graphene to produce bulk materials. In contrast, the Marcano method is ideal for thin layer electronic film applications [8].

Ikram, et al. (2020) stated that selecting an appropriate precursor is fundamental to the quality of graphene and the success of graphene products on a larger industrial scale. Graphene precursors can be grouped into five, namely conventional (graphite and graphite oxide), plant and animal derivatives (cheese, butter, lard), chemicals (synthetic polymers, silicon carbide, carbon dioxide, ethanol and other alcohols), fossil fuels (coal, methane, and LPG), and advanced starting materials (CNT and carbon ions) [2].

Graphene synthesis can also be done using biomass. Some of the biomass used [synthesis technique] are as follows: rice husk [microwave plasma irradiation], bagasse [oxidation in the muffled atmosphere], camphor leaf [hot pyrolysis], and waste paper [degradation-oxidation of carbon nanosphere]. Graphite is the most frequently used precursor for the synthesis of graphene. However, other precursors were used, and the results compared quality and yield [11]. This carbon precursor will be needed especially for the large-scale production of graphene. The precursors used in the synthesis of graphene can use industrial waste, such as plastic waste and batteries, newspapers, biomass, chitosan, coal, insect waste, and others.

In the synthesis of graphene, a large number of strong acids are used, such as sulfuric acid and citric acid. The reduction technique of the material uses the reducing agent: hydrazine and sodium borohydride. Both materials have a very high toxicity. The risk of the synthesis technique is higher. The type of synthesis technique chosen must also consider the scale of the amount of graphene to be produced. For industrial-scale graphene production, more attention is needed regarding the method selected because the level of danger for both humans and the environment is higher. A review of various graphene synthesis techniques has been published by Ikram et al., 2020 [2].

4. Risk Analysis in Application of Graphene

There is a limitation about the widespread use of graphene because the challenges in industrial-scale production are still limited. Even so, graphene has been used for electronic equipment, batteries, sensors, and others. The application of graphene in various equipment is in a reasonably stable form, namely composites and thin layers, and does not directly contact humans and or the environment [12].

The use of graphene-based materials is very prospective, especially from a biological context. Therefore, this requires a detailed discussion of the toxicity of this material. Graphene has been used for nanobiotechnology applications, such as environmental, biomedicine and biotechnology. Despite the widespread application of this material, graphene can pose a hazard to humans. Moreover, different forms of graphene (Few Layer Graphene, Graphene Oxide, reduced Graphene Oxide, graphene nanosheet) will have other physicochemical properties. It has different toxicological effects. It will lead to inaccuracies and trigger misunderstandings, so the expert cannot make a generalization [3].

In vivo studies of graphene materials are increasing recently. The in vivo study of graphene exposure on rats and the results observed inflammation in the lungs. Another study that is often carried out is in vivo tests with zebrafish objects. This zebrafish is an essential pre-clinical model due to its homologous resemblance to the human genome. In addition, zebrafish embryos are more sensitive to chemical changes [3].

The toxicity test of graphene in the environment is known by using a test on vegetable plants (cabbage, tomato, spinach, lettuce) that are exposed to graphene. The test results showed that graphene
had a negative effect related to the growth of roots and shoots. ROS levels also increased. The mechanism of toxicity that occurs is oxidative stress (3).

Related to the toxicity of graphene material in the environment, researchers began to look for ways to overcome it. Zhang, et al. (2018) demonstrated the role of humic compounds in reducing the ecotoxicity of graphene-based materials. This test was carried out in clean water overgrown with microalgae Scenedesmus obliquus [13]. The presumed mechanism of the humic role is:
1. Reducing the contact of graphene with algae by regulating the structure and surface negative charge.
2. Preventing physical penetration and damage by deposition of graphene on cells by interacting with humic acid.
3. Reacting as an antioxidant with intracellular ROS and extracellular hydroxyl radicals.

### Table 1. Comparison of neurotransmitters, antioxidant activity, and oxidative stress content in zebrafish brain tissues quantified using Enzyme-Linked Immunosorbent Assay (ELISA) after exposure of graphene. The data are displayed as the mean with SD. Statistical significancies were labelled as *p<0.05 and **p<0.01 (n=5) [14]

| Biomarkers | Control 0.1 ppm | 0.5 ppm | Unit |
|------------|-----------------|---------|------|
| 5-HT       | 27.03±7.697     | 34.39±5.905 | 0.1305 | 15.23±2.767** | 0.0232 | ng/total protein (mg) |
| Ach        | 3.147±0.874     | 3.622±0.831 | 0.4040 | 1.956±0.458*  | 0.0354 | ng/total protein (mg) |
| KISS       | 6.920±2.315     | 8.59±1.437 | 0.2138 | 5.001±0.805   | 0.1409 | ng/total protein (mg) |
| AChE       | 19.13±7.015     | 22.57±4.635 | 0.3910 | 20.85±5.443   | 0.6772 | ng/total protein (mg) |
| DA         | 13.58±4.321     | 12.3±1.369 | 0.5668 | 6.816±1.036*  | 0.0229 | pg/total protein (mg) |
| OT         | 14.19±4.802     | 20.43±3.686 | 0.0520 | 13.60±1.827   | 0.8100 | pg/total protein (mg) |
| Cortisol   | 217.7±52.48     | 217.9±36.46 | 0.9955 | 90.49±14.81** | 0.0043 | pg/total protein (mg) |
| ROS        | 75.17±16.97     | 101.6±18.34* | 0.0456 | 68.30±14.63   | 0.5127 | IU/total protein (mg) |

