

## Review Article

# Applications of *Daphnia Magna* in Ecotoxicological Studies: A Review

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## I N F O

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## A B S T R A C T

*Daphnia magna*, a small freshwater crustacean, has emerged as a crucial model organism in toxicological research due to its sensitivity to various environmental stressors and its ecological relevance. Its biological characteristics, such as short life cycles, rapid reproduction, and transparent exoskeleton, make it an ideal subject for studying toxicological effects. These features allow for efficient observation and measurement of toxic responses, facilitating the assessment of acute and chronic toxicity of various substances. The immobilization, feeding, and reproductive behaviors of *Daphnia magna* can be sensitive indicators of toxic effects, providing valuable insights into lethal and sub-lethal impacts on survival, growth, and reproduction. Toxicity tests utilizing *Daphnia magna* play a crucial role in evaluating the potential hazards of chemicals, pharmaceuticals, pesticides, heavy metals, and nanoparticles in aquatic environments. The standardized protocols, like OECD guidelines, ensure consistent and reliable toxicological assessments, aiding in risk assessment and regulatory decision-making. Additionally, *Daphnia magna*'s responses can be used to elucidate mechanisms of toxicity, allowing a deeper understanding of how contaminants affect biological processes at the organismal and molecular levels. Furthermore, the ecological relevance of *Daphnia magna* as a keystone species in freshwater ecosystems emphasizes the importance of studying its responses to pollutants. Insights gained from *Daphnia magna* studies can contribute to the protection and preservation of aquatic biodiversity and the sustainable management of freshwater resources. In short, *Daphnia magna*'s application as a model organism in toxicological research is instrumental in advancing our understanding of environmental toxicity and promoting effective environmental risk assessment and management strategies.

**Keywords:** *Daphnia Magna*, Bio-Indicators, Eco-Sensitivity Comparative Toxicology, Biomonitoring, Genomics Research

## Introduction

*Daphnia magna*, commonly known as water flea, is a widely used model organism in various fields of research, including ecology, toxicology, genetics, and environmental science. Its popularity as a model organism stems from its unique characteristics, ease of culturing, and sensitivity to environmental changes. *Daphnia magna* is a small freshwater crustacean belonging to the cladoceran group. Transparent exoskeleton allows for easy observation of internal structures and processes. Size and morphology make it suitable for microscopic study and experimental manipulation.<sup>1</sup> It has short generation time and rapid reproduction rate. Clonal reproduction via parthenogenesis, producing genetically identical offspring. Sexual reproduction occurs under certain environmental conditions, facilitating genetic diversity.<sup>2</sup> They are highly responsive to variations in temperature, pH, pollutants, and other environmental factors. They are widely used in ecotoxicology to assess the effects of various chemicals and pollutants on aquatic organisms.<sup>3</sup> *Daphnia magna* is a key model organism for assessing the toxicity of substances in aquatic ecosystems. Acute and chronic toxicity tests provide valuable data on lethal and sublethal effects of pollutants.<sup>4</sup> Availability of genomic resources and molecular tools for genetic studies and functional genomics. Genome sequencing and gene expression studies enhance understanding of underlying biological mechanisms of *Daphnia magna*.<sup>5</sup> *Daphnia magna*'s characteristics and its sensitivity to environmental changes make it an invaluable model organism for studying ecological and toxicological processes, ultimately contributing to our understanding of aquatic ecosystems and environmental health.

It has a short life cycle (figure 1), allowing researchers to conduct toxicity tests and observe the effects of environmental contaminants over multiple generations quickly.<sup>6</sup> *Daphnia magna* can be easily cultured in the laboratory providing researchers with a consistent and readily available test organism for toxicological experiments. *Daphnia* is used to assess the toxicity of various environmental contaminants, including heavy metals, pesticides, herbicides, pharmaceuticals, and nanoparticles.<sup>7</sup> This water biomarker's position as a key component of freshwater food webs makes it a relevant model for understanding the impacts of contaminants on aquatic ecosystems.<sup>8</sup> *Daphnia magna*'s sensitivity, ease of use, and ecological relevance make it a valuable tool in assessing and understanding the effects of environmental contaminants, contributing to improved environmental management and protection. *Daphnia* toxicity data are often used in environmental risk assessments and regulatory decision-making processes.<sup>9</sup>

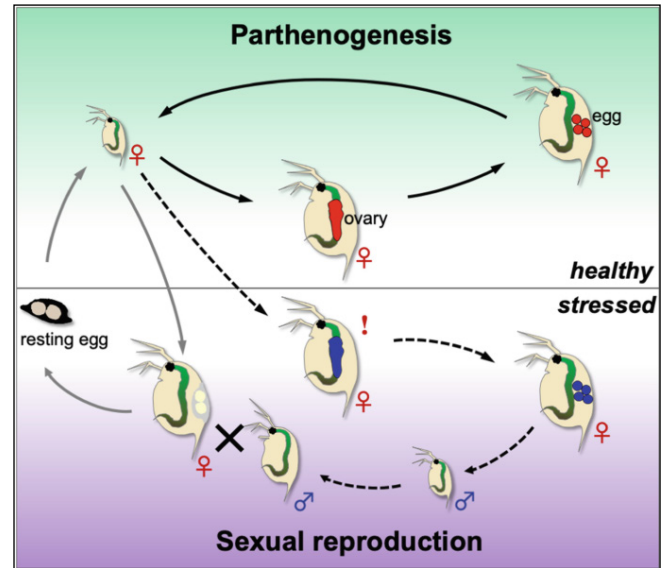


Figure 1. Life cycle of *Daphnia magna*

## *Daphnia magna*: An Ideal Model Organism

*Daphnia magna*, a small freshwater crustacean has evolved over the years to become a significant model organism in various scientific disciplines, particularly in toxicological and environmental studies. Early mentions of *Daphnia*-like organisms date back to the 17th century, with early microscopists and naturalists noting their presence in freshwater habitats. The classification and taxonomy of *Daphnia* species started to take shape in the 18th century, with scientists like Carl Linnaeus contributing to their systematic description. Initially recognized for its ecological importance in aquatic ecosystems, *Daphnia magna*'s biological features, sensitivity to environmental changes, and ease of maintenance in laboratory settings have made it an ideal organism for studying responses to environmental stressors, pollutants, and ecological dynamics.<sup>10</sup> In the late 19th and early 20th centuries, *Daphnia magna* gained attention as a subject for ecological and physiological research due to its pivotal role in freshwater food chains and its sensitivity to changes in water quality and pollutants. Researchers began to appreciate its rapid reproductive cycle, transparency, and accessibility, allowing for detailed observations of its life cycle and interactions.<sup>5</sup> As scientific tools and methodologies advanced, the use of *Daphnia magna* expanded beyond ecology into toxicology and genetics. Its role as a model organism further solidified in the late 20th century with the advent of molecular biology, genomics, and high-throughput technologies. The availability of its complete genome and the understanding of its gene functions propelled *Daphnia magna* to the forefront of toxicological research, aiding in the assessment of environmental contaminants and their impact on both aquatic ecosystems and human health.<sup>11</sup> Today, *Daphnia magna* continues to be a vital model organism, offering

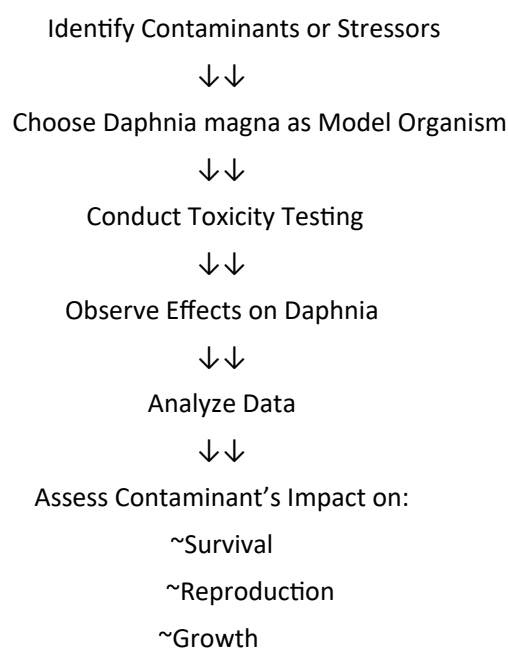
valuable insights into the effects of environmental stressors, climate change, and pollutants on ecosystems. Its evolutionary journey from an ecological subject to a sophisticated model organism underscores its importance in advancing scientific understanding and shaping regulatory and environmental policies.

