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氧化石墨烯抗菌性及生物安全性研究进展

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摘要: 氧化石墨烯是一种表面有丰富含氧官能团的石墨烯衍生物,具有与石墨烯相似的二维蜂窝状晶格结构,从而导致了其具有电学、光学、力学特性和良好的生物相容性,被广泛应用于材料学、生物医学和药物传递等诸多领域。因氧化石墨烯日益增多的生产和使用,其在空气、水和土壤中大量积累,引发了人们对其生物安全性的高度关注。以微生物、陆生动植物和水生动植物为分类标准,综述了近几年氧化石墨烯对微生物、动物和植物的毒性影响,总结并分析了三者的毒性机理,比较了不同生存环境下其对动植物毒性影响的不同,旨在更加全面地揭示氧化石墨烯的生物安全性,为氧化石墨烯安全使用剂量和其功能化应用提供一定的参考。

关键词: 氧化石墨烯;抗菌性;生物安全性
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Progress on Antibacterial and Biosafety Research of Graphene Oxide

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Abstract: Graphene oxide is a graphene derivative with abundant oxygen-containing functional groups on its surface. It has a two-dimensional honeycomb lattice structure similar to that of graphene, which leads to its electrical, optical, and mechanical properties and a good biocompatibility. Graphene oxide has been widely used in many fields, such as materials science, biomedicine, and drug delivery. Due to the increasing production and usage of graphene oxide products, this chemical is substantially accumulated in air, water, and soil, which undoubtedly increases the possibility of direct human contact. Therefore, the biosafety of graphene oxide has been brought to the public's

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attention. By using microorganisms, terrestrial organisms, and aquatic organisms as three main categories, this paper reviewed recent studies about the toxic effects of graphene oxide on those organisms and summarized the mechanisms of the respective toxicities. Further, we compared the effects of different living environments on the toxicity to plants and animals. This paper, hopefully, can reveal the biosafety of graphene oxide more comprehensively and provide a reference to the safe dosage and functional application of graphene oxide.

Keywords: graphene oxide; antibacterial; biological safety

氧化石墨烯(graphene oxide, GO)是单层碳原子以 sp^2 杂化形式存在的二维蜂窝状晶格结构[1],是石墨烯的衍生物,故具备了石墨烯易导热、强机械特性等卓越的性能;同时因其表面含有丰富的含氧官能团,如羟基、羧基和环氧基等,使得 GO 具有了大比表面积且表面易修饰的优良特性,也使得其在水和大多数极性有机溶剂中具有良好的分散稳定性、亲水性和生物相容性[2]。由于 GO 的诸多优良特性,目前已被批量生产并广泛应用于光伏电池、抗菌和生物医学等方面[3-5],尤其在生物医学方面,GO 已在成像、癌症治疗和药物输送、疫苗增强剂等领域成为研究热点[6]。但大规模的使用使得 GO 不可避免地流入空气、水或土壤等环境介质和生物体内,不仅生态安全受到了影响,人类健康也受到了威胁,引发了公众对 GO 生物安全性问题的高度关注。本文综述了近几年 GO 的抗菌性和生物安全性的研究进展,旨在更全面地揭示其生物安全性,为 GO 有效安全的使用作出指导。

1 GO 的抗菌性研究(Antibacterial research of GO)

1.1 GO 的抗菌性

近年来,大量研究表明,GO 对细菌、真菌和病毒均具有较强的抑菌效果,如表 1 所示。

由表 1 可知,GO 对 3 种微生物的毒性影响来看,其对细菌的毒性最大,且均具有浓度依赖性。

但在某些情况下,GO 对菌种的生长却显示出良好的促进作用。Ruiz 等[11]首次于 2011 年证明,GO 可作为细菌黏附的骨架,促进其生长、增殖和形成细菌生物膜。Varela 等[12]将 GO 纳米颗粒给予流感嗜血杆菌后,GO 诱导了菌落形成单位(CFU)的增加。Hui 等[13]发现,200 $\mu g \cdot mL^{-1}$ 的 GO 在 LB 培养基中与大肠埃希菌作用 3 h 后,细菌存活率可达 97.95%。实验证实,GO 可以通过非共价吸附 LB 培养基中的蛋白、牛血清蛋白和色氨酸等成分,这些蛋白物质覆盖 GO 后导致其丧失抗菌能力。

