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Metal nanoparticles – a real threat or harmless companions for the thyroid gland? A review

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ABSTRACT. The application of metal nanoparticles (NPs) in various industries is increasingly common due to their numerous beneficial properties. However, recent studies have demonstrated that NPs can also pose significant environmental risks, adversely affecting living organisms. The thyroid gland is one of the organs whose functioning can be impaired by metal NPs. Evidence from many studies indicates a possible toxic effect of metal NPs, mainly through the activity of reactive oxygen species on the functioning of the endocrine system, including the thyroid gland. Such disruptions may be manifested as altered organ mass, induction of inflammation, oxidative stress, genotoxic effects, and changes in the functioning of the hypothalamic-pituitary-thyroid axis. Moreover, the large specific surface area of NPs can increase the bioavailability of certain pollutants, thereby affecting the thyroid function of aquatic organisms. It is essential to note that the potential toxicity of NPs is influenced by numerous chemical and physical factors, and the resulting effects are also dependent on the research model and methodology employed. This review highlights the problem of the possible influence of NPs on thyroid gland function and the need for further research in this area.

Introduction

The introduction of new technological solutions in various areas of our lives leads to an increased frequency of their contact with organisms. This can be potentially dangerous, especially in the case of insufficiently tested chemical compounds. Endocrine-disrupting chemicals (EDCs) belong to a group of compounds that may negatively affect endocrine system homeostasis. According to the definition established by the Endocrine Society in 2012, these compounds are chemicals or mixtures of chemicals of external origin that can interact with any aspect of hormone action (Zoeller et al., 2012). The organisms of most living beings function as complex entities, relying on the cooperation of all bodily systems, including the endocrine network. Disruptions in this delicate equilibrium can result in a variety of health issues. One organ whose improper function due to EDCs can have serious health consequences is the thyroid gland (Zoeller et al., 2012).

Despite their wide range of applications, many EDCs, including nanoparticles (NPs) can negatively impact the surrounding environment by forming unexplored chemical combinations or exerting toxic effects on cells, tissues or entire organisms (Zoeller et al., 2012; Martínez et al., 2021). Recent literature points to the possible toxicity of many NPs, even those already implemented in different modern technologies (El-Kady et al., 2023). This raises concerns about future interactions between NPs and living organisms, particularly regarding delicate systems or organs such as the thyroid gland, which is known to be sensitive to external chemicals.

Therefore, in this review, we would like to focus on the action of factors that can interfere with thyroid function, and specifically on the potentially toxic effects of commonly used metal NPs.

Thyroid gland

The thyroid gland is a component of the multilevel (HPT) axis, which regulates various physiological processes, including energy balance, thermal homeostasis, growth, and development (Ortiga-Carvalho et al., 2016). In amphibians, the thyroid gland plays a crucial role in a process known as metamorphosis, making these animals valuable research models for studying thyroid disorders (Pickford, 2010; Brent, 2012).

Thyrotropin-releasing hormone (TRH), produced by TRH neurons in the hypothalamus, is the primary hormone of the HPT axis. It stimulates the synthesis and release of thyroid-stimulating hormone (TSH) from the anterior pituitary (Gavrila and Hollenberg, 2019). TSH action is mediated by the TSH receptor (TSHR), expressed mainly on thyroid follicular cells, where it stimulates the synthesis and release of thyroid hormones (THs) (Brent, 2012). Thyroglobulin (Tg) is a precursor and storage form of THs, whose synthesis occurs via iodination of tyrosine residues in thyroid follicles (Brent, 2012; Stathatos, 2019). THs form a negative feedback loop for two hormones: TRH and TSH (Bianco and da Conceição, 2018). Two important THs are thyroxine (T4) and triiodothyronine (T3), which exist in either free forms (fT3, fT4) or bound to proteins (Hoermann et al., 2016). While T4 is the main product of the thyroid gland, T3 is the more active hormone, and both exert their effects through binding to thyroid hormone receptors (THRs) (Ortiga-Carvalho et al., 2016; Bianco and da Conceição, 2018). One of the roles of THs is the regulation of the HPT axis. THRs are present in many tissues, allowing THs to influence various regulatory pathways through the modulation of transcriptional activity of specific genes (Ortiga-Carvalho et al., 2016). Furthermore, THRs can exist in different isoforms, such as THRα or THRβ, making these regulatory pathways even more complex (Nappi et al., 2022). The genes encoding these isoforms are *THRA* and *THRB*, respectively. The activity of the THs can be regulated, among others, by enzymes known as deiodinases. Three types of these enzymes can be distinguished: deiodinase type 1 (Dio1), deiodinase type 2 (Dio2) and deiodinase type 3 (Dio3), each of which exhibits varying expression levels in different tissues. They can also modulate the activity of THs either by activating or deactivating them through the catalysis of iodine release from their structures (Ortiga-Carvalho et al., 2016). THs enter cells via thyroid hormone membrane transporters, which include various types such as monocarboxylate transporters (MCTs), L-amino acid transporters or organic anion transporters (Ortiga-Carvalho et al., 2016). Among these, MCT8 is considered one of the most important, being a specific TH transporter, also involved in the secretion of these hormones (Brent, 2012; Bianco and da Conceição, 2018).