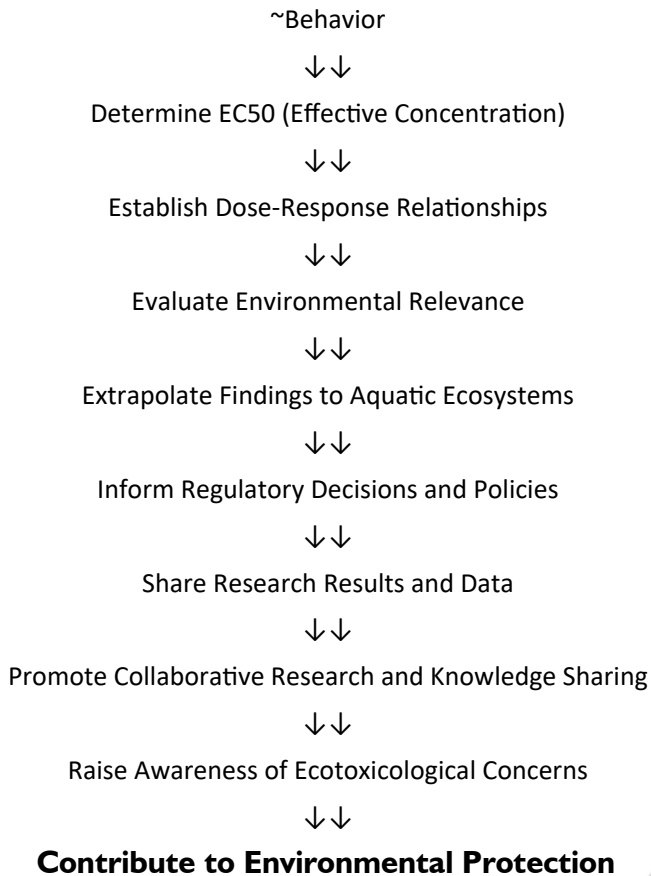
*Daphnia magna* exhibits a translucent body with a characteristic elongated shape and a carapace that covers the head and thorax.<sup>12</sup> It is primarily parthenogenic, meaning females can reproduce without mating, producing genetically identical offspring under favorable conditions. However, sexual reproduction can occur in response to environmental cues, leading to the production of dormant eggs known as ephippia.<sup>13</sup> The life cycle of *Daphnia magna* typically consists of several stages, including neonates (young individuals), juveniles, and adults. The rapid development and short generation time make it an ideal model organism for experiments.<sup>14</sup> *Daphnia magna* is a filter feeder, primarily consuming algae and small particles suspended in the water. Its feeding behavior is crucial in aquatic ecosystems as it helps regulate phytoplankton populations. One of the key attributes of *Daphnia magna* is its sensitivity to environmental changes and contaminants. It responds rapidly to variations in water quality, temperature, and exposure to toxins, making it a valuable organism for assessing ecological health and toxicological studies.<sup>15</sup> It is relatively easy to culture in laboratory settings, making it a convenient model organism for experiments. Cultures can be maintained in controlled conditions, allowing researchers to manipulate various environmental factors. Due to its sensitivity and ecological relevance, *Daphnia magna* is commonly used in Ecotoxicological studies to assess the impacts of pollutants, including pesticides, heavy metals, and nanoparticles, on aquatic ecosystems.

Its physical attributes are essential to its role as a model organism in various scientific studies, particularly in the field of toxicology.<sup>16</sup> *Daphnia magna* typically measures around 1 to 3 millimeters in length, making them easily observable under a microscope. These organisms are transparent, which allows for detailed observation of their internal structures and physiological processes. *Daphnia magna* possess two long antennae used for sensory perception and movement through water. They have a protective carapace, or exoskeleton, that covers their body, resembling a miniature helmet. *Daphnia magna* also have several appendages, including thoracic legs and a large abdominal appendage called the "brood pouch" used for carrying eggs. A conspicuous beating heart, visible through their transparent body, is a distinctive feature that aids in toxicological studies, particularly for assessing the effects of substances on heart rate. *Daphnia magna* have a single compound eye that is sensitive to changes in light intensity.<sup>17</sup> These physical attributes, particularly their

transparency and relatively large size for a microscopic organism, make *Daphnia magna* an excellent choice for studying the impact of environmental contaminants and toxins on various physiological processes.<sup>18</sup> Their sensitivity to changes in their aquatic environment allows researchers to use them as a valuable bioindicator species. *Daphnia magna*, a widely used model organism in environmental and toxicological studies, displays a unique and fascinating life cycle. Reproduction in *Daphnia magna* is both sexual and asexual, allowing for rapid population growth and genetic diversity.<sup>18</sup> *Daphnia* begin their life cycle as neonates, hatching from dormant eggs or ephippial eggs. Neonates mature into juveniles, characterized by the presence of a single brood chamber. Juveniles grow into adults, developing two brood chambers for reproduction. *Daphnia* reproduce asexually through parthenogenesis, wherein unfertilized eggs develop into clones of the mother.<sup>19</sup> In response to environmental cues or stress, sexual reproduction occurs, resulting in the production of resting eggs (ephippial eggs). These resting eggs can remain dormant until conditions become favorable for hatching. Environmental conditions, particularly temperature and photoperiod, influence the ratio of asexual to sexual reproduction in *Daphnia magna*.<sup>20</sup> Adequate food availability promotes asexual reproduction, while scarcity or poor quality of food often triggers sexual reproduction. *Daphnia* undergo aging, eventually reaching a stage of senescence characterized by reduced reproduction and increased mortality.<sup>21</sup> Senescent individuals may produce ephippial eggs, contributing to resting egg banks in aquatic habitats.

#### Schematic diagram of *Daphnia* Ecotoxicological studies





### Advantages of using *Daphnia magna* in toxicological studies

**Sensitivity to environmental changes:** *Daphnia magna* is a highly sensitive and responsive aquatic organism when it comes to environmental changes. This sensitivity is a key feature that makes it an exceptional model organism for studying the effects of various environmental stressors, including contaminants and climate-related factors. *Daphnia* exhibits sensitivity to temperature fluctuations. Changes in water temperature can influence their metabolic rates, reproductive patterns, and overall life history traits.<sup>22</sup> Researchers have utilized this sensitivity to assess the impacts of temperature on aquatic ecosystems and species interactions (e.g., predator-prey dynamics).<sup>23</sup> One of the primary reasons *Daphnia magna* is widely used in toxicological studies is its exceptional sensitivity to chemical contaminants, including pesticides, heavy metals, and pharmaceuticals. Even low concentrations of pollutants can affect their survival, reproduction, and behavior, making them a reliable indicator of water quality.<sup>24</sup> *Daphnia magna* is also sensitive to changes in dissolved oxygen levels. Oxygen depletion in aquatic habitats can significantly impact their distribution and abundance, leading to disruptions in the food web.<sup>25</sup> Variations in pH levels and water acidity can influence *Daphnia magna*'s physiology and survival. Acidic

conditions can negatively affect their carapace structure and reproductive success, illustrating their sensitivity to changes in water chemistry.<sup>22</sup> *Daphnia magna* is vulnerable to ultraviolet (UV) radiation, particularly in clear water environments. Increased UV radiation due to factors like ozone depletion can reduce their survival rates and affect population dynamics.<sup>26</sup>

