Open Journal of Nano
ISSN: 2147-0081
(2023) 7–1
Research Article

Toxicological Aspects and Bioanalysis of Nanoparticles: Zebrafish Model

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Submission Date: 15.10.2022 Acceptation Date: 29.11.2022

Abstract - Nanoparticles increase their availability and diversity in the environment day by day with the natural formation processes of the world geography and the development of advanced technological industry. Due to their intelligent and kaleidoscopic physico-chemical structural forms, they can cause toxic effects in various metabolic steps (in structural proteins, genetic structure, organelles, cells, tissues, organs, metabolic systems) in the organism. Despite these harmful situations some magnetite nanoparticles such as gold nanoparticles, silver nanoparticles, nanodiamonds, dendrimers, polymeric and liposomic smart nanoparticles can be used in medical studies, pharmaceutical industry, nanotheranostic studies and molecular methods. Zebrafish (*Danio rerio*), which is used a model species in many study disciplines, has been used in many studies to reveal the potential toxic effects and positive effects of the tested nanoparticles. Both *in vivo* and *in vitro* test systems and interdisciplinary studies conducted in recent years were analyzed and evaluated via the traditional review method in the current study. Besides, many studies were grouped in order to obtain fast and efficient results on the characterization of nanoparticles and understanding their mechanism of action. A systematic search was conducted based on the keywords of this study in databases such as PubMed, Google Scholar, Web of Science and Carrot², in May 2022. In addition to recognizing the toxic effects of nanoparticles, several studies were emphasized, in which the utilitarian status of nanoparticles in medical, pharmaceutical, molecular and genetic applied studies was understood more clearly day by day.

Keywords: Model species, Nanoparticle, Toxic, Zebrafish

Nanopartiküllerin Toksikolojik Yönleri ve Biyo-Analizleri: Zebra Balığı Modeli

Öz - Nanopartiküller, dünya coğrafyasının doğal oluşum süreçleri ve ileri teknolojik sanayinin gelişimi ile çevredeki bulunurluklarını ve çeşitliliğini her geçen gün arttırmaktadır. Akıllı ve sürekli değişen fiziko-kimyasal yapısal formları nedeniyle organizmada çeşitli metabolik basamaklarda (yapı proteinlerinde, genetik yapıda, organellerde, hücrede, dokuda, organlarda, metabolik sistemlerde) toksik etkilere neden olabilmektedirler. Bu zararlı durumlara karşın altın nanopartiküller, gümüş nanopartiküller, nanoelmaslar, dendrimerler, polimerik ve lipozomik akıllı nanopartiküller gibi bazı manyetit nanopartiküller medikal çalışmalarda, eczacılık endüstrisinde, nanoteranostik çalışmalarda ve moleküler yöntemlerde kullanılabilmektedir. Birçok çalışma disiplininde model tür olarak kullanılan zebra balığı (*Danio rerio*) test edilen nanopartiküllerin potansiyel toksik etkileri ile pozitif etkilerini ortaya çıkarmak için bir çok çalışmada kullanılmıştır. Halihazırdaki bu çalışmayla son yıllardaki hem *in vivo* hem de *in vitro* test sistemleri ile interdisipliner boyutlu çalışmalar geleneksel derleme yöntemiyle araştırılmış ve değerlendirilmiştir. Ayrıca nanopartiküllerin karakterizasyonları ile etki mekanizmalarını anlamak konusunda hızlı ve verimli sonuçlar almak için birçok çalışma gruplandırılmıştır. Mayıs 2022'de PubMed, Google Scholar, Web of Science ve Carrot² gibi veri tabanlarında bu çalışmanın anahtar kelimeleri baz alınarak sistematik bir tarama yapıldı. Nanopartiküllerin toksik etkilerinin anlaşılmasının yanı sıra medikal, eczacılık, moleküler ve genetik uygulamalı çalışmalarda nanopartiküllerin faydacı durumlarının her geçen gün daha da anlaşıldığı çeşitli çalışmalar vurgulanmıştır.

Anahtar kelimeler: Model tür, Nanopartikül, Toksik, Zebra balığı

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Open Journal of Nano
ISSN: 2147-0081
(2023) 7–1
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1. Introduction

While science has focused on optical and electronic devices with nanoparticle structure for the past half-century, it has now begun to include a large amount of research including nanoparticle-based biological studies and biological measurements made by nanoparticle methods. As the perspective of use of nanoscale materials in advanced technological studies expands, examining their possible toxic effects has become vital in terms of environmental welfare and public health [1]. Nanotechnology has modulated the chemical, physical and optical properties of metals at nanoscale and thus, gained great momentum in the 21st century. The term nano means one billionth of a meter, and these tiny dust particles formed the basis of two- or three-dimensional technological materials [2,3]. Nanoparticles can take many forms such as nanofibers, nanotubes, nanocomposites and nanostructured material [4]. In the use of nanomaterials, it has been shown in many studies that nanomaterials are classified according to their shape and morphology, as well as their composition, agglomeration, size, surface reactivity and uniformity [5,6]. Nanoparticles reach the environment through corrosion processes that occur naturally in the earth's structure, anthropogenic ways and advanced technological studies. Therefore, in recent years, we have had to encounter nanoparticles more and more in our daily lives. Two or three-dimensional particles smaller than 100 nanometers are called nanoparticles and are simply classified into three main groups including carbon-based nanoparticles, metal-based and metal-oxide nanoparticles, and semiconductor-based nanoparticles [3]. Toxic effects of nanoparticles in aquatic ecosystems are increasing in parallel with the increase in advanced technologies [7]. It is stated in many reports that industrial facilities and domestic wastes emitted to wetlands, and nano-sized materials in these wastes also mix with aquatic environments [8]. Many animal models have been used to determine the effect of nanoparticles. Zebrafish (*Danio rerio*), as an important animal model in recent years, has been found suitable for both *in vivo* and *in vitro* studies [9,10,11]. In this systematic review, the studies covering the effects of different types of nanoparticles, which are widely used in many industrial areas, on zebrafish, a member of aquatic ecosystems, in the years between 2007-2022 were discussed. In addition, the application areas, toxicological aspects and bio-measurements of nanoparticles in recent years have been examined. This review was to aim the forming basis for the logistics and problem-based selection of suitable nanoparticles for future studies.