5-HT=serotonin. Ach=acetylcholin. KISS=kisspeptin. AChE=acetylcholinesterase. DA=dopamine. OT=oxytocin. ROS=reactive oxygen species

The results of the biomarker expression test after exposure to graphene material are shown in Table 1 [14]. The increasing concentration of graphene exposure resulted in decreased neurotransmitter levels. Significant changes occur in serotonin, acetylcholine, dopamine, and cortisol. The difference is shown by ROS levels which increased with increasing exposure to graphene, theoretically increasing ROS levels with increasing graphene exposure. Nonetheless, in general, the exposure to graphene exhibits decreased function of neurotransmitters based on investigations in zebrafish brain tissue.

### 5. Solution

Some of the recommendations given as a solution to the risks described above are as follows:

- In carrying out laboratory/larger-scale work involving the use of graphene nanomaterials, researchers are required to use complete Personal Protective Equipment (PPE) with adequate specifications, such as gloves, masks, goggles, and long-sleeved laboratory coats.
- Risk reduction by handling graphene properly, namely by taking the following steps [15]:
  - Limiting the number of nanomaterials or batch sample size reduces the risk of air contamination and skin hazard.
  - Place graphene in a closed container surrounded with the necessary protective material to prevent spillage.
  - When preparing the dispersion, include as much graphene as possible, especially when the material is sonicated.
  - Inform other colleagues who work in the exact location that there are risks that must be prevented in handling this material.
The safe-by-design strategy changes the biological activity by reducing toxicity, which will result in high effectiveness in the risk control hierarchy, which is very suitable for engineering nanomaterials.

- Use of environmentally friendly reducing agents: ascorbic acid [1], Oolong tea extract [16], green tea extract [17], coconut water [18], and other natural compounds. This extract is used to replace hydrazine as a reducing agent, which is highly toxic. There is also a pomegranate juice ingredient used not only as a reducing agent but also as a capping agent [19]. The use of tea polyphenols has been started since 2011 [20].
- In the synthesis process, it is better to replace NaNO₃ by increasing the amount of H₂SO₄. NaNO₃ can produce NO₂ and N₂O₄ gases which are hazardous chemicals [21]. To replace NaNO₃, H₂SO₄ can be used in the synthesis process with a specific ratio. Luo, et al. (2019) has used less acid in the graphene synthesis process [22]. This step is followed by the use of dry ice to control the reaction temperature so that the mixture remains stable and the risk of Mn₂O₇ explosion can be avoided.
- Industrial-scale production of graphene must pay attention to material precursors. It is possible to use industrial waste [11]. The use of industrial waste makes the price of raw materials cheaper and overcomes the problem of waste generated.
- In large-scale production, the recommended synthesis method is electrochemical exfoliation/electrolytic oxidation using water as a medium [23]. The efficiency of the synthesis technique can be conducted with the help of a microwave as an alternative. This will result in a reduction in the use of toxic and hazardous materials in the synthesis process.
- When applying graphene in various fields, it is essential to pay attention to the life cycle of graphene and the use of graphene in direct contact with humans. Provision of antidotes against graphene toxicity is an alternative that can be studied to reduce risks to environmental safety and human safety [13].

6. Conclusions
To sum up, it is essential to have a comprehensive understanding of graphene material. The study relates to the handling of materials and chemicals involved in the synthesis process and its application. In the synthesis process, the synthesis method uses environmentally friendly practices with good product quality and high yield, and also scaleability is the priority. These purposes lead to the direction of synthesizing methods according to this aspect. In addition, the use of graphene in humans can be used safely through preliminary tests and conducting a study of published research results.

Acknowledgement
The first author gratefully acknowledges the Indonesian Endowment Fund for Education (LPDP) for its financial support in the doctoral study.