**Short generation time:** The short generation time of *Daphnia magna* is a key attribute that makes it a valuable model organism in various fields of research, particularly in toxicology and ecological studies. This trait allows researchers to conduct experiments and observe multiple generations within a relatively short period. Several key points highlight the significance of *Daphnia magna*'s short generation time.<sup>6</sup> *Daphnia magna* is capable of reproducing both sexually and asexually. Under favorable conditions, they can reproduce asexually, producing genetically identical offspring through parthenogenesis. This asexual reproduction mechanism results in the rapid production of offspring, with each individual giving rise to a new generation quickly.<sup>27</sup> The short generation time enables researchers to perform experiments, toxicity tests, and studies on the effects of environmental factors in a timely manner. This efficiency is especially important when assessing the impact of contaminants or changes in environmental conditions on populations.<sup>28</sup> While asexual reproduction dominates under stable conditions, *Daphnia magna* can switch to sexual reproduction in response to environmental stressors. This transition introduces genetic variability into populations, allowing researchers to explore the effects of genetic diversity on adaptation and evolution.<sup>29</sup> *Daphnia magna*'s rapid generation turnover facilitates long-term studies and observations. Researchers can track changes in populations and assess the impact of chronic exposures or changing conditions over extended periods.<sup>30</sup>

**Ease of culturing and maintenance:** The Organism is widely recognized as an excellent model organism for toxicological and ecological studies, in part because of its ease of culturing and maintenance in laboratory settings. This species offers several advantages that make it an ideal choice for researchers studying various aspects of ecology, toxicology, and genetics.<sup>31</sup> The rapid reproduction allows for the establishment of sizable populations in the laboratory in a relatively short time frame.<sup>32</sup> *Daphnia magna* can reproduce asexually under favorable conditions, producing genetically identical offspring. This simplifies the maintenance of genetically homogeneous cultures, facilitating controlled experiments. The dietary needs are relatively uncomplicated. They primarily feed on microalgae, yeast, or other small particulate matter present in the culture medium. This simplicity in diet makes it cost-effective and straightforward to provide adequate nutrition.<sup>17</sup> Its cultures do not demand a large amount of



space, making them suitable for laboratories with limited resources. Cultures can be maintained in containers such as beakers or culture flasks.<sup>33</sup> *Daphnia magna* thrives in stable temperature and light conditions. Most laboratories can easily maintain the required environmental parameters, ensuring the health and longevity of the culture.<sup>34</sup> *Daphnia magna*'s transparent exoskeleton allows for easy observation of internal organs and physiological processes without the need for dissection or invasive procedures. This feature is especially valuable for toxicological studies.<sup>5</sup> Many commercial suppliers offer *Daphnia magna* cultures, providing researchers with a convenient source for initiating and replenishing cultures (Table 1).

### Applications in Toxicological Studies

*Daphnia magna* is a valuable organism for toxicological studies, offering insights into the potential risks that chemicals and pollutants pose to aquatic ecosystems and human health. Its use in the following studies helps to inform environmental protection efforts and regulatory decisions.

**Assessment of environmental contaminants:** *Daphnia magna* has played a pivotal role in toxicological research, especially when it comes to assessing the impact of environmental contaminants on aquatic life. This tiny crustacean's remarkable sensitivity to pollutants and its position as a keystone species in freshwater ecosystems make it an invaluable model organism for these investigations.<sup>35</sup>

**Sensitivity to Environmental Contaminants:** It exhibits acute sensitivity to a wide range of environmental contaminants, including heavy metals, pesticides, herbicides, industrial chemicals, and nanoparticles. This sensitivity allows researchers to detect even subtle changes in its physiology and behavior when exposed to these substances.<sup>36</sup>

**Bioassays and Toxicity Testing:** The use of *Daphnia magna* in bioassays and toxicity testing is well-established. These assays provide valuable insights into the potential harm caused by contaminants, helping researchers assess the risk to aquatic ecosystems.<sup>37</sup>

**Acute and Chronic Toxicity Assays:** Researchers conduct both acute and chronic toxicity assays using *Daphnia magna* as a model. Acute assays focus on short-term exposure and immediate effects, while chronic assays examine longer-term impacts, such as reproductive and developmental changes.<sup>38</sup>

**Relevance to Aquatic Ecosystems:** It is a keystone species in many freshwater ecosystems, serving as a primary food source for fish and other aquatic organisms. Therefore, its response to contaminants can have cascading effects throughout the food web, making it a crucial indicator of environmental health.<sup>39</sup>

**Assessing Heavy Metal Toxicity:** Short-term exposure to

varying concentrations of heavy metals to determine lethal concentrations (LC50) and sublethal effects. Longer-term exposure to low levels of heavy metals to assess effects on reproduction, growth, and development.<sup>40</sup> Observations of *Daphnia* behavior, including swimming patterns, feeding, and avoidance responses, can provide insights into sublethal effects.<sup>41</sup> Assessing changes in gene expression related to stress response, detoxification, and oxidative stress.<sup>39</sup> Measurement of biomarkers such as metallothioneins, superoxide dismutase, and glutathione-S-transferase to gauge cellular responses to heavy metals. *Daphnia* can accumulate heavy metals from their diet, providing insights into the potential for biomagnification in food webs.<sup>42</sup> Toxicity assessments on *Daphnia* help predict the impact of heavy metal pollution on aquatic ecosystems, including effects on primary consumers and higher trophic levels. Assessing heavy metal toxicity on *Daphnia magna* is a valuable tool in environmental toxicology. It allows for the identification of potential risks associated with heavy metal pollution and aids in the development of mitigation strategies. The sensitivity, rapid reproduction, and molecular tools available make *Daphnia* an essential model organism in understanding the impact of heavy metals on aquatic ecosystems (Table 2).

**Assessing Pesticides and herbicides Toxicity:** Its sensitivity to environmental stressors and rapid reproduction makes it an ideal candidate for such evaluations. It exhibits high sensitivity to a wide range of pesticides and herbicides commonly used in agriculture and aquaculture. This sensitivity allows for the detection of sublethal effects, making it a useful bio-indicator for environmental contamination.<sup>43</sup> Studies have shown that exposure to pesticides like neonicotinoids and herbicides such as glyphosate can result in adverse effects on *Daphnia*, including altered behavior, reduced reproductive capacity, and impaired growth. Even subtle alterations in water quality can have noticeable effects on the survival and reproduction of these organisms.<sup>44</sup> Researchers employ acute and chronic toxicity assays to evaluate the short-term and long-term impacts of pesticides and herbicides on *Daphnia magna*. These experiments provide valuable data on mortality rates, reproductive impairments, and developmental abnormalities.<sup>45</sup> The ecological relevance of the studies cannot be overstated. *Daphnia* are essential components of aquatic ecosystems, serving as a crucial food source for various organisms, including fish. Any disruption in *Daphnia* populations can have cascading effects on higher trophic levels.<sup>46</sup> Toxicity assessments with *Daphnia magna* help gauge the potential ecological risks posed by pesticide and herbicide contamination in aquatic environments. Findings from toxicity studies involving *Daphnia magna* contribute to regulatory decisions regarding the approval and use of pesticides and herbicides. They are instrumental in establishing safe usage

limits and mitigation strategies.<sup>47</sup> Regulatory agencies such as the Environmental Protection Agency (EPA) in the United States and the European Food Safety Authority (EFSA) in Europe often require toxicity data on *Daphnia magna* as part of pesticide registration. Findings from *Daphnia magna* toxicity studies can be extrapolated to predict the effects of pesticides and herbicides on entire aquatic ecosystems. As primary consumers in freshwater food webs, *Daphnia magna* influences the transfer of toxins through trophic levels.<sup>44</sup> Data obtained from *Daphnia magna* toxicity assessments play a crucial role in the registration and regulation of pesticides and herbicides. Regulatory agencies worldwide use this information to establish safe usage guidelines and restrictions (Table 3). The use of *Daphnia magna* in toxicity studies raises ethical considerations related to animal welfare.<sup>39</sup> Researchers aim to minimize harm and employ alternatives when possible, such as using fewer organisms or developing in vitro assays.<sup>48</sup>