2. Classification of Nanoparticles

2.1. Carbon-based nanoparticles

Carbon-based particles are mostly composed of carbon nanotubes, fullerenes and their derivatives, carbon black, nano diamonds, graphite nanoparticles, graphene nanoparticles and graphene oxide [12]. Fullerenes and multi-walled carbon nanotubes are in these nanoparticles and extremely insoluble in water. In a drug development study, the *in vivo* and *in vitro* effects of various nanoparticles were compared. Accordingly, it was observed that carbon nanoparticles (other than C60 fullerene) such as multi-walled carbon, single-walled carbon and mixed carbon nanotubes caused more stimulation of thrombocyte aggregation than conventional particle (SRM1648, size: 1.4 µ) and accelerated vascular thrombosis in the carotid arteries [13]. Nanodiamonds with their functional convenience and high biocompatibility [14], which have been used in drug design and gene transfer studies in recent years, and carbon C60 nanotubes [15,16] with their antioxidant capacity utilized in environmental, cosmetic and drug design have become very important. Unprocessed forms of nanoparticles, which are increasing in the environment day by day due to their usage areas in the electronics, informatics and aeronatics industries, remain in the air because they are very light. Hence, it has been determined that nanoparticles have the potential to affect living creatures toxicologically by respiration [17].

2.2. Metal-based and metal-oxide nanoparticles

Gold nanoparticles are widely used in biomedical fields due to their various advantages and properties such as adjustable size, easy synthesis, easy modification and strong optical properties [18]. It has been shown that nanoparticles in the cobalt-chromium mixture cause more free radicals, DNA damage, aneuploidy and cytotoxicity in soft tissue cultures than other nanoparticles [19]. The antibacterial, antifungal, antiviral and antifloral properties of silver nanoparticles lead to the use of these particles for biomedical purposes such as antimicrobial agents, drug delivery, molecular imaging, biomedical sensing and even cancer photodynamic therapy [20,21,22,23]. Zinc oxide is generally recognized as safe by the U.S. Food and Drug Administration (FDA) and is called GRAS for short [3]. Some researchers have stated that aluminum oxide (AlO) is less toxic than other metal-based nanomaterials [24,25]. However, crude aluminum has long been known to be a potential neurotoxin [26]. Aluminum oxide (AlO) nanoparticles have been shown to induce cell death by disrupting cellular components both *in vivo* and *in vitro* [27,28]. Testicular damage and changes in gene expression profiles of organisms induced by intragastric administration of titanium dioxide (TiO_2) nanoparticles were investigated. Accordingly, it was observed that TiO_2 nanoparticles crossed the blood-testicular barrier to reach the testis and caused testicular lesions, sperm malformations and changes in serum sex hormone levels. Therefore, the production and application of TiO_2 nanoparticles should be done carefully, especially by people of reproductive age [29]. It is believed that platinum (Pt) nanoparticles can release platinum ions through surface oxidation, which may contribute to their anti-cancerous properties [30]. Superparamagnetic iron oxide nanoparticles (SPION) have great utilitarian potential in a variety of biomedical applications, including magnetic resonance imaging (for example, as contrast agents), drug delivery, hyperthermia, transfections, *in vivo* cell tracking, and tissue repair [31,32,33,34].

2.3. Semiconductor-based and metal-oxide nanoparticles

Quantum dots are processed smart materials that are very important in medical imaging, biomedicine, nanobiotechnology, and microelectronics [35]. Quantum dots are nanometer-scale semiconductor crystals composed of group elements II-VI or III-V. They are defined as particles whose physical dimensions are smaller than the Bohr radius of the exciton [36].

Dendrimers are being used in drug delivery systems due to their flexible dimensions and sizes ranging from 1-100 nm [37]. Silica nanoparticles, also known as silicon dioxide, have received great attention due to their large surface area-volume ratios, adjustable pore size, and unique properties such as connectivity, biocompatibility, and ease of surface modification [38,39]. These features have made them attractive tools for biomedical and biotechnological fields such as medical diagnosis, drug delivery, gene therapy, biomolecule detection, photodynamic therapy, and bioimaging [40]. However, it has been shown that exposure to silica nanoparticles can induce the formation of ROS and cause oxidative stress [41]. Polymeric nanoparticles have been recognized as excellent drug carriers in cancer therapy due to their distinguished pharmacokinetic properties such as drug loading, drug release, structure stability and nanoparticle degradation [42].