1.2 GO 的抗菌机制

GO 的抗菌机制主要体现在 5 个方面。(1)GO 片层上的大量含氧基团能与组成细胞壁的糖类或者蛋白质形成氢键,将细胞包裹起来使胞内外物质交换受阻,从而使细胞缺乏营养物质而死亡。(2)GO 锐利的边缘能够破坏细胞膜,大量胞质流出而产生空腔结构,最终能破坏细菌或真菌结构和功能的完整性导致细菌死亡[14]。(3)GO 可以通过非活性氧机制诱发氧化压力,导致细胞物质被氧化,破坏细胞内部组成致细胞死亡[15-16]。(4)氧化应激。高浓度能完全包裹菌体,主要依赖于隔绝营养物质来发挥抗菌性,但低浓度下,GO 不能完全包裹菌体,所以主要靠氧化应激对壁和膜的破坏来抗菌[6]。(5)高浓度(>3 000 $mg \cdot L^{-1}$)GO 还可能通过改变细菌细胞的离子通道而使离子溶出细胞体外,从而导致细菌细胞因生理活性丧失而死亡[17]。

相比于对 GO 抗菌机理的研究,人们对其抗病毒机理的研究较少,有学者认为 GO 的抗病毒机理主要依赖于其特有的单层结构及其所带的负电荷,带负电荷的 GO 在病毒进入细胞前与病毒静电结合,其单层片状结构所致的锋利边缘导致了病毒结构的破坏[10]。

此外,GO 的抗菌、抗病毒性能还与其尺寸有关。大尺寸 GO 更容易覆盖在细菌细胞表面,从而限制细胞膜上的生命活动,比横向尺寸小的 GO 抑菌效果好[18]。

2 GO 的陆生生物毒性研究(Terrestrial biological toxicity of GO)

2.1 GO 的陆生植物毒性效应研究

2.1.1 GO 对陆生植物的毒性

GO 的排放会对环境中的植物产生一定影响。有研究显示,土壤会阻隔 GO 的运输,所以低浓度的 GO 对植物毒性是不明显的,但在一些有阳离子存在的土壤中,GO 因吸附作用会与之产生联合毒性,从而影响植物的正常生长,而高浓度的 GO 会大量

表 1　氧化石墨烯(GO)对细菌、真菌和病毒的毒性

Table 1　Toxicity of graphene oxide (GO) to bacteria, fungi, and viruses

分类 Classification	受试生物 Subject organism	暴露剂量 Exposure dose	毒性效应 Toxic effects	参考文献 Reference
细菌 Bacteria	大肠杆菌(*E. coli*) (G^-菌 G^- bacteria) 绿脓杆菌(*P. aeruginosa*) (G^-菌 G^- bacteria) 金黄色葡萄球菌 (*Staphyloccocus aureus*) (G^+菌 G^+ bacteria)	≤100 μg·mL^{-1}	当浓度≤100 μg·mL^{-1}时,GO 对 G^-菌的抗菌活性高于 G^+菌;而当浓度达到200 μg·mL^{-1}时,GO 对 G^-菌和 G^+菌均表现出较高的抗菌活性 When the concentration of GO was less than 100 μg·mL^{-1}, the antimicrobial activity of GO against G^- bacteria was higher than G^+ bacteria, and when the concentration reached 200 μg·mL^{-1}, GO showed higher antimicrobial activity against G^- bacteria and G^+ bacteria.	[4]
	恶臭假单胞菌 (*Pseudomonas putida* (Trevisan) Migula) (G^-菌 G^- bacteria)	50 μg·mL^{-1}	随 GO 浓度的增加,其对恶臭假单胞菌生长的抑制作用增加,且 GO 对恶臭假单胞菌的主要影响是膜完整性的丧失 The inhibition of GO on the growth of *Pseudomonas putida* increased with the increase of GO concentration, and the main effect of GO on *Pseudomonas putida* was the loss of membrane integrity.	[7]
真菌 Fungus	巴斯德毕赤酵母 (*Pichia pastoris*)	1 000 mg·L^{-1}	GO 对真菌的毒性具有剂量依赖性,当 GO 浓度高于 1 000 mg·L^{-1}时,对巴斯德毕赤酵母的毒性主要是膜损伤和氧化损伤 The toxicity of GO to fungi was dose-dependent. When the concentration of GO was higher than 1 000 mg·L^{-1}, the main toxicity to *Pichia pastoris* was membrane damage and oxidative damage.	[8]
	酿酒酵母 (*Saccharomyces cerevisiae*)	0～600 μg·mL^{-1}	细胞增殖受到显著抑制,少数细胞变形并收缩,半数效应浓度(LC_{50})值为 352.704 mg·L^{-1} Cell proliferation was significantly inhibited, and a few cells deformed and contracted. The median lethal concentration (LC_{50}) value was 352.704 mg·L^{-1}.	[9]
病毒 Viruses	猪流行性腹泻病毒 (PEDV) Porcine epidemic diarrhea virus (PEDV)	200 μg·mL^{-1}	GO 能够抑制病毒增殖 Virus proliferation can be inhibited by GO.	[10]