References
[1] Tewatia K, Sharma A, Sharma M and Kumar A 2021 Synthesis of Graphene Oxide and Its Reduction by Green Reducing Agent Materials Today: Proceedings 44(6) 3933-3938
[2] Ikram R, Jan BM and Ahmad W 2020 An Overview of Industrial Scalable Production of Graphene Oxide and Analytical Approaches for Synthesis and Characterization Journal of Materials Research and Technology 9(5) 11587-11610
[3] Seabra AB, Paula AJ, Lima R, Alves OL and Duran N 2014 Nanotoxicity of Graphene and Graphene Oxide Chemical Research in Toxicology 159-168
[4] Gurunathan S and Kim JH 2016 Synthesis, Toxicity, Biocompatibility, and Biomedical Applications of Graphene and Graphene-Related Materials International Journal of Nanomedicine 11 1927-1945
[5] Arvidsson R, Boholm M, Johansson M and Montoya ML 2018 “Just Carbon”: Ideas about Graphene Risks by Graphene Researchers and Innovation Advisors Nanoethics 12 199-210
[6] Material Safety Data Sheet (MSDS) for CamGraph@Graphene Powder, Cambridge Nanosystems.

[7] Safety Data Sheet PureGraph™ Graphene Powder, Australia-WHS

[8] Pelin M, Sosa S, Prato M and Tubaro A 2018 Occupational Exposure to Graphene Based Nanomaterials: Risk assessment Nanoscale 10 15894-15903

[9] Awodele MK, Adedokun O, Bello IT and Akinrinola O 2018 Graphene and Its Health Effect, International Journal of Nanotechnology and Nanomedicine 3(2) 1-5

[10] Lujan LS, Roman SV, Toledo C, Parejo OS, Mansour AE, Abad J, Amassian A, Benito AM, Maser, WK and Urbina A 2019 Environmental Impact of the Production of Graphene Oxide and Reduced Graphene Oxide Springer Nature Switzerland 1(179)

[11] Ikram R, Jan BM and Ahmad W 2020 Advances in Synthesis of Graphene Derivatives Using Industrial Wastes Precursors; Prospects and Challenges Journal of Materials Research and Technology 9(6), 15924-15951

[12] Park MVDZ, Bleeker EAJ, Brand W, Cassee FR, Elk M, Gosens I, Jong WHD, Meesters JAJ, Peijnenburg WJGM, Quik JTK, Vandebriel RJ and Sips AJAM 2017 Considerations for Safe Innovation: The Case of Graphene ACS Nano 11 9574-9593

[13] Zhang Y, Meng T, Guo X, Yang R, Si X and Zhou, J 2018 Humic Acid Alleviates the Ecotoxicity of Graphene-Family Materials on the Freshwater Microalgae Scedesmus obliquus Chemosphere 197: 749-758

[14] Audira G, Lee JS, Siregar P, Malhotra N, Rolden MJM, Huang JC, Hsu HS, Hsu Y, Ger TR and Hsiao CD 2021 Comparison of the Chronic Toxicities of Graphene and Graphene Oxide toward Adult Zebrafish by Using Biochemical and Phenomic Approaches Environmental Pollution 278 1-14

[15] Mohan VB 2019 Handling and Risk Mitigation of Nanoscale Graphene and Related Materials: Some considerations and recommendations Journal of Carbon Research 5(36) 1-9

[16] Cheong MF, Liu WW, Khe CS, Hidayah NMS, Lee HC, Teoh YP, Foo, Voon CH, Zaaba N, Adelyn PYP 2018 Green Synthesis of Reduced Graphene Oxide Decorated with Iron Oxide Nanoparticles Using Oolong Tea Extract AIP Conf. Proc. 2045 020031-1-020031-8

[17] Tai MJY, Liu WW, Khe CS, Hidayah NMS, Teoh YP, Voon CH, Lee HC, Adelyn PYP 2018 Green Synthesis of Reduced Graphene Oxide Using Green Tea Extract AIP Conf. Proc. 2045, 020032-1-020032-7

[18] Kartick B, Srivastava SK and Srivastava I 2013 Green Synthesis of Graphene Journal of Nanoscience and Nanotechnology 13 4320-4324

[19] Tavakoli F, Niasari MS, Badiei A and Mohandes F 2015 Green Synthesis and Characterization of Graphene Nanosheets Materials Research Bulletin 63 51-57

[20] Wang Y, Shi ZX and Yin J 2011 Facile Synthesis of Soluble Graphene via a Green Reduction of Graphene Oxide in Tea Solution and Its Biocomposites Applied Materials & Interfaces 3 1127-1133

[21] Andrijanto E, Shoelarta S, Subiyanto G and Rifki, S 2015 Facile Synthesis of Graphene from Graphite Using Ascorbic Acid as Reducing Agent AIP Conf. Proc. 1725 020003-1-020003-4

[22] Luo D, Zhang F, Ren Z, Ren W, Yu L, Jiang L, Ren B, Wang L, Wang Z, Yu Y, Zhang Q and Ren Z 2019 An Improved Method to Synthesize Nanoscale Graphene Oxide Using Much Less Acid Materials Today Physics 9 1-6

[23] Pei S, Wei Q, Huang K, Cheng HM and Ren W 2018 Green Synthesis of Graphene Oxide by Seconds Timescale Water Electrolytic Oxidation Nature Communications 9(145) 1-9