**Assessing Nanoparticle Toxicity:** Assessing nanoparticle toxicity on *Daphnia magna*, a widely used model organism in ecotoxicology, is an essential aspect of understanding the potential environmental impact of nanoparticles. Nanoparticles can have various physicochemical properties that influence their toxicity, including size, shape, surface charge, and composition. The physicochemical properties of nanoparticles play a significant role in their toxicity. Size, in particular, is a critical factor. Smaller nanoparticles often exhibit increased toxicity because they can enter cells and tissues more easily. Surface charge and composition also affect toxicity. For example, nanoparticles with reactive surfaces may induce oxidative stress.<sup>49</sup> *Daphnia magna* can be exposed to nanoparticles through various routes, including waterborne exposure (dispersed in water), dietary exposure (ingesting nanoparticles with food), and dermal exposure (contact with nanoparticle-contaminated surfaces).<sup>50</sup> Common endpoints for assessing toxicity in *Daphnia magna* include mortality, reproduction, growth, behavior, and biochemical markers of stress such as oxidative stress indicators (e.g., reactive oxygen species). These endpoints provide insights into the overall health and well-being of the organisms.<sup>51</sup> Proper experimental design is crucial. Studies should consider nanoparticle concentration, exposure duration, and the choice of control groups. Positive and negative controls are essential to ensure the validity of results.<sup>52</sup>

**Bioaccumulation Studies:** Bioaccumulation of contaminants in *Daphnia magna* is evaluated to understand how contaminants move through the food chain.<sup>53</sup>

**Multi-Chemical Assessments:** Some studies assess the effects of mixtures of contaminants to understand potential synergistic or antagonistic interactions.<sup>54</sup>

**Size and Surface Characteristics:** The size and surface

characteristics of nanoparticles play a significant role in their toxicity to *Daphnia magna*.<sup>55</sup> Smaller nanoparticles and those with specific surface coatings may have different toxic effects compared to larger or uncoated particles.<sup>7</sup>

**Mechanisms of Toxicity:** Researchers have explored the mechanisms underlying nanoparticle toxicity, including oxidative stress, genotoxicity, and interactions with cell membranes. These mechanisms shed light on how nanoparticles can disrupt normal physiological processes in *Daphnia magna*.<sup>51</sup>

**Toxicological Endpoints:** Toxicity studies on *Daphnia magna* often focus on various endpoints, including acute toxicity, chronic toxicity, reproductive toxicity, and behavioral changes. These endpoints help researchers understand the full range of impacts nanoparticles can have on these organisms.<sup>55</sup>

**Exposure Duration and Concentrations:** Researchers typically expose *Daphnia magna* to a range of nanoparticle concentrations over specific timeframes. Short-term acute exposure tests (e.g., 48-96 hours) are common, but longer-term chronic exposure tests may also be necessary to assess sublethal effects. Concentrations should span a range, including environmentally relevant levels.<sup>51</sup>

**Toxicity Endpoints:** Toxicity endpoints are measurable responses that indicate the impact of NPs on *Daphnia magna*. Common endpoints include mortality, immobilization, and reproductive impairments. Sublethal endpoints like altered behavior, growth, and biochemical responses should also be considered.<sup>7</sup>

**Methodology:** Various methodologies are employed to conduct toxicity tests, including static and flow-through systems. The Oecd *Daphnia magna* acute toxicity test guideline (Oecd 202) provides standardized protocols for conducting tests. In recent years, there has been an emphasis on developing more ecologically relevant tests that consider the organism's life cycle and behavior.<sup>51</sup>

**Characterization of Nps:** Detailed characterization of nanoparticles is critical, including size, shape, surface charge, and agglomeration state. This information helps correlate nanoparticle properties with observed toxicity.<sup>56</sup>

**Mechanistic Studies:** To gain a deeper understanding of NP toxicity, mechanistic studies can be conducted. This involves investigating the pathways and cellular/molecular interactions through which nanoparticles exert their effects on *Daphnia magna*.<sup>56</sup>

**Environmental Relevance:** Results from toxicity tests should be interpreted in the context of environmental conditions and concentrations of NPs found in natural waters. Assessing NP toxicity under realistic exposure scenarios is essential.<sup>57</sup>

**Regulatory Implications:** Findings from nanoparticle tox-

icity studies can inform regulatory decisions regarding the safe use and disposal of NPs. Regulatory agencies, such as the Environmental Protection Agency (EPA) in the United States, may use such data in risk assessments (Table 4).<sup>58</sup>

### Bioassays and toxicity testing

**Acute and chronic toxicity assays:** Acute toxicity assays involve exposing *Daphnia magna* to a high concentration of a substance over a short duration, typically within 24 to 48 hours. The primary objective is to determine the immediate effects and lethal concentrations (LC50) of the tested substance on the organisms.<sup>59</sup> Observations during acute assays often include mortality rates, changes in behavior, and physiological alterations, allowing researchers to assess the substance's acute toxicity level. Acute assays are crucial for identifying highly toxic substances and assessing the immediate risk they pose to aquatic life. They are valuable in regulatory assessments and can guide emergency response actions. Chronic toxicity assays, on the other hand, involve prolonged exposure of *Daphnia magna* to lower concentrations of a substance over an extended period, usually spanning multiple generations of *Daphnia*.<sup>46</sup> These assays provide insights into sublethal effects, such as growth, reproduction, and development, which are critical for understanding the long-term impacts of the tested substance. Chronic toxicity assessments contribute to a comprehensive understanding of the potential ecological consequences and help establish acceptable exposure levels over extended periods.<sup>60</sup> Employing both acute and chronic assays can offer a comprehensive view of a substance's toxicity profile. Acute tests identify immediate risks, while chronic tests reveal subtle, long-term effects. Regulatory agencies often require data from both acute and chronic tests for the approval and classification of substances, emphasizing their importance in risk assessment.<sup>61</sup> These tests help us understand the immediate and long-term impacts of contaminants on aquatic ecosystems, providing valuable data for regulatory decisions and environmental protection efforts. Researchers, environmental agencies, and industries alike rely on these assays to ensure the safety of our aquatic environments.