积累在植物根部,少量会进入植物体内的其他部位,最终影响植物的干重、含水量和叶绿体等的指标,如表 2 所示。

2.1.2　GO 对陆生植物的毒性机制

GO 对植物的毒性机制总结为以下 6 个方面:(1)氧化应激;(2)通过调节植物激素对植物产生毒性;(3)GO 和植物细胞之间的相互作用,引起与关键生物过程相关物质代谢的紊乱;(4)在植物根部大量富集,阻碍了植物对营养物质的吸收与利用[19];(5)高浓度 GO 的富集可能会使环境中的渗透压增大,从而降低根皮质细胞的直径并导致细胞收缩和变形;(6)GO 抑制了碳水化合物(例如吡喃葡萄糖和麦芽糖)和氨基酸(例如缬氨酸和脯氨酸)代谢,使得不饱和脂肪酸和饱和脂肪酸的比例增加[25]。

2.2　GO 的陆生动物毒性效应研究

2.2.1　GO 对陆生动物的毒性

GO 一旦进入动物体内会随着血液流通到身体的各个部位,不同部位对 GO 的敏感程度是不同的,

表2 GO对陆生植物的毒性

Table 2 Toxicity of GO to terrestrial plants

受试生物 Subject organism	暴露剂量 Exposure dose	毒性效应 Toxic effects	参考文献 Reference
拟南芥 (*Arabidopsis thaliana*)	10～1 000 g·L^{-1}	表观上显示GO对拟南芥的生长无影响,但很容易被拟南芥的根毛吸收,沉积在根部的薄壁细胞中,不易运转到茎叶等其他部位;受到独特环境的胁迫时会影响植物的生长 Apparently, GO had no effect on the growth of *Arabidopsis thaliana*, but it was easily absorbed by the root hairs of *Arabidopsis thaliana*, deposited in parenchyma cells of the root, and not easily transported to other parts such as stems and leaves. The growth of plants is affected by GO under the stress of unique environment.	[19]
小麦 (*Triticum aestivum*)	1.0 g·L^{-1}	GO主要积累在根中,并会缓慢从根部排除 GO accumulates mainly in roots and is slowly removed from roots.	[20-21]
裸燕麦 (*Arrhenatherum elatius*)	0～2.0 g·L^{-1}	水培条件下,GO主要积累在裸燕麦的根部,但土培条件下,土壤的阻隔作用会减少了GO与根之间的接触,使毒性大大减小 Under hydroponic conditions, GO mainly accumulated in the roots of *Arrhenatherum elatius*, but under soil culture conditions, the barrier effect of soil would reduce the contact between GO and roots, and the toxicity would be greatly reduced.	[22]
甘蓝 (*Brassica oleracea*)、西红柿 (*Lycopersicon esculentum*)	500～2 000 mg·L^{-1}	植物叶片数量和大小以剂量依赖的方式减少,细胞坏死性病变的可见症状和细胞死亡呈浓度依赖性增加 The number and size of plant leaves decreased in a dose-dependent manner, the visible symptoms of cell necrotic lesions and cell death increased in a concentration-dependent manner.	[23]
水稻 (*Oryza sativa*)	50 mg·kg^{-1}	对水稻的苗高和根长有不利影响,水稻根系中抗氧化酶包括超氧化物歧化酶(SOD)和过氧化物酶(POD)活性也显著增加,且根部的激素也明显增加 It has an adverse effect on the seedling height and root length of *Oryza sativa*, the activities of superoxide dismutase (SOD) and peroxidase (POD) in *Oryza sativa* roots were also significantly increased, and the hormones in roots were also significantly increased.	[24]