3. Utilization and Importance of *Danio rerio* in Toxicity Studies

Nanotechnological elements are preferred in proteomic and genomic analyses using various biomarkers, in various imaging techniques such as ultrasonography (USG), in radiological fields such as magnetic resonance (MR), fluorescent imaging, nuclear and computed tomography, in molecular imaging techniques, in pharmacological studies including targeted cancer treatments and drug development studies, and in many other fields of research based on many other reasons [20,21,22,18,23]. In addition to such useful technological contributions to environmental welfare and human health, various studies have been carried out to determine the toxic effects of nanotechnological elements on respiratory, hematological, neurophysiological, gastrointestinal and dermatological systems due to their advantages in molecular structures [43].

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ISSN: 2147-0081
(2023) 7–1
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Animal models are useful in investigating the possible physiological, molecular and biochemical effects of such advanced technological products [44]. Not only do zebrafish have many physiological and genetic similarities to human beings including the brain, digestive system, musculature, vasculature, and innate immune system, but also 70% of human disease genes are functionally similar to those of zebrafish [10,11]. In these studies, it was stated that fish was an extremely important vertebrate model in various fields of developmental biology, genetics and toxicology [9].

Many topics have been studied on the effects of a wide variety of nanoparticles on the activities of tissues and organs of *Danio rerio*. These are basically the effects of nanoparticles on fish mortality, fertility, embryo development, oxidative damage, gene expression, hemostasis, body growth, exposure status, swimming performance, histological and biochemical changes followed by cancer diagnosis, drug development, fluorescence emission studies [45,46,47,48,49,50,51,52,53] (Figure 1-2).

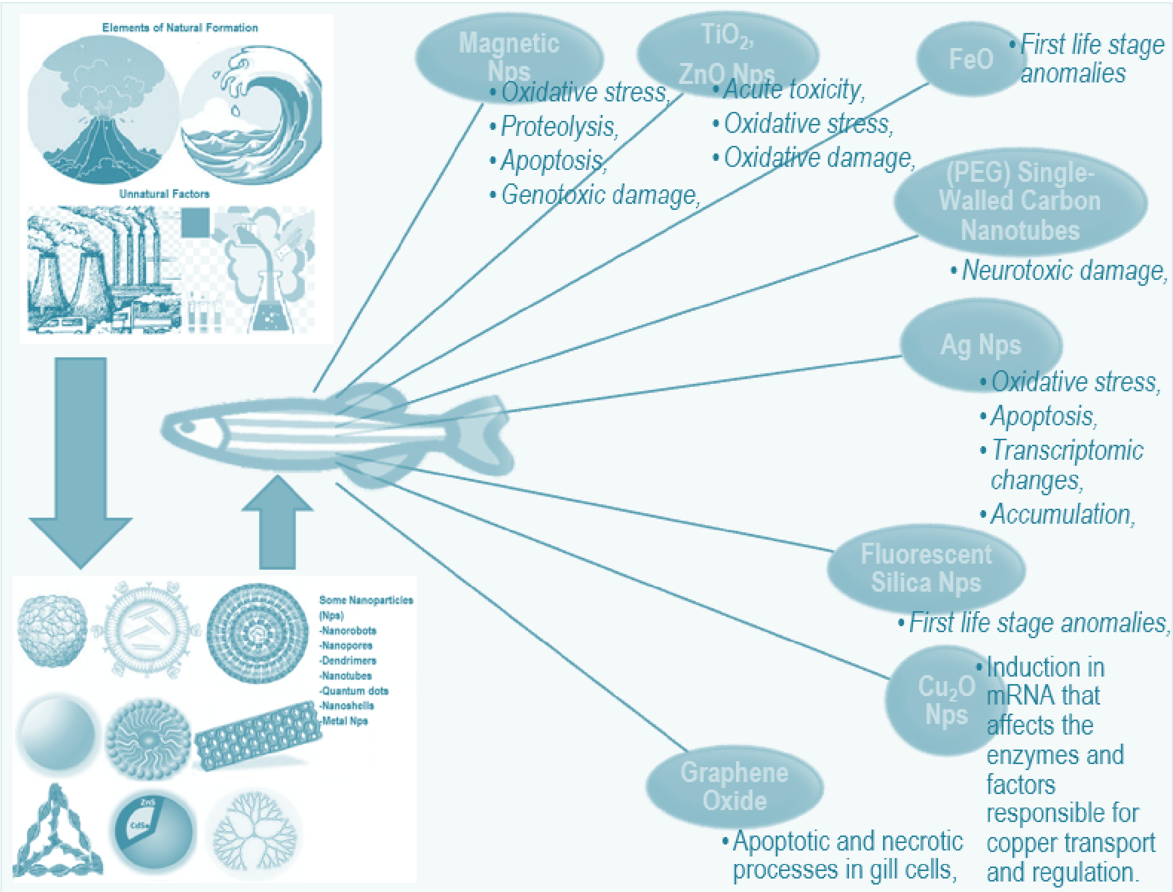

Figure 1. Intoxications of various nanoparticles on *Danio rerio*.