**Reproductive and developmental assays:** Reproductive and developmental assays on *Daphnia magna* are essential components of toxicological studies, providing insights into how various environmental contaminants can impact the reproductive success and growth of this widely used model organism. Reproductive and developmental assays with *Daphnia magna* offer researchers a means to evaluate the sublethal effects of pollutants, providing insights into potential risks to aquatic ecosystems and human health.<sup>17</sup> Reproductive assays in *Daphnia magna* focus on various endpoints related to reproduction, including Fecundity (The number of offspring produced per female over a defined

period.) Timing of Reproduction (Assessing the timing of the first brood release and subsequent reproductive cycles) Brood Size (Evaluating the size of individual broods produced by exposed *Daphnia*).<sup>62</sup> Developmental assays investigate the impact of contaminants on the growth and development of *Daphnia magna*, with a focus on Growth Rate (Monitoring the growth and size of juvenile *Daphnia* exposed to pollutants) Molt Frequency (Assessing how contaminants affect the molting frequency and integrity of the exoskeleton) Developmental Abnormalities (Identifying and quantifying any abnormalities in the structure or development of *Daphnia*).<sup>63</sup> Reproductive and developmental assays are crucial for evaluating the toxicity of environmental pollutants such as heavy metals, pesticides, and pharmaceuticals (table no. 5).<sup>64</sup> They provide critical data for risk assessment and regulatory decision-making to protect aquatic.<sup>65</sup>

### Daphnia studies relate to human health

**Relevance to Aquatic Ecosystems:** This Aquatic insect serves as a significant link in the trophic cascade of freshwater ecosystems. It feeds on phytoplankton, controlling their populations and influencing water clarity. This, in turn, affects the availability of light and nutrients, thereby shaping the composition of aquatic flora and subsequent trophic levels.<sup>32</sup> *Daphnia magna*'s sensitivity to environmental changes, pollutants, and toxins makes it a valuable bio indicator species. Changes in its population, behavior, and reproductive capabilities can reflect alterations in water quality, pollution levels, and overall ecosystem health. Monitoring. It's population aids in assessing the impact of human activities on aquatic ecosystems.<sup>66</sup> The abundance and health of its populations are indicative of ecosystem resilience and stability. Well-balanced populations of *Daphnia magna* help maintain a healthy nutrient cycle by controlling algal blooms, promoting water clarity, and ensuring the availability of food for higher trophic levels.<sup>67</sup> Its life cycle is temperature-dependent, making it highly responsive to climate change. Changes in temperature can alter its reproductive patterns and population dynamics, potentially disrupting trophic interactions and affecting the overall structure and function of aquatic ecosystems.<sup>68</sup> Understanding the ecological role of *Daphnia magna* is essential for conservation efforts and ecosystem management. Research on its interactions with other organisms, response to pollutants, and adaptations to environmental stressors provides valuable insights into mitigating the impacts of anthropogenic activities on aquatic habitats.<sup>69</sup>

**Insights into Human Health Risks:** Studies involving *Daphnia* have shed light on the toxicity and effects of various substances, allowing us to infer potential risks to humans exposed to similar substances in their environment. It's sensitivity to a range of environmental contaminants, including



heavy metals, pesticides, and pharmaceuticals, allows for detailed toxicity assessments.<sup>3</sup> By understanding the effects of these substances on *Daphnia magna*, researchers can make informed comparisons to potential effects on human health, considering similarities and differences in physiological responses. Research on bioaccumulation and trophic transfer of pollutants in *Daphnia* informs us about how contaminants can accumulate within organisms and move through the food chain. Understanding these processes provides insights into potential human exposure through consumption of contaminated food, water, or other environmental sources.<sup>70</sup> Studies on how pollutants affect reproduction, growth, and development in *Daphnia* can provide insights into potential risks to human reproductive health and early-life development, as there are often parallels in the biological pathways and mechanisms affected by environmental stressors.<sup>71</sup> Advances in genomics and molecular tools have allowed researchers to investigate the genetic and molecular responses of *Daphnia magna* to contaminants. Comparative genomics can reveal conserved genetic pathways, helping researchers draw parallels between *Daphnia magna* and humans, potentially identifying shared molecular mechanisms of toxicity.<sup>72</sup> The field of translational toxicology aims to bridge the gap between environmental toxicology and human health. *Daphnia magna* studies can provide valuable data points for developing predictive models and conducting risk assessments that incorporate human health considerations.<sup>73</sup> While *Daphnia magna* research primarily addresses environmental impacts and aquatic ecosystems, the knowledge gained indirectly informs our understanding of how pollutants might affect human health.<sup>74</sup> Interdisciplinary approaches that bridge research on *Daphnia magna* and human health are essential to draw more direct connections and enhance our ability to anticipate and mitigate risks to both aquatic life and humans.

### Genomic and Molecular Tools

Genomic resources for *Daphnia magna* have expanded significantly in recent years, providing valuable insights into the biology and ecology of this model organism. The sequencing of the *Daphnia magna* genome has been a major milestone in understanding its biology. The *Daphnia* Genomics Consortium has led efforts in sequencing and annotating the genome.<sup>5</sup> Transcriptomic studies have provided valuable information on gene expression patterns under different environmental conditions and life stages. These datasets are important for understanding how *Daphnia* responds to environmental changes.<sup>75</sup> Understanding genomic variation within *Daphnia* populations is crucial for studying adaptation and evolution. Whole-genome resequencing projects have shed light on genetic diversity within *Daphnia magna* populations.<sup>76</sup> Epigenetic modifications play a role in the response of *Daphnia magna* to environmental changes.

Research on DNA methylation and histone modifications in *Daphnia* contributes to understanding epigenetic regulation.<sup>77</sup> Functional genomics research includes studies on gene knockdowns, RNA interference (RNAi), and gene expression profiling to elucidate the functions of specific genes in *Daphnia magna*.<sup>78</sup> Tools for genetic manipulation and functional studies in *Daphnia magna* are continually developing, including methods for transgenesis and gene editing using CRISPR/Cas9.<sup>79</sup> Studies on population genomics and evolution in *Daphnia magna* have utilized genomic data to investigate adaptation, speciation, and responses to changing environments.<sup>80</sup> Metabolomics provides a comprehensive analysis of small molecules (metabolites) in *Daphnia magna*. It can reveal changes in metabolic pathways in response to toxicants and help identify biomarkers of exposure and effects.<sup>81</sup> These genomic resources for *Daphnia magna* have opened up avenues for research in various fields, including ecology, evolution, and environmental toxicology, and have the potential to provide insights into broader ecological and evolutionary questions. Researchers can access these resources to better understand the responses of *Daphnia magna* to environmental changes and stressors.

Omics studies of *Daphnia* species, including genomics, transcriptomics, and proteomics, have significantly advanced our understanding of this model organism's biology and its responses to various environmental conditions and stressors. The genome of *Daphnia magna* was first sequenced and published in 2011 by.<sup>5</sup> This landmark study provided a high-quality reference genome for the species, enabling further genomics research. Researchers have used techniques such as RNA sequencing (RNA-seq) and gene expression profiling to study the functional genomics of *Daphnia magna*. These studies have helped understand gene expression changes in response to environmental stressors.<sup>82</sup> Comparative genomics involving *Daphnia magna* and other *Daphnia* species have shed light on the evolutionary processes and adaptive mechanisms in response to different ecological niches.<sup>83</sup> Genomics research has helped elucidate the ecological interactions of *Daphnia magna*, including its response to predation, toxins, and temperature changes.<sup>84</sup> Transcriptomics provides insights into changes in gene expression patterns in response to various stressors, allowing for the identification of key genes and pathways involved in toxicological responses.<sup>5</sup> Transcriptomic studies help identify genes and pathways involved in *Daphnia magna* responses to environmental stressors, toxicants, and other factors affecting their physiology and development.<sup>77, 85</sup> Proteomics techniques, such as mass spectrometry-based proteomics, enable researchers to study changes in protein expression and post-translational modifications in *Daphnia magna*. This helps in understanding the downstream effects of gene



expression changes.<sup>86</sup> Proteomic studies in *Daphnia magna* offer insights into protein expression and regulation in response to environmental stimuli, providing a deeper understanding of the molecular mechanisms underlying their responses to stressors.<sup>87</sup> Omics approaches, including genomics, transcriptomics, and proteomics, have revolutionized our understanding of *Daphnia magna* biology,

including responses to environmental stressors, mechanisms of adaptation, and evolutionary processes. These studies contribute to environmental risk assessment and help bridge the gap between molecular responses in *Daphnia magna* and potential impacts on aquatic ecosystems, thus indirectly linking to human health through ecosystem health and function.