如表3所示,所以近几年人们通过对动物的呼吸、生殖、感官和循环等各大系统的大量研究来评估GO的动物毒性及其毒性机理。

2.2.2 GO对陆生动物的毒性机制

GO对动物的毒性机制可以从以下2个方面来阐明。从细胞水平上来看,GO对动物的毒性机制总结为以下3点。(1)质膜损伤:GO可以从双层质膜中抽取磷脂分子造成膜损伤,导致孔形成和水分子流入膜中[39];(2)氧化应激:这2点在GO诱导的动物毒性中起到了关键性作用[40];(3)GO的疏水区域和膜的脂质尾部之间的强分散相互作用[41]。从分子水平上,GO的动物毒性机制可能跟其与蛋白质、RNA等生物大分子相互作用有关。具体的毒性机制总结为以下5点。(1)与细胞膜上的蛋白受体直接作用,封闭蛋白活性位点,影响细胞正常的信号通路,抑制蛋白功能的正常发挥[35];(2)GO暴露通过调节某些基因在遍在蛋白连接酶复合体(APC)中的表达、调节某些蛋白的正常表达来破坏主要和次要靶器官的功能[42];(3)形成GO-溶菌酶复合物,破坏溶菌酶蛋白的二级结构[43];(4)干扰酶基因的转录过程;(5)抑制氨基酸代谢和不饱和脂肪酸与饱和脂肪酸的比例。

此外,石墨烯基材料的大小、形状、表面性质、化学性质,浓度、聚集、剂量和制备也是可能导致毒性的生物学活性的决定因素。比如,较大尺寸的GO比小尺寸的GO毒性更大[44];GO纳米带(GONRS; 310 nm×5 000 nm)比GO纳米片(GONPS; 100 nm×100 nm)的毒性更强[45]。

表 3　GO 对陆生动物的毒性

Table 3　Toxicity of GO to terrestrial animals

分类 Classification	受试生物 Subject organism	暴露剂量 Exposure dose	毒性效应 Toxic effects	参考文献 Reference
呼吸系统 Respiratory system	小鼠肺部(*Mus musculus* liver)、人肺癌上皮细胞(Human lung adenocarcinoma epithelial cells)	10 $g \cdot L^{-1}$	GO 可引发肺功能变化,且会出现“低促高抑”的现象 GO can cause changes in lung function, and there will be a phenomenon of “low promotion, high inhibition”	[26-27]
生殖系统 Reproductive system	小鼠胚胎成纤维细胞(Mouse embryonic fibroblasts)、哺乳期雌鼠(Female mice during lactation)、体外精子(Sperm *in vitro*)	0.5 $g \cdot L^{-1}$	高浓度 GO 对雌性动物的生殖系统具有显著毒性,并会通过母体传给子代,且氧化程度越低,毒性越大;对于雄性生殖系统,有研究者认为几乎没有影响;但也有研究显示对精子有低促高抑的作用 High GO concentration has significant toxicity to the reproductive system of female animals, and will pass through the mother to the offspring. The lower the degree of oxidation, the greater the toxicity. For the male reproductive system, some researchers believe that it has little effect, but some studies show that it has a low concentration promoting and high concentration suppressing effect on sperm.	[28-31]
感官系统 Sensory system	模拟皮肤(Skin simulation)、角膜上皮细胞(Cornea epithelial cells, CECs)	0.09 $g \cdot L^{-1}$	GO 对皮肤的毒性具有时间依赖性;会引起角膜上皮细胞形态变化、甚至细胞凋亡 The toxicity of GO to skin is time-dependent; it can cause morphological changes and even apoptosis of corneal epithelial cells.	[32-33]
循环系统 Circulatory system	心肌细胞(Cardiomyocyte)、原代人外周血 T 淋巴细胞(Primary human peripheral blood T lymphocyte)	(652.1±1.2) $mg \cdot L^{-1}$	心肌细胞活力降低;浓度低于 25 $\mu g \cdot mL^{-1}$ 时,GO 对 T 淋巴细胞具有良好的生物相容性 The viability of cardiac myocytes was decreased, and GO had good biocompatibility with T lymphocyte when the concentration of GO was lower than 25 $\mu g \cdot mL^{-1}$.	[34-35]
消化系统 Digestive system	蟋蟀(*Chinensis gryllulus*)	200 $mg \cdot kg^{-1}$	蟋蟀的肠细胞中有大量的退行性改变 There are many degenerative changes in intestinal cells of *Chinensis gryllulus*.	[36]
	秀丽隐杆线虫(*Caenorhabditis elegans*)	100 $mg \cdot L^{-1}$	GO 暴露可以通过抑制 DAF-16 在胰岛素信号通路中的表达对线虫的寿命、运动行为和肠功能产生毒性作用 GO exposure can inhibit the expression of DAF-16 in insulin signaling pathway, which may have toxic effects on the longevity, motor behavior and intestinal function of *Caenorhabditis elegans*.	[37]
神经系统 Nervous system	大鼠(*Rattus norvegicus*)	100 $\mu g \cdot mL^{-1}$	还原氧化石墨烯(rGO)会影响大鼠星形胶质细胞和大脑内皮细胞的细胞活性 Reduced graphene oxide (rGO) can affect the activity of *Rattus norvegicus* astrocytes and cerebral endothelial cells.	[38]