The functioning of the thyroid gland can be easily disrupted by both external and internal factors. This disruption may lead to various health issues, including infertility and carbohydrate metabolism disorders, owing to the close interrelationship between the HPT axis and other biological processes (Hage et al., 2011; Unuane and Velkeniers, 2020). The thyroid gland is strongly linked to the liver, a major metabolic organ responsible for detoxification, macronutrient metabolism and the mediation of immunological functions (Lepionka et al., 2021; Zhang et al., 2023a). Given its function, the organ is particularly exposed to EDCs, including nanomaterials. Some thyroid functional disorders, such as hypothyroidism or hyperthyroidism, may affect functioning of the liver (Piantanida et al., 2020). Approximately 22% of patients with nonalcoholic fatty liver disease also suffer from hypothyroidism (Vidal-Cevallos et al., 2023). The liver expresses THRβ1, one of the isoforms of THR, which enables THs to exert their peripheral effects through both genomic and non-genomic pathway. Additionally, the liver is the main source of T3 due to the presence of Dio1 and Dio3, where activation and inactivation of THs takes place (Gavrila and Hollenberg, 2019, Piantanida et al., 2020). Moreover, the liver synthesises the majority of proteins responsible for the transport of THs, including thyroxine-binding globulin (TBG), albumin (A) and transthyretin (TTR) (Figure 1) (Piantanida et al., 2020).

Nanomaterials

Nanotechnology is a field of science, that involves the use of a diverse range of nanomaterials, defined as substances or materials measuring less 100 nanometres (nm) in at least one dimension (El-Kady et al., 2023). Due to their nanoscale and shape changes, nanomaterials can gain new properties, making them eagerly used in various industries. The significant reduction in size can lead to changes in the physicochemical properties of materi-

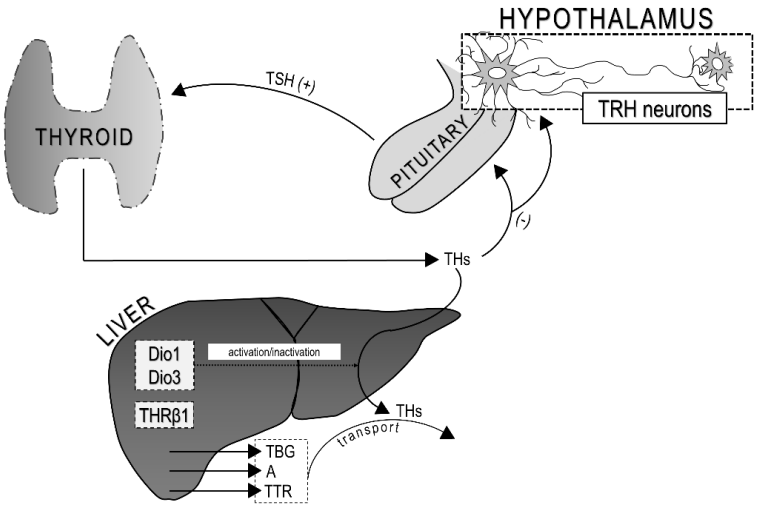

Figure 1. Diagram showing the interregulation of the hypothalamus, pituitary, thyroid and liver

TRH – thyrotropin-releasing hormone, TSH – thyroid-stimulating hormone, Dio1 – type 1 deiodinase, Dio3 – type 3 deiodinase, THRβ1 – thyroid hormone receptor β1, TBG – thyroxine-binding globulin, A – albumin, TTR – transthyretin, THs – thyroid hormones

als, including their optical, chemical, electrical and mechanical characteristics (Saleh, 2020). Numerous compounds have been synthesised at the nanoscale to explore their unique properties. This, in turn, has contributed to their introduction in industries such as electronics, agriculture, medicine, nutrition and many other fields (Ibrahim et al., 2020; El-Kady et al., 2023).