**Table 1. Evolution of *Daphnia* Genomics Research**

Year	Advancement In Genomics Research	Reference
2005	Daphnia Genomic Sequencing Initiative Begins.	[5]
2007	Sequencing of the <i>Daphnia pulex</i> genome.	[5]
2011	Comparative Genomics of <i>Daphnia</i> .	[5]
2013	Functional Annotation of <i>Daphnia</i> Genes.	[88]
2015	Epigenomic Studies in <i>Daphnia</i> .	[89]
2015	Single-cell RNA sequencing (scRNA-seq), enabling researchers to study gene expression at the single-cell level in <i>Daphnia</i> .	[90]
2016	Development of <i>Daphnia</i> genomics resources.	[76]
2017	Comparative genomics and adaptation studies.	[91]
2018	Epigenetic studies using techniques like DNA methylation profiling.	[92]
2018	Epigenomics and epigenetic regulation in <i>Daphnia</i> .	[92]
2020	Advances in Single-Cell RNA Sequencing.	[93]
2020	Advances in functional genomics, including RNA interference (RNAi) techniques and gene knockdowns.	[94]
2020	Integration of genomics and ecological studies.	[95]

**Table 2. Metals and Their Adverse Effects on *Daphnia magna***

Metal	Effect on <i>Daphnia magna</i>	Reference
Copper (Cu)	Inhibition of growth, impaired reproduction, and altered behavior.	[114]
Lead (Pb)	Growth inhibition, altered behavior, and impaired reproduction.	[115]
Zinc (Zn)	Impaired reproduction and altered behavior.	[116]
Cadmium (Cd)	Growth inhibition, altered behavior, and impaired reproduction.	[117]
Mercury (Hg)	Growth inhibition, altered behavior, and impaired reproduction.	[118]
Chromium (Cr)	Impaired reproduction and altered behavior.	[119]

**Table 3. Pesticides and Their Adverse Effects on *Daphnia magna***

Pesticide	Effect on <i>Daphnia magna</i>	Reference
Chlorpyrifos	Decreased mobility	[96]
Glyphosate	Reduced reproduction rate	[97]
DDT	Acute toxicity, mortality	[98]
Malathion	Impaired swimming ability	[99]
Neonicotinoids	Altered behavior	[100]
Imidacloprid	Impaired growth and reproduction	[101]
Atrazine	Disruption of reproduction	[102]
DDT	Strong negative impact on survival and growth of <i>Daphnia magna</i>	[103]

**Table 4. Nanoparticles and Their Adverse Effects on Daphnia magna**

Nanoparticle Type	Effect on Daphnia magna	Reference
Silver nanoparticles (Ag)	Acute toxicity, reduced mobility, mortality.	[120]
Titanium dioxide (TiO <sub>2</sub> )	Altered behavior, growth inhibition, mortality.	[121]
Zinc oxide (ZnO)	Developmental delays, mortality.	[122]
Carbon nanotubes (CNTs)	Growth inhibition, oxidative stress.	[123]
Gold nanoparticles (Au)	Altered behavior, mortality.	[124]
Polystyrene nanoparticles	Reduced feeding, altered growth.	[125]
Copper nanoparticles (Cu)	Oxidative stress, mortality.	[126]

**Table 5. Pharmaceutical Products and Their Adverse Effects on Daphnia magna**

Pharmaceutical	Effect on Daphnia magna	Reference
Ibuprofen	Reduced heart rate and mobility, altered behavior, and reproduction inhibition.	[104]
Fluoxetine	Altered behavior, reduced feeding, and decreased reproductive output.	[105]
Ciprofloxacin	Altered behavior, reduced survival, and growth inhibition.	[106]
Ethinyl estradiol	Interference with reproduction and feminization of male Daphnia.	[107]
Atenolol	Altered heart rate and decreased reproductive success.	[108]
Sertraline	Altered behavior and reproduction, leading to population-level effects.	[109]
Acetaminophen	Altered behavior, reduced reproduction, oxidative stress.	[110]
Carbamazepine	Altered behavior, reduced feeding, growth inhibition.	[111]
Gemfibrozil	Growth inhibition, altered lipid metabolism.	[112]
Atorvastatin	Reduced feeding, growth inhibition	[113]

## Future Perspectives

Research on *Daphnia magna* continues to evolve, with emerging trends and future perspectives focusing on various aspects of ecology, toxicology, genomics, and the application of novel technologies. Other fields will contribute to a deeper understanding of the ecological and biological processes involving *Daphnia* and their significance in aquatic ecosystems. These trends offer exciting opportunities to address environmental challenges and inform conservation and management efforts.

**Omics Approaches for Comprehensive Analysis:** Future research is likely to increasingly employ omics approaches such as genomics, transcriptomics, proteomics, and metabolomics to comprehensively analyze the molecular and biochemical responses of *Daphnia magna* to environmental stressors. These techniques allow for a deeper understanding of the mechanisms underlying various physiological processes and stress responses in *Daphnia*.<sup>5</sup>

**Ecological Genomics and Evolutionary Adaptations:** Studying the genomics of *Daphnia magna* populations in diverse habitats can provide insights into their evolutionary adaptations to environmental conditions. Researchers will likely focus on understanding how natural selection and genetic variations influence *Daphnia*'s ability to adapt to

changing environments and the implications for ecosystem dynamics.<sup>76</sup>

**Endocrine Disruption and Chemical Exposure:** Future research will likely delve deeper into the effects of endocrine-disrupting chemicals (EDCs) on *Daphnia magna*. Understanding the impacts of EDCs on reproduction, development, and behavior can provide valuable insights into potential risks posed by these chemicals to aquatic organisms and, by extension, to human health.<sup>39</sup>

**Climate Change and Environmental Stressors:** Given the increasing concern about climate change, future research is expected to focus on the interactions between climate change stressors (e.g., temperature, pH changes) and other environmental stressors (e.g., pollutants). This research will shed light on how multiple stressors interact and amplify their effects on *Daphnia magna* and aquatic ecosystems.<sup>127</sup>

**Micro plastics and Nanoparticles:** Research into the effects of Microplastic and nanoparticles on *Daphnia magna* is likely to increase. Understanding how these emerging pollutants impact *Daphnia magna*'s physiology, behavior, and reproductive capabilities will be crucial for assessing the risks associated with their presence in aquatic ecosystems.<sup>128</sup> By focusing on these emerging trends, researchers aim to deepen our understanding of *Daphnia magna* and

its responses to various stressors, ultimately contributing to better environmental management and safeguarding human health in aquatic ecosystems. Top of Form

**Integration with other model organisms and technologies:** Research involving *Daphnia magna* often benefits from integration with other model organisms and cutting-edge technologies. These integrations can enhance our understanding of *Daphnia* biology and its relevance to broader ecological and toxicological contexts. Integrating genomics and transcriptomics with *Daphnia* research allows scientists to explore the molecular mechanisms underlying various responses and adaptations. Comparative genomics with model organisms like *Drosophila* and *Caenorhabditis elegans* can provide insights into the evolution of stress response pathways in *Daphnia*.<sup>5</sup> Studying the metabolome and proteome of *Daphnia* can reveal metabolic pathways and protein expression patterns under different conditions. Integration with technologies used in human health research can help identify biomarkers of stress, toxicity, or disease in *Daphnia*.<sup>129</sup> The CRISPR-Cas9 system can be applied to *Daphnia* research to create targeted gene knockouts or modifications. This technology allows scientists to investigate the functional roles of specific genes in *Daphnia* biology and responses to environmental stressors.<sup>130</sup> Advanced microscopy techniques, including fluorescence microscopy and live imaging, enable researchers to observe dynamic processes in *Daphnia*, such as embryo development, behavior, and response to toxicants, with high spatial and temporal resolution.<sup>131</sup> Integration with ecological modeling allows researchers to simulate and predict the population dynamics of *Daphnia magna* in response to changing environmental conditions. Such models can provide insights into how *Daphnia* populations influence and are influenced by other aquatic organisms.<sup>132</sup> Collaboration between *Daphnia* researchers and human health experts can lead to a better understanding of the relevance of *Daphnia* studies to human health. For example, understanding the mechanisms of chemical toxicity in *Daphnia* can provide insights into potential risks to human health from exposure to similar chemicals.<sup>71</sup> Integrating *Daphnia magna* research with other model organisms and advanced technologies enhances the comprehensiveness and applicability of findings. This interdisciplinary approach can lead to a more holistic understanding of ecological and toxicological processes, with potential implications for both environmental and human health.