Zebrafish have become popular in the last quarter-century and have been used for a wide variety of purposes. To summarize some of them; it has played an important role in the diagnosis of diseases, parallel studies in genetics and experimental embryology, studies of embryonic axis formation, examination of organogenesis and neural networks, as it allows the development of transparent embryos to be observed in the embryonic development of such vertebrate organisms [54]. The timing of spawning of fish is provided under control with a molecular circadian timer in experimental studies, providing experimental convenience and control [55]. In adult zebrafish, both the fin and heart can quickly repair themselves after an experimental lesion. Zebrafish have optimized cost in all aspects

DOI: 10.56171/ojn.1189800

(time, labor, financial support) due to their ease of maintenance, small size and practicality of the experimental design, the ability to obtain hundreds of embryos per day, and the accessibility of early developmental stages and experimental speed, thus providing various advantages over other model species [54].

4. Bioanalysis Applications of Nanoparticles

A total of 454 articles with a nanoparticles content, which were published between 2007 and 2022 in different disciplines, were searched in web search engines such as PubMed (biomedical database), Google Scholar (full text or metadata of scientific literature), WOS (Web of Science) and Carrot² (cluster database), and the effects of nanoparticles on zebrafish were investigated in May 2022. Accordingly, a total of 155 articles on the effects of different nanoparticles on zebrafish and their distribution over the years were examined. In this study, the theme-based distribution of studies on zebrafish (*Danio rerio*) with a nanoparticles content was visualized by the cluster engine and Office software. Nanoparticular studies on zebrafish were carried out at most in 2021 (18.72%) and at least in 2012 (0.88%) (Figure 3). Although one may assume that as it is a fish species, the literature review about zebrafish should largely include zoological studies, the review of studies from different disciplines in this study revealed that the most studies were indeed conducted in the field of toxicology (40.53%) and the least in the field of zoology (6.17%) (Figure 4). This shows that zebrafish is an important experimental animal in research and development studies such as toxicity studies [56], drug development [57], nanotheranostic studies [58], and medical-molecular methods [59].

In studies on zebrafish in the fields of toxicology, environmental science, biochemistry and molecular biology, aquatic biology and zoology between 2007 and 2022, the most examined nanoparticles were silver-containing nanoparticles (29.03%), followed by titanium (14.84%), copper (12.90%) and metal-based particles such as zinc oxide (7.10%). The nanoparticles that were studied the least or whose effects aroused the least curiosity were identified to be polymethylmethacrylate (1.29%), fluorescent silica (1.29%), zero-valent iron (0.65%), chromium oxide (1.29%), lead sulfide (1.29%), cobalt coride (1.29%), ponatinib loaded PLGA-PEG-PLGA (1.29%), BSA/ASN/POL (407) (1.29%), cerium oxide (1.29%), palladium (1.29%), ferrous magnetites (1.29%), chitosan and those containing chitosan (1.29%) (Figure 5). Moreover, despite the low use of zero-valent iron nanoparticles in zebrafish, it was stated that zero-valent iron nanoparticles caused developmental toxic disorders in early life stages at a higher rate than oxidation products in another fish species, *Oryzias latipes* [60].

Various analyse methods were performed to determine the effects of different nanoparticles on zebrafish. The most common types of bioanalysis tests conducted were zebrafish's liver glutathione activity (GSH) (14.19%), apoptosis mechanism (10.97%), fish larval development anomalies (10.32%), polymerase chain reaction (qRT-PCR) (7.1%), and neurotoxicity (5.81%). Food chain (1.94%), environmental risk assessment (1.29%), fish skeletal development (1.94%), and mRNA-enabled studies of the first life stage (1.94%) were among the least studied subjects (Figure 6). Despite the limited number of studies conducted on the trophic transmission of TiO_2 [61] and ZnO [62] nanoparticles to zebrafish, trophic-level environmental monitoring studies were based only on certain nanoparticles. In fact, there was very little information about these substances due to many reasons such as the lack of methods in distinguishing the size and form of nanoparticles, and the uncertainty of their long-term toxicological effects. As a result, it becomes difficult to follow the studies on this subject in environmental welfare and public health studies [63].

In a study, in which an inulin-based fructan obtained from chicory plant extract was structured and characterized with selenium nanoparticles (CIP-SeNPs) to improve antitumor effects, CIP-SeNPs were found to significantly suppress the proliferation and spread of tumors as well as angiogenesis in transgenic zebrafish in the concentration range of 1-4 μg/mL [57]. It was shown that PLGA-PEG-PLGA nanoparticles loaded with ponatinib at a concentration of 001 mg/ml were not cardiotoxic in

Open Journal of Nano
ISSN: 2147-0081
(2023) 7–1
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the conventional zebrafish xenograft model [64]. It was also revealed that many nanoparticle therapies used in the diagnosis and treatment of various types of cancer could be usefully utilized as an initial step (Figure 7). In addition, gold nanocluster-based ratiometric fluorescent probes were used as a sensitive biosensor for rapid biological detection and imaging of mercury ions in zebrafish [65]. Titanium dioxide nanoparticles (TiO_2) were reported to alleviate cardiotoxicity by reducing the bioconcentration of azoxystrobin and changes in cardiac-related gene expression in zebrafish larvae.