Nanomaterials can differ significantly in their properties and classifications. One common method of categorisation is based on dimensionality. Zero-dimensional nanomaterials have all their dimensions within the nanoscale range. One-dimensional and two-dimensional nanomaterials have one or two dimensions that extend beyond the nanoscale, respectively. With respect to three-dimensional nanomaterials, all three dimensions exceed the nanoscale, but are composed of multiple nanoscale blocks (Mekuye and Abera, 2023). Another classification involves categorising nanomaterials according to their chemical and electromagnetic properties, which are often influenced by their constituent compounds. For example, carbon is the structural foundation for a broad category of carbon nanomaterials, including carbon nanotubes, fullerenes or graphene. Other types of nanomaterials may include metal NPs, metal oxides and many others, providing various possibilities for their practical applications (Saleh, 2020).

Silver nanoparticles

Silver nanoparticles (SNPs), also known as nanosilver or colloidal silver, measure between 1 and 100 nm in at least one dimension. SNPs are mostly known for their antibacterial, antiviral and anti-inflammatory activity (Ge et al., 2014). These distinct chemical and physical characteristics have led to their extensive use in fields such as medicine, cosmetics, textiles, paints or packaging industry. SNPs can be also found in products that come into more direct contact with the body, such as toothpastes or contraceptive products (Ge et al., 2014; Zhang et al., 2022). However, recent research has highlighted concerns regarding the potential toxicity of SNPs. Scientific data indicate high contamination of both terrestrial and aquatic environments with silver, including SNPs (Blaser et al., 2008). Additionally, possible uncontrolled transformations of these NPs in the environment may exacerbate their toxic potential (Ge et al., 2014; McShan et al., 2014).

SNPs can enter the body through various routes, including inhalation, ingestion, skin contact or medical injections (Ferdous and Nemmar, 2020). Due to their exceptionally small size, when inhaled, SNPs easily pass through the lung epithelium to the blood, facilitating their transport to other tissues and organs. Permeation through the skin can occur in both damaged and healthy skin, raising concerns, especially given the widespread use of skin products containing SNPs (Zhang et al., 2022). Moreover, data indicate that SNPs can penetrate from the bloodstream into tissues such as testes, thymus, liver, lungs, or brain, affecting multiple regulatory pathways, including thyroid gland function. These effects have been observed in *in vitro* and *in vivo* studies in organisms such as rodents, frogs, and chickens (Carew et al., 2015; Kulak et al., 2018; Ferdous and Nemmar, 2020; Katarzyńska-Banasik et al., 2021).

Table 1. *In vivo* effects of silver nanoparticles (SNPs) on the thyroid gland and thyroid-related genes and hormones

Research object	Route	Period	Effect	Source
Male rats	IP injections	21 days	Elevated serum TH and TSH levels	Al-Bishri (2018)
Male rats	IP injections	14 days	Elevated serum T3 levels	Parang and Davood (2019)
Immature male rats	Oral administration	38 days	Elevated serum T3 levels, increased hypothalamic *Trh*, *Thra1*, *Thra2* and *Thrb2* and reduced *Dio2* mRNA expression. Reduced mRNA expression of *Mct8* and *Dio2* in the pituitary. Reduced mRNA expression of *Mct8* in the heart and liver.	de Oliveira et al. (2020)
Female rats	IP injections	20/30 days	Decreased serum T4 levels and histological thyroid changes	Sulaiman et al. (2018)
Frog tadpoles	Surrounding exposure	Two 28-days exposures	Disruption of TH-responsive targets	Carew et al. (2015)
Hens	Oral administration	14 days	Elevated serum T3 levels	Katarzyńska-Banasik et al. (2021)
Hens	Oral administration	14 days	Elevated serum T3 levels, increased *DIO3* mRNA expression in the thyroid gland, upregulated *DIO2* and downregulated *MCT10* mRNA expression in the liver	Katarzyńska-Banasik et al. (2024)
Male broiler chickens	Oral administration	12 days	Elevated plasma T3 levels	Saleh and El-Magd (2018)

IP – intraperitoneal; TH – thyroid hormone; TSH – thyroid-stimulating hormone; T3 – triiodothyronine; T4 – thyroxine; *Trh* – thyrotropin-releasing hormone; *Thra1*, *Thra2*, *Thrb2* – thyroid hormone receptors a1, a2, b2, respectively; *Mct8, MCT10* – monocarboxylate transporter genes; *Dio2* – type 2 deiodinase gene; *DIO2*, *DIO3* – type 2 and 3 deiodinase genes, respectively