低浓度GO对细胞活性的促进作用机制尚不明确,可能与GO材料本身具有丰富的含氧官能团,水溶性好,吸附性强有关,还需在以后的研究中进一步证明。

3 GO的水生生物毒性研究(Aquatic biological toxicity of GO)

3.1 GO的水生植物毒性效应研究

3.1.1 GO对水生植物的毒性

GO的大量使用与生产,导致其在制造、运输、使用和处置过程中被释放到土壤中影响陆生生物的同时,也不可避免地释放到水中,对水生生物造成重大不利影响,甚至影响到水生生态系统,将近期典型研究成果总结于表4中。

3.1.2 GO对水生植物的毒性机制

GO对水生植物的毒性机制如下。(1)氧化应激,在这一点上与陆生植物是相似的。(2)GO不会改变水生植物的鲜重,但会严重抑制干物质的增加,导致更高的持水率,还会抑制叶绿素的含量,上调叶绿素a/b的比值,从而影响光合作用。(3)GO还会对水生植物的微观结构和超微结构造成干扰,诱导细胞壁和细胞膜的分离以及叶片表面微孔的形成[49]。(4)GO能够显著下调光系统Ⅱ的活性;作为半透明涂层附着于藻细胞表面,造成细胞壁和膜完整性的损伤,从而抑制了藻类的生长和光合作用[50]。(5)GO大量富集在水生植物根部,逐渐积累形成一层覆盖物,阻碍根对营养物质的吸收利用[48],不过这一机制尚需深入研究。(6)GO可以吸附藻类中的大量营养素