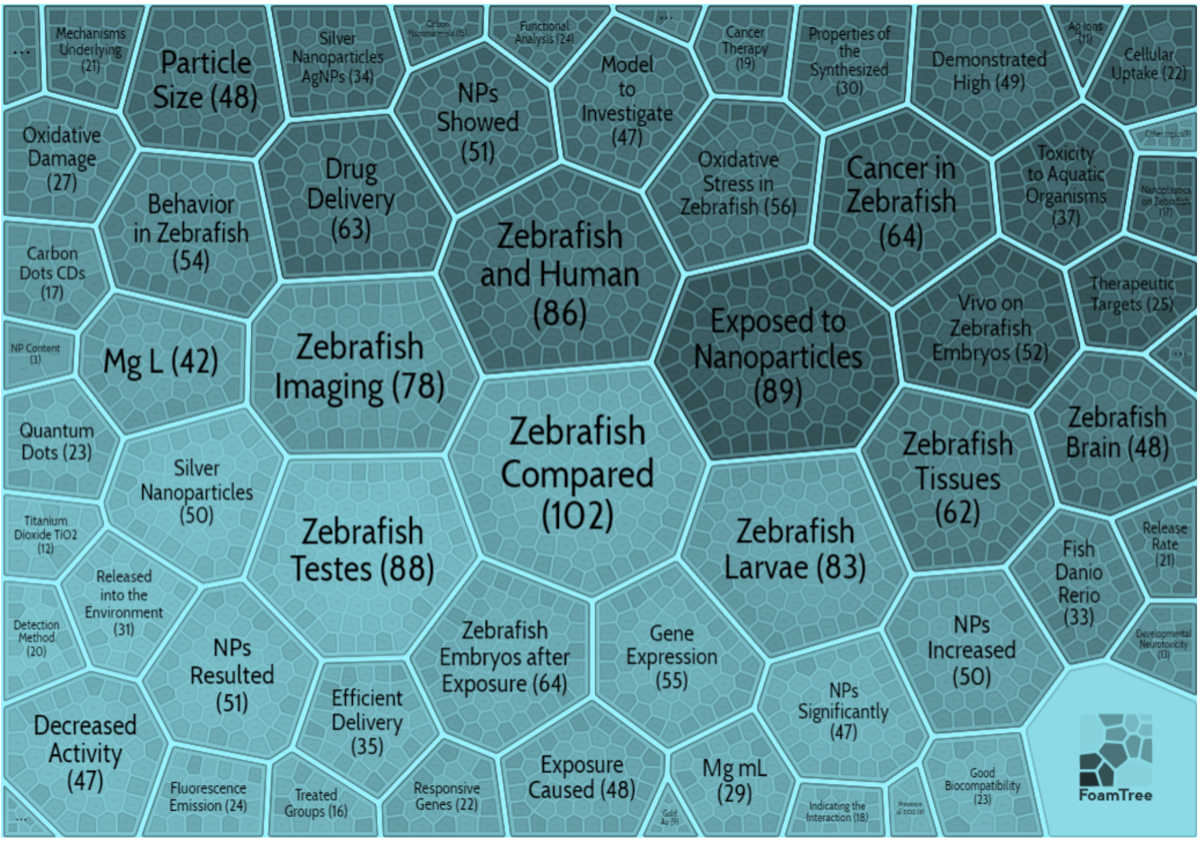

Figure 2. Thematic distribution of nanoparticle studies applied on *Danio rerio*.

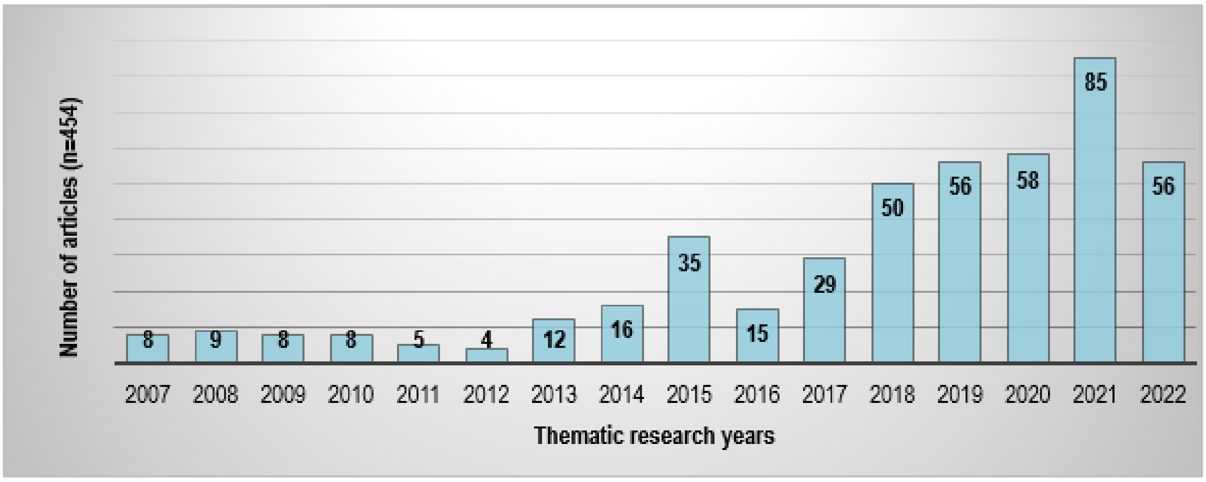

Figure 3. Distribution of nanoparticle-containing studies *Danio rerio* by years between 2007-2022.