Due to the widespread presence of SNPs in various environments, studies analysing their effects have been conducted in a variety of animal species. Despite the diversity of animal models and methodologies, similar conclusions have been drawn, indicating that SNPs may exert a disruptive effect on thyroid function (Table 1). *In vitro* studies, as well as experiments on rodents and birds, have consistently shown that SNP exposure can lead to increased blood levels of T3 or disruption of its signalling (Hinther et al., 2010; Al-Bishri, 2018; Saleh and El-Magd, 2018; Parang and Davood, 2019; Katarzyńska-Banasik et al., 2021). Elevated T4 levels were also observed following intraperitoneal (IP) SNP injections (Al-Bishri, 2018). On the other hand, in a similar procedure, Sulaiman et al. (2018) observed reduced T4 levels in rats' blood, with a concomitant increase in thyroid mass and its histological changes. Other reports have demonstrated that SNPs can affect TH signalling and disrupt T3-mediated transduction cascades in tail fin biopsy cultures derived from premetamorphic *Rana catesbeiana* tadpoles (Hinther et al., 2010). The study using both premetamorphic and prometamorphic tadpoles also indicated potential disturbances caused by relatively low, chronically administered SNP doses. These disruptions included bioaccumulation of nanomaterials, physical changes and impairments in the TH-responsive targets, such as altered mRNA expression of two peroxidase genes, indicating the involvement of reactive oxygen species (ROS) (Carew et al., 2015).

In addition to affecting THs, several reports showed the influence of SNPs on the activity of deiodinases. For instance, it was found that SNPs increased *DIO3* mRNA expression in the thyroid gland of Hy-line brown hens, which could cause TH degradation. This phenomenon was accompanied by elevated T3 levels in the blood. The same study also reported an increase in *DIO2* and a decrease in *MCT10* mRNA expression in the liver (Katarzyńska-Banasik et al., 2024). In a separate experiment on prepubertal male rats, SNPs affected the activity of deiodinases in various tissues: *Dio2* mRNA expression decreased in both the hypothalamus and pituitary, while *Dio3* mRNA expression increased in the heart and liver. Moreover, the expression of many marker genes related to the HPT axis, including *Trh, Thra1, Thra2, Thrb2 and Mct8* mRNA was examined. The results indicated that even relatively low doses of SNPs during puberty could alter the functioning of the HPT axis, thereby affecting thyroid function (de Oliveira et al., 2020).

Zinc oxide nanoparticles

Zinc, both in its bulk form and as zinc oxide (ZnO), is a metal widely utilised in many industries. Additionally, it is involved in numerous physiological processes, being an important factor in immune response and oxidative stress. Currently, ZnO is classified as a safe compound, which, combined with its properties, contribute to its widespread use,

also in the form of NPs (Fujihara and Nishimoto, 2024). Due to its effective protection against UVA and UVB radiation, ZnO is frequently included in sunscreens, where its nano form is preferred due to its less visible whitening effect after application (Sruthi et al., 2018). Another interesting characteristic of ZnO NPs is their antibacterial effect, which result from their photocatalytic activity and ability to generate ROS (Sruthi et al., 2018). Moreover, scientific reports indicate the potential antifungal properties of these NPs (Król et al., 2017). Consequently, in addition to sunscreen creams, ZnO NPs are incorporated into various everyday products, including cosmetics, toothpastes or food packaging. They also find extensive application or have the potential to be used in multiple medical fields. Examples include areas such as dentistry, bioimaging, drug and gene delivery, biosensors, and even cancer therapy, which can be attributed in part to Zn's involvement in many cellular pathways related to protection, such as activation of DNA repair processes (Elshama et al., 2018).

The classification of zinc as relatively safe pertains to its bulk form, which does not provide sufficient evidence for the safety of ZnO NPs. This is primarily due to the characteristic feature of NPs, which is their ability to obtain new properties (Fujihara and Nishimoto, 2024). Moreover, ZnO NPs can be synthesised using many biological, physical or chemical methods, and this multitude may also potentially affect the properties of NPs (Król et al., 2017). Scientific reports have indicated a possible toxic effect of ZnO NPs on numerous bacteria, as evidenced by their ability to inhibit their growth, or photosynthetic processes in cyanobacteria (Król et al., 2017).