表4 GO对水生植物的毒性

Table 4 Toxicity of GO to aquatic plants

受试生物 Subject organism	暴露剂量 Exposure dose	毒性效应 Toxic effects	参考文献 Reference
盐生杜氏藻 (*Dunaliella salina*)	72 h-EC_{50}: 13.04 mg·L^{-1}	当GO浓度达100 mg·L^{-1}时,超出藻细胞的自我调节能力,光合色素分子受损或合成代谢受阻,导致含量降低;高、低浓度GO暴露均能显著增加藻细胞蛋白质和总脂含量 When GO concentration reached 100 mg·L^{-1}, exceeding the self-regulating ability of algae cells, photosynthetic pigment molecular damage or anabolism is blocked, resulting in reduced content. High and low concentration GO exposure could significantly increase the protein and total lipid content of algae cells.	[46]
海水微绿球藻 (*Nannochloris oculata*)	72 h-EC_{50}: 79.10 mg·L^{-1}		
斜生栅藻 (*Scenedesmus obliquus*)	72 h-EC_{50}: 25.63 mg·L^{-1}		
淡水微绿球藻 (*Nannochloris oculata*)	72 h-EC_{50}: 48.44 mg·L^{-1}		
薄尾藻 (*P. tenuifolia*)	96 h-EC_{50}: 3.76 mg·L^{-1}	抑制薄尾藻的生长,提高丙二醛(MDA)含量和抗氧化酶活性;并会抑制原生动物的光利用 Inhibiting the growth of *P. tenuifolia*, increasing the content of malondialdehyde (MDA) and the activity of antioxidant enzymes. It also inhibits the light use of protozoa.	[47]
浮萍 (*Lemna minor*)	10 mg·L^{-1}	对浮萍叶片数和湿重的抑制率分别约为35.3%和26.1%,显著抑制了浮萍的生长(P<0.05);低浓度的GO对浮萍抗氧化酶却无明显影响 The inhibition rates of leaf number and wet weight of duckweed were about 35.3% and 26.1% respectively, which significantly inhibited the growth of *Lemna minor* (P<0.05); low concentration of GO had no significant effect on the antioxidant enzymes of *Lemna minor*.	[48]
白苔藓 (white moss *Leucobryum glaucum*)	0.04~4 mg·mL^{-1}	GO可影响白苔藓光合作用,严重抑制干重增长;高浓度的GO可导致白苔藓细胞壁的破坏,使叶片表面微孔形成 GO can affect the photosynthesis of white moss *Leucobryum glaucum* and seriously inhibit the growth of dry weight. High GO concentration can cause cell wall damage and micropore formation on the leaf surface of white moss *Leucobryum glaucum*.	[49]

注:EC_{50}表示半数效应浓度。

Note: EC_{50} is concentration for 50% of maximal effect.

(N、P、Mg 和 Ca),从而导致营养物质耗竭[51]。另外,和陆生植物相似,GO 对水生植物也会出现“低促高抑”现象,可能是由于低浓度的 GO 暴露能显著增加藻细胞蛋白质和总脂含量;还能刺激藻细胞内碳水化合物的合成;促进光合色素产生,使光合作用增强[46]。

3.2　GO 的水生动物毒性效应研究

3.2.1　GO 对水生动物的毒性

为了更全面了解 GO 对水体生态系统的潜在危害,近几年也有研究者报道了 GO 对水生动物的毒性,结果总结于表 5 中。

3.2.2　GO 对水生动物的毒性机制

GO 对水生动物的毒性机制与其对陆生动物的毒性机制是大体相同的,GO 纳米片引起质膜的内陷和穿孔,并在血细胞的胞质溶胶和内溶酶体囊泡中发现 GO,从而造成膜损伤,其毒性是活性氧族(ROS)介导的氧化损伤[59]。但由于二者在身体构造上的细微差异与所处环境的不同,导致 GO 对水生动物的毒性机制还可能是消化道堵塞,也可能是水