In further studies, it was determined that azoxystrobin and TiO_2 nanoparticles together reduced total-ATPase and Ca^{2+} ATPase, Na^+/K^+-ATPase activities [66]. As a new approach to

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ISSN: 2147-0081
(2023) 7–1
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ecological risk management, this and similar studies are important in creating a biosensor in the organism as a protection method against the toxicity caused by the common relationships of pollutants in the environment and examining the changes in gene expression and enzyme activities as a response to the various effects of pollutants (Figure 7).

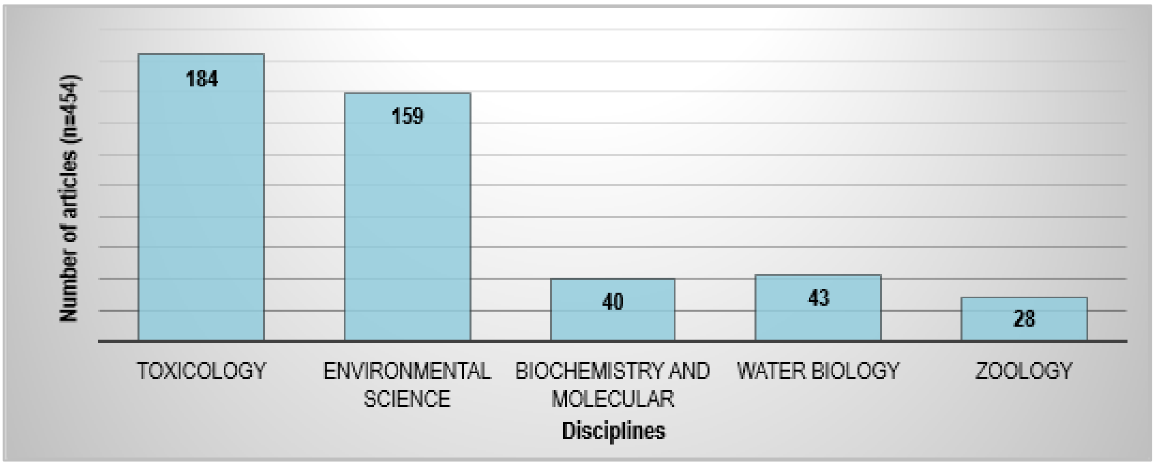

Figure 4. Distribution of nanoparticles studied on *Danio rerio* between 2007-2022 according to scientific disciplines.