**Potential contributions to understanding environmental and health risks:** Research on *Daphnia magna* continues to be important for understanding environmental and health risks, and future contributions can have a significant impact on these areas. Here are some potential contributions and areas of focus in its research in the future. With the increasing use of nanoparticles in consumer products

and industrial applications, there is a need to understand their potential environmental and health risks. *Daphnia magna* can serve as a valuable model organism for studying the toxicity of nanoparticles, including their effects on reproduction, behavior, and long-term ecological consequences.<sup>7</sup> As climate change affects aquatic ecosystems, *Daphnia magna* research can help elucidate how rising temperatures, altered precipitation patterns, and changing nutrient dynamics impact their populations and interactions with other organisms. This knowledge is crucial for predicting ecosystem responses and potential health risks associated with climate-induced changes.<sup>133</sup> Micro plastics and emerging contaminants pose significant challenges to aquatic ecosystems. Research on how *Daphnia magna* interacts with and accumulates micro plastics, as well as its responses to emerging contaminants like pharmaceuticals and endocrine disruptors, can provide insights into their potential impacts on food webs and human health through the consumption of contaminated aquatic organisms.<sup>134</sup> Advances in genomics and molecular biology techniques allow for a deeper understanding of *Daphnia magna*'s responses to environmental stressors. Future research can focus on genomics, transcriptomics, and proteomics to identify key molecular pathways involved in stress responses, reproduction, and immune function, providing a foundation for targeted toxicity assessments and health risk evaluations.<sup>5</sup> Establishing long-term monitoring programs involving *Daphnia magna* populations in various ecosystems can provide valuable data on population dynamics, trends, and responses to multiple stressors over time. Such studies can help us identify emerging risks and assess the effectiveness of environmental management strategies.<sup>135</sup>

**Policy implications and regulatory applications:** Research involving *Daphnia magna* has important policy implications and regulatory applications, particularly in the context of environmental protection and chemical safety assessments. *Daphnia magna* studies are commonly used in the environmental risk assessment of chemicals, pesticides, and pollutants. Regulatory agencies, such as the Environmental Protection Agency (EPA) in the United States and the European Chemicals Agency (ECHA) in Europe, often require toxicity data on *Daphnia* as part of chemical registration and approval processes.<sup>136</sup> Research on *Daphnia magna* helps establish and update water quality standards. These standards are used to set permissible pollutant levels in surface waters to protect aquatic ecosystems and human health. *Daphnia*-based toxicity data contribute to the development of scientifically sound and protective standards.<sup>137</sup> *Daphnia magna* research can lead to the classification and labeling of chemicals based on their hazardous properties. For instance, chemicals that are highly toxic to *Daphnia* may be labeled accordingly, and this information is important for safe handling and disposal.<sup>138</sup> Regulatory agencies of-

ten require detailed *Daphnia magna* toxicity data as part of chemical registration dossiers. These data are used to assess the risks associated with chemical substances and to determine whether they can be authorized for use in various applications.<sup>139</sup> Policies related to the conservation and management of aquatic ecosystems may be informed by research on *Daphnia magna* and their role in food webs. Understanding the ecological significance of *Daphnia* can influence the design of protected areas and sustainable management practices.<sup>140</sup> International organizations, such as the Organization for Economic Co-operation and Development (OECD), work to harmonize guidelines and data requirements for chemical testing. *Daphnia magna* is a model organism used in these efforts to ensure consistency in regulatory approaches globally.<sup>39</sup> Research involving *Daphnia magna* is crucial for shaping policies and regulations related to chemical safety, environmental protection, and ecosystem management. *Daphnia*-based studies provide valuable data that help safeguard both aquatic ecosystems and human health by informing the assessment and management of chemical risks in the environment.

### Challenges and Limitations

**Factors influencing *Daphnia magna* research:** Factors influencing *Daphnia magna* research encompass a range of variables that can affect the outcome and interpretation of studies using this model organism. Understanding these factors is crucial for designing effective experiments and drawing meaningful conclusions. However, several factors can influence research involving *D. magna*, impacting the reliability and applicability of study findings. The physicochemical characteristics of the environment in which *D. magna* are cultured or studied can significantly influence research outcomes. Factors such as temperature, pH, water quality, and nutrient availability can affect *D. magna*'s growth, reproduction, and response to toxins.<sup>3</sup> Genetic diversity within *D. magna* populations can lead to variability in responses to toxicants. Different genotypes may exhibit varying levels of sensitivity or resistance to pollutants, emphasizing the importance of maintaining genetic diversity in laboratory cultures.<sup>141</sup> The life stage of *D. magna* used in experiments can influence research outcomes. Neonates, juveniles, and adults may respond differently to toxicants due to variations in their physiological and metabolic processes.<sup>70</sup> Prior exposure to a specific toxicant or environmental condition can result in acclimation or adaptation of *D. magna* populations. This phenomenon can lead to altered sensitivity to subsequent exposures, affecting the accuracy of toxicity assessments.<sup>142</sup> Experimental factors such as the duration of exposure, concentration of toxicants, and choice of endpoints can influence research outcomes. Careful consideration of these factors is essential to ensure the relevance and reliability of study results.<sup>143</sup> In natural ecosystems, *D. magna* interacts

with various species, including predators, competitors, and parasites. These interactions can modify *D. magna*'s responses to toxicants and should be considered when extrapolating laboratory findings to real-world scenarios.<sup>144</sup> Ethical concerns related to the treatment and welfare of *D. magna* should be addressed in research. Proper care and handling of the organisms, as well as adherence to ethical guidelines, are essential.<sup>145</sup> Understanding and accounting for these factors is critical in designing and interpreting studies involving *D. magna* to ensure that research findings accurately represent the complex dynamics of this model organism in response to environmental stressors.

**Variability in Sensitivity in research:** it's important to recognize that there can be variability in sensitivity among *Daphnia* populations and even within individuals, which researchers must consider when conducting toxicological research. *Daphnia magna* populations can exhibit variability in their sensitivity to toxins and environmental stressors. This variation may be due to genetic differences, local adaptations, or historical exposure to contaminants.<sup>146</sup> Researchers often collect *Daphnia* from different locations to assess how different populations respond to specific substances.<sup>147</sup> Even within a single *Daphnia* population, individual organisms can vary in their sensitivity to toxins. Factors such as age, size, reproductive status, and genetic diversity can contribute to these differences.<sup>148</sup> This individual-level variability can complicate toxicity testing, as responses may not be uniform across all tested organisms. The presence of sensitivity variability in *Daphnia magna* emphasizes the importance of using statistically relevant sample sizes and conducting multiple replicates in toxicological experiments. This ensures that research findings are robust and representative of the population under investigation.<sup>148</sup> Researchers should consider the potential for local adaptations in *Daphnia* populations when conducting studies in different geographic regions.<sup>147</sup> This knowledge can help in assessing how specific populations may respond differently to environmental stressors. Toxicological studies often need to account for individual variation in *Daphnia* responses. This can be done by conducting experiments with a diverse range of individuals or by statistically analyzing data to identify factors contributing to variability.<sup>146</sup> Variability in sensitivity within and among *Daphnia* populations is a crucial consideration in toxicological research. Recognizing and addressing this variability enhances the reliability and applicability of findings in assessing the ecological and human health risks associated with environmental contaminants.