表 5　GO 对水生动物的毒性

Table 5　Toxicity of GO to aquatic animals

受试生物 Subject organism	暴露剂量 Exposure dose	毒性效应 Toxic effects	参考文献 Reference
大型溞 (*Daphnia magna*)	1～84.2 mg·L^{-1}	GO 对大型溞 48 h 急性毒性的 LC_{50} 为 84.2 mg·L^{-1},当 GO 浓度达到 1 mg·L^{-1} 时能够显著推迟母溞的头胎出生时间,抑制母溞头胎幼溞数、单胎最高产溞数和总产溞数 The acute toxicity of GO to *Daphnia magna* for 48 hours was 84.2 mg·L^{-1}. When GO concentration reached 1 mg·L^{-1}, the first birth time of *Daphnia magna* was significantly delayed, and the number of babies, the highest number of babies per single fetus and the total number of babies were inhibited.	[52]
淡水枝角类网纹水蚤 (*Ceriodaphnia dubia*)	1.25 mg·L^{-1}	GO 暴露会降低水蚤的摄食率,慢性暴露会导致新生蚤数量显著减少;非致死性 GO 浓度会导致生物体中促红细胞生成量的增加 GO exposure can reduce the feeding rate of water fleas, and chronic exposure can lead to a significant reduction in the number of newborns. Non-lethal GO concentration can increase erythropoiesis in organisms.	[53]
卤虾 (Halogenated shrimp)	48h-EC_{50}: 0.16 mg·mL^{-1} 48 h-LC_{50}: 0.65 mg·mL^{-1}	GO 被卤虾摄取并集中在肠道中,并附着在其体表,导致其游动受到浓度依赖性的抑制,还有一部分原因是 GO 造成了卤虾酶活性的改变 GO is ingested by halogenated shrimp and concentrated in the intestines, and adheres to its body surface, causing its swimming to be concentration-dependently inhibited. In addition, GO causes changes in the enzymatic activity of halogenated shrimp.	[54]
丰年虾 (Prawn)	400、600 mg·L^{-1}	包囊和解囊囊肿的孵化率显著降低(P<0.01);幼虫的个体干重也随剂量的增加而降低;GO 附着在孢囊和幼虫表面,对幼虫体表面造成不可逆的损伤 The hatching rate of encapsulation and decapsulation cysts decreased significantly (P< 0.01); the dry weight of larvae decreased with the increase of dosage; GO attached to the surface of cysts and larvae caused irreversible damage to the surface of larvae.	[55]
斑马鱼 (*Danio rerio*)	1～100 μg·L^{-1}	未观察到明显的急性毒性;然而,通过肝脏和肠道的组织分析,检测到许多细胞有空泡形成,细胞排列松散,组织分解和细胞边界崩解 No obvious acute toxicity was observed. However, tissue analysis of the liver and intestine revealed that many cells had vacuole formation, loose cell arrangement, tissue decomposition and disintegration of cell boundaries.	[56-57]
大型水蚤 (*Daphnia magna*)	72 h-EC_{50}: 44.3 mg·L^{-1} 72 h-LC_{50}: 45.4 mg·L^{-1}	水蚤具有较高的亲水性,水蚤对 GO 的吸收/释放速率比对其他碳纳米材料更快,更容易净化 GO,这可能有助于解释 GO 对水蚤的非重度毒性 *Daphnia magna* has high hydrophilicity, and its uptake/release rate of GO is faster than that of other carbon nanomaterials. It is easier to purify GO, which may help to explain the non-severe toxicity of GO to *Daphnia magna*.	[57-58]

生动物吸收 GO 以后产生的分泌物(GOBS)造成的影响[60],有研究显示,因为分泌物独特的纳米板形貌,厚度约为 10 nm,横向长度为 19.5 ~ 282 nm,尺寸比 GO 纳米流体(GONS)小,表现出比 GONS 更多的负表面电荷和更低的聚集状态,使斑马鱼 β-半乳糖苷酶上调和斑马鱼胚胎线粒体膜电位损失,导致斑马鱼畸形甚至死亡。

4 总结与展望(Summary and prospect)

综上所述,GO 能够有效抑制细菌、真菌和病毒,并且 GO 的抑菌性和潜在毒性均与石墨烯基材料的大小、形状、聚集、剂量、浓度、制备、表面性质和化学性质等密切相关,且释放到空气中的 GO 大多数情况下并不是单一发挥毒性作用,而是与环境中的金属、金属离子等物质相互结合,从而产生更大的毒性,对环境的影响与人类的潜在危害是不容忽视的,但已有研究的一些结论是相互矛盾的,仍需要进一步的研究。且如何在极大地发挥 GO 应用潜力的同时,又能够减少其对人体健康的毒害,成为摆在研究者面前的首要问题。有研究者将 GO 功能化,将一些基团(羧基、聚乙二醇(PEG)、氨基化聚乙二醇(PEG-NH_2)和丝素等)交联到 GO 上,产生的功能化 GO 毒性减小,具有良好的生物相容性[61-63]。因此,将 GO 功能化有望成为大剂量并安全使用 GO 的一种新方法。虽然大多数研究表明,不论是对动物还是对植物,GO 的主要毒理性机制都是氧化应激,但是还有一些其他的毒性机制有待深入的研究和进一步的确定。本综述将为后续 GO 的毒理性机制研究提供参考,进而找到相应的控制和解决办法,为 GO 的临床应用提供安全性评价资料,并在此基础上建立全面的、切实可行的 GO 使用安全评价标准。

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