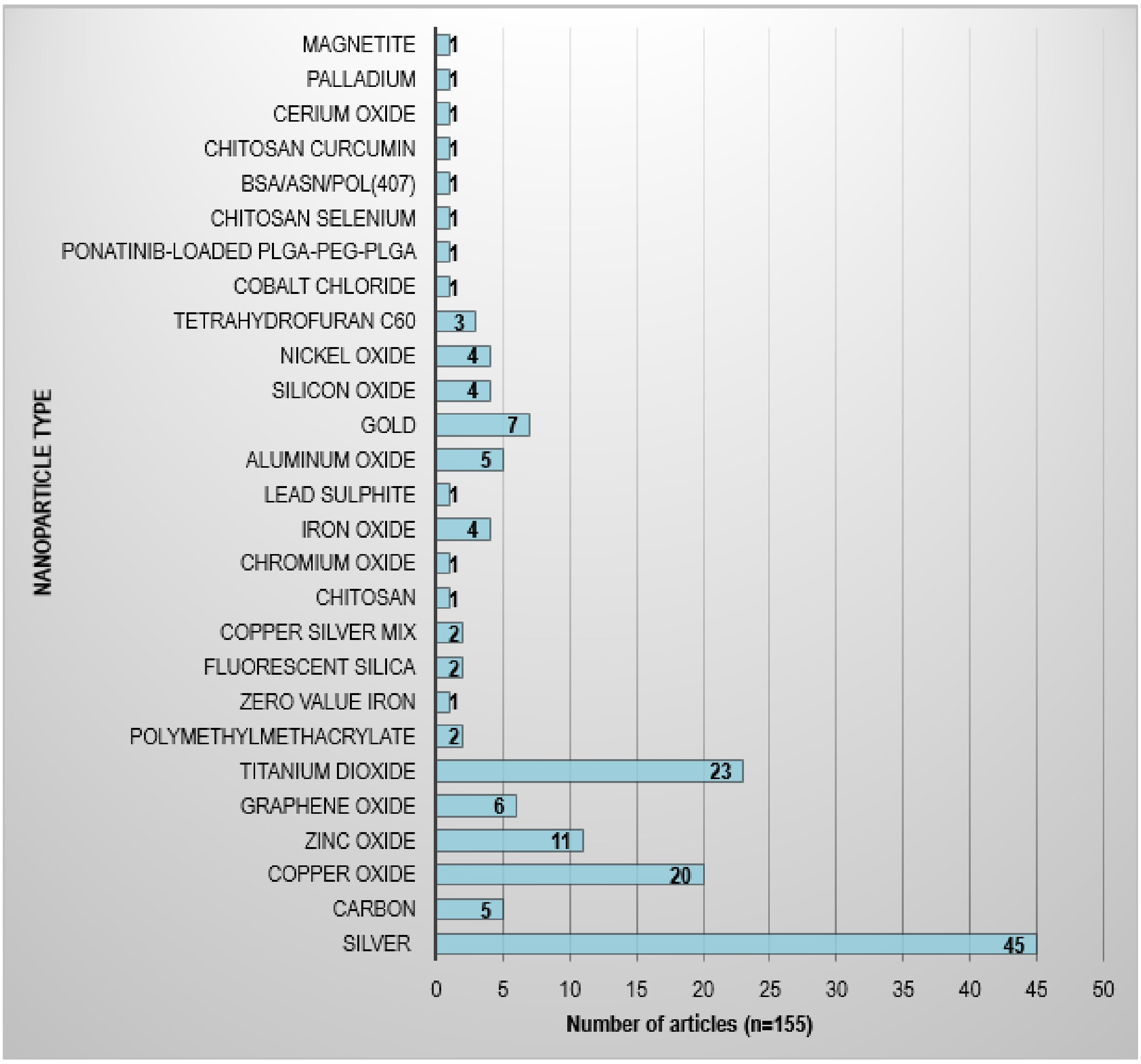

Figure 5. Number and types of nanoparticles studied on *Danio rerio* by years between 2007-2022.

DOI: 10.56171/ojn.1189800

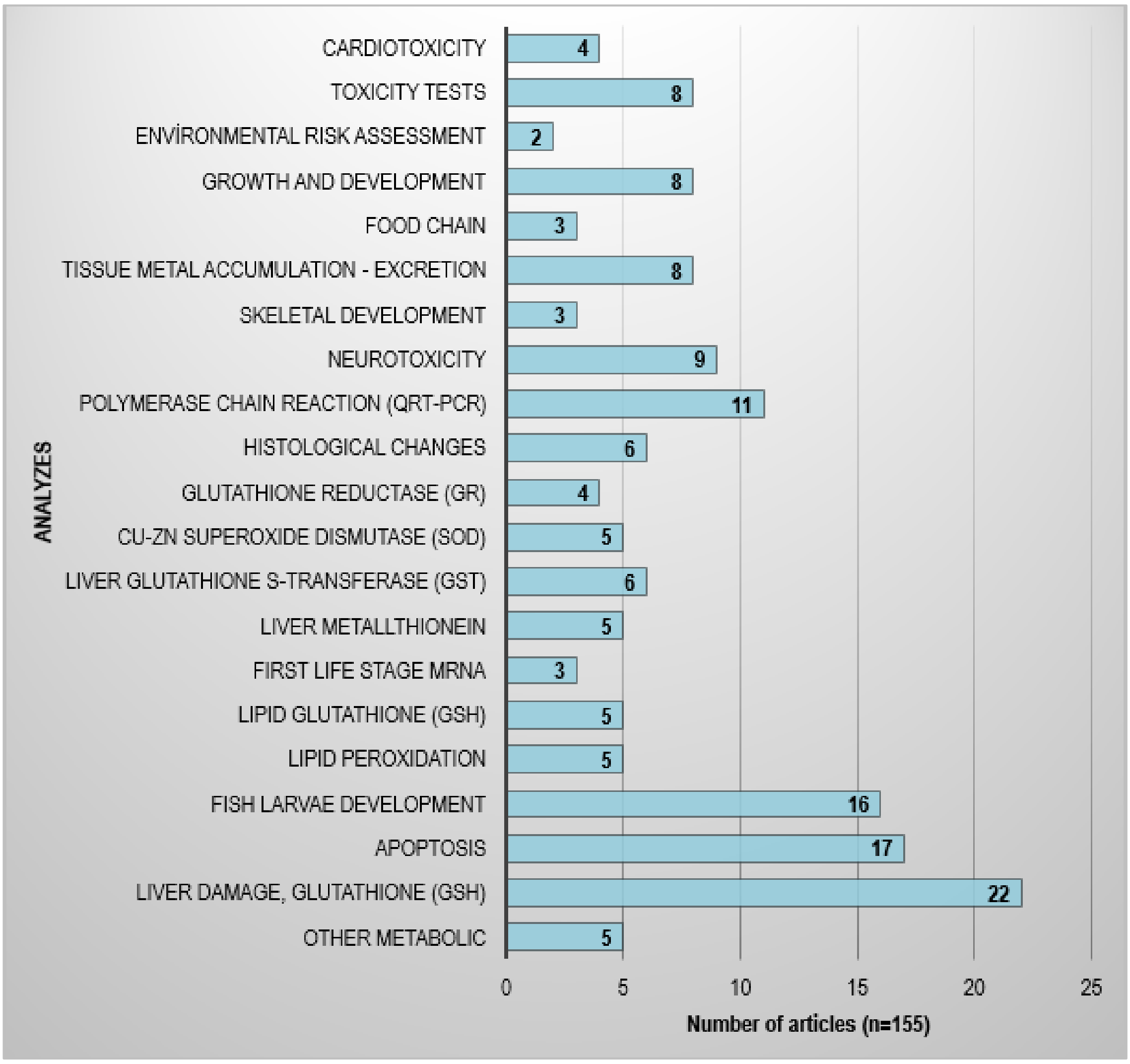

Figure 6. Analysis of the nanoparticle effects on *Danio rerio* in recent years.