**Complex interactions:** *Daphnia magna*, a keystone species in freshwater ecosystems, engages in complex interactions with various biotic and abiotic factors. These interactions have far-reaching consequences for the structure and functioning of aquatic ecosystems. *Daphnia magna* are



primary consumers in aquatic food webs and serve as prey for a variety of predators, including fish, insects, and other zooplankton. The intensity of predation can have cascading effects on Daphnia populations, affecting their abundance and size distribution. This, in turn, can influence phytoplankton abundance and community structure.<sup>22</sup> They often coexist with other species of zooplankton in aquatic environments. Competitive interactions for resources like algae can shape community structure. Understanding the competitive abilities of Daphnia in relation to other species is crucial for predicting community dynamics.<sup>149</sup> Daphnia can indirectly affect the abundance and composition of phytoplankton communities through their grazing activity. By reducing the density of specific algal species, Daphnia can facilitate the dominance of other phytoplankton taxa, leading to complex shifts in community composition.<sup>20</sup> Daphnia can alter their environment through the release of chemical cues or allelopathic substances. These chemicals can influence the growth and behavior of other aquatic organisms, including phytoplankton and competing zooplankton species.<sup>150</sup> Temperature is a key abiotic factor that influences Daphnia magna's life history traits, including growth, reproduction, and metabolic rates. Climate change can alter water temperatures in freshwater ecosystems, affecting the phenology and distribution of Daphnia populations, which can have cascading effects on trophic interactions.<sup>133</sup> In natural ecosystems, Daphnia magna's interactions with other organisms and environmental conditions are highly dynamic and context-dependent. These interactions are central to understanding the functioning and stability of freshwater ecosystems, which are essential for maintaining water quality, biodiversity, and the services provided by these ecosystems.

**Predation and Grazing Impact:** Daphnia magna is a key prey item for many aquatic predators, including fish and insects. Predation pressure can significantly influence Daphnia population dynamics, behavior, and life history strategies.<sup>151</sup> It feeds on algae, and the abundance and composition of algal communities can affect Daphnia growth, reproduction, and survival. Competition for food resources among Daphnia and other herbivores also plays a crucial role in their interactions within the ecosystem.<sup>20</sup> Nutrient availability, particularly phosphorus and nitrogen, affects Daphnia growth and reproduction. Understanding nutrient cycling and how Daphnia respond to nutrient fluctuations is critical for understanding their role in ecosystem dynamics.<sup>152</sup> Temperature is a significant environmental factor affecting the physiology, growth, and reproductive rates of Daphnia magna. Changes in temperature due to climate change can have profound effects on Daphnia populations and their interactions with other organisms in the ecosystem.<sup>153</sup> Daphnia can detect chemical cues released by predators and adjust their behavior and morphology accordingly to

reduce predation risk. These chemical interactions have implications for Daphnia population dynamics and community structure.<sup>154</sup> Understanding these complex interactions involving Daphnia magna is essential for comprehending the dynamics of aquatic ecosystems and predicting how environmental changes may impact both Daphnia populations and the broader ecological communities they are a part of. Additionally, these insights can help in assessing potential implications for human activities, such as fisheries and water resource management.

**Ethical considerations and animal welfare:** The use of Daphnia magna in scientific research raises important ethical considerations and concerns about animal welfare. While Daphnia are small invertebrates and often considered to be less sentient than vertebrate animals, ethical guidelines and principles should still be followed to ensure their well-being and minimize harm. Researchers should take measures to minimize harm to Daphnia magna during experiments. This includes using non-lethal endpoints whenever possible, avoiding unnecessary stress, and providing appropriate care and housing conditions.<sup>155</sup> Adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement) is essential. Researchers should actively seek alternatives to animal testing (Replacement), use the minimum number of Daphnia required to achieve research objectives (Reduction), and refine experimental procedures to minimize suffering and distress (Refinement).<sup>156</sup> Studies involving Daphnia magna should undergo ethical review and oversight. Institutional Animal Care and Use Committees (IACUCs) or similar ethics committees should evaluate the proposed research to ensure that it meets ethical standards and legal requirements.<sup>157</sup> Investigators should be transparent about their methods and results, including any adverse effects on Daphnia magna. This transparency is essential for the scientific community to assess the ethical conduct of experiments and the validity of the research.<sup>158</sup> Individuals conducting experiments with Daphnia magna should receive appropriate education and training in animal welfare and ethical considerations. This ensures that researchers are knowledgeable about the best practices for minimizing harm to these organisms.<sup>159</sup> Whenever possible, scientists should explore alternative testing methods that do not involve live animals. For example, computer modeling, cell culture techniques, and in vitro assays can sometimes replace the use of Daphnia magna in toxicity testing.<sup>160</sup> Researchers should also be aware of relevant legal and institutional guidelines to ensure the welfare of Daphnia and other animals used in scientific studies.

## Conclusion

Daphnia studies are crucial for assessing the toxicity of substances in aquatic environments, providing insights into potential impacts on aquatic ecosystems and human health.

Daphnia serve as sensitive indicators of water quality, helping monitor environmental changes and evaluate potential risks to both aquatic ecosystems and human health. Research on Daphnia helps understand the effects of PPCPs on aquatic organisms, addressing concerns about human exposure through water consumption and potential health risks. Daphnia's role in nutrient cycling and food webs is essential for understanding energy transfer within aquatic ecosystems, indirectly influencing human nutrition and health. Daphnia magna is highly sensitive to a wide range of environmental stressors, including chemical pollutants, heavy metals, pesticides, and other toxic substances. This sensitivity allows researchers to detect adverse effects even at low concentrations, making Daphnia a valuable model organism for assessing the toxicity of various environmental contaminants. It provides an early warning system for potential ecological and human health risks associated with exposure to these substances. Daphnia magna has a rapid reproductive cycle, with individuals producing offspring asexually. This characteristic allows for quick and cost-effective toxicity testing, enabling researchers to observe the effects of contaminants on reproduction, growth, and survival within a relatively short timeframe. It is particularly useful for assessing chronic and sublethal effects. Daphnia magna is transparent, which makes it easy to observe internal structures and physiological processes under a microscope. Researchers can assess developmental abnormalities, organ damage, and other physiological responses to toxicants with high precision. This transparency simplifies data collection and analysis. Daphnia magna occupies a crucial position in aquatic food webs as a primary consumer of phytoplankton. Toxicological studies using Daphnia help determine the potential impacts of contaminants on entire aquatic ecosystems. Understanding how these substances affect Daphnia can inform ecological risk assessments and contribute to the preservation and management of aquatic environments. Daphnia magna has been widely adopted as a standardized test organism in ecotoxicological research. Various international regulatory agencies, such as the U.S. Environmental Protection Agency (EPA) and the Organization for Economic Co-operation and Development (OECD), recommend the use of Daphnia magna in toxicity testing protocols. This standardization ensures consistency and comparability of results across different studies and laboratories. Using Daphnia magna in toxicity testing is more ethical and practical than testing on vertebrate animals, as it involves fewer ethical concerns and does not require extensive ethical approvals. Additionally, maintaining and culturing Daphnia is relatively straightforward and cost-effective, reducing the financial burden of toxicological research. Daphnia magna toxicity tests have been shown to have a good predictive value for assessing the potential toxicity of substances to other aquatic organisms, including fish. This

predictive capacity is valuable in regulatory decision-making and risk assessment processes.

## Compliance with ethical standards

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### Disclosure of conflict of interest

The author of this article declares that he has no conflicts of interest that could potentially influence the results or interpretations presented herein. Top of Form

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