5. Conclusions

The studies on the subject in pure zoology, biochemistry and molecular-based fields, which form the basis of applied sciences, are less than the others (Figure 4). It is thought that especially the small number of nanoparticles indicated in Figure 5 will provide advantages and innovations in medicine-based molecular studies and health technology (Figure 7). There is no homogeneous distribution between the environmental risk analyzes, metabolic function of the organism and mRNA studies of the analyzes of napoparticles and the oxidative stress parameters that have been studied much (Figure 6). It should be ensured that bioanalyses are functionalized in application areas with a holistic perspective.

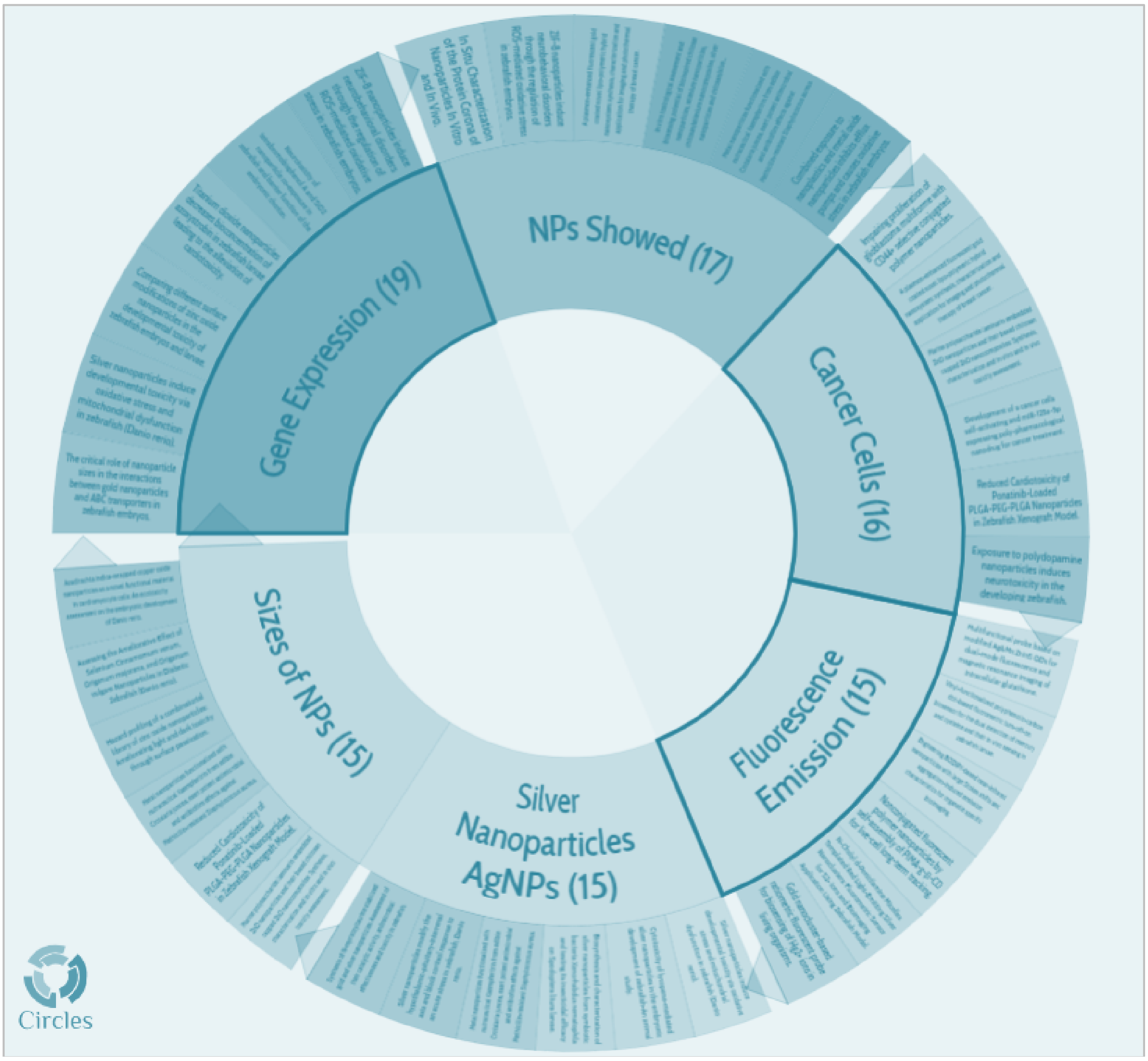

Figure 7. Emerging studies in the discipline of medicine and molecular biology.

The fact that the applicability and accuracy of similar standard test protocols of nanoparticles examined in various toxicological studies are not completely standardized may cause long-term misconceptions about reliability. For this reason, there is a need to standardize experimental protocols or use a new test system in risk assessment studies of nanoparticle. As a result, the toxic effects of nanoparticles on zebrafish have been observed in many *in vivo* and *in vitro* studies. Nanotechnological applications are increasing day by day, which means more contact of organisms with nanoparticles. Therefore, further research on the physiological, molecular and biochemical states of nanoparticles in living systems and specific standardizations in the comparison of these experimental studies are required. Consequently, the confusion about which particles have an effect and how they are characterized will be avoided and more results will be obtained in the studies of nanoparticles.

Peer-review: Externally peer - reviewed.

Author contributions: Concept – B.Y.; Data Collection &/or Processing - B.Y.; Literature Search - B.Y.; Writing - B.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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