In addition to the aforementioned toxic effects of ZnO NPs, there have also been reports regarding their negative influence on animals. For instance, after oral administration in rats, ZnO NPs undergo a chemical reaction that releases Zn^{2+} into the circulation. A similar reaction can occur in the acidic lung lining fluid of rodents upon exposure to ZnO NPs in the respiratory tract (Fujihara and Nishimoto, 2024). When administered orally, the liver is particularly sensitive to their accumulation, both after single and chronic administration. Moreover, the accumulation of ZnO NPs has been observed in various tissues, including the bones, spleen, brain, lungs and heart of rodents (Fujihara and Nishimoto, 2024). Currently, data suggest that the potential accumulation of ZnO NPs as a result following dermal administration is limited, indicating that this route of absorption into the body may be less significant. However, Osmond-McLeod et al. (2014) observed that repeated application of sunscreen creams containing ZnO NPs in mice could lead to increased Zn concentrations, primarily in the liver, but also in other organs. Contact with ZnO NPs can also induce changes in body and organ weights, a pro-inflammatory response, damage organs, and even lead to DNA fragmentation, as observed in studies involving rodents and rabbits (Yousef et al., 2019; Sakr and Steenkamp, 2021; Ashfaq et al., 2022; Fujihara and Nishimoto, 2024).

There are numerous reports on various animal species showing the possible impact of ZnO NPs on the functioning of the thyroid gland (Table 2). A work by Samy et al. (2022), conducted on broiler chickens, showed that ZnO NPs, applied as a dietary additive, did not affect TH levels and the T3:T4 ratio, either in green or chemically synthesised form. Some studies have indicated stimulation of thyroid function in rats after oral or IP administration of ZnO NPs, resulting in elevated TH levels, and decreased TSH levels in the blood (Yousef et al., 2019;

Table 2. *In vivo* effects of zinc oxide nanoparticles (ZnO NPs) on the thyroid gland and thyroid-related genes and hormones

Research object	Route	Period	Effect	Source
Male rats	Oral administration	75 days	Increased plasma TH and decreased TSH levels	Yousef et al. (2019)
Male rats	IP Injection	1 week 2 weeks 4 weeks	Decreased serum TH and decreased TSH levels	Luaibi and Zayed (2020)
Male rats	Oral administration (gastric intubation)	30 days	Decreased serum TH and decreased TSH levels. Increased weight and slight histological changes of the thyroid gland	Sakr and Steenkamp (2021)
Male rats	IP Injection	21 days	Increased serum TSH levels	Espanani et al. (2013)
Female rats	IP injection	1 week 2 weeks 4 weeks	Increased serum TH levels and decreased Tshr mRNA expression in thyroid gland. Additionally, after 4 weeks, decreased serum TSH levels	Lazim et al. (2021)
Male broiler chickens	Oral administration	35 days	No differences in TH levels and the T3:T4 ratio	Samy et al. (2022)

IP – intraperitoneal; TH – thyroid hormone; TSH – thyroid-stimulating hormone; T3 – triiodothyronine; T4 – thyroxine

Lazim et al., 2021). In addition, a decrease in *Tshr* mRNA expression was observed in the thyroid gland (Lazim et al., 2021). Similar results regarding the reduction of TSH levels in the blood after ZnO NP administration were also reported by other research groups conducting experiments on rats (Luaibi and Zayed, 2020; Sakr and Steenkamp, 2021). However, other studies indicated a decrease in THs levels in the blood after ZnO NP administration, possibly due to differences in the duration of administration, concentration, or size (Luaibi and Zayed, 2020; Sakr and Steenkamp, 2021). An increase in thyroid gland weight and slight histological changes were also observed (Sakr and Steenkamp, 2021). Conversely, research conducted by Espanani et al. (2013) found that in rats, IP injections of ZnO NPs led to an increase in TSH levels.

Gold nanoparticles

Gold has been known and widely used for millennia. It is considered chemically neutral to living organisms and shows relatively high biocompatibility and stability (Adewale et al., 2019; Hammami et al., 2021). Similarly to numerous other NPs, gold NPs (AuNPs) are used in practice due to their interesting physicochemical properties that differ from their bulk form (Niżnik et al., 2024). AuNPs can form many shapes, such as gold nanotubes, nanorods, nanospheres, nanocages or nanostars, which can influence their various properties (Hammami et al., 2021). The potential applications of AuNPs are broad, and they have already been introduced into many fields, including biomedicine. They have received approval for various biomedical applications, such as drug delivery, cancer therapy or biosensing (Sani et al., 2021). Additionally, they can be employed in bioimaging, radiotherapy, gene therapy and many other areas. Their anti-inflammatory potential is also widely recognised. Furthermore, these nanoparticles can be used for environmental purposes, such as water purification (Sani et al., 2021). Given their extensive use and high potential for functionalisation, there is a significant need for intensive research of AuNPs, particularly due to their regular contact with living organisms.

Studies analysing the safety of AuNPs yield inconclusive results. While many reports indicate their safety, there is also evidence of potential toxicity. These findings have been obtained in both *in vitro* and *in vivo* studies, using various cell lines, animal models and different forms of AuNPs, including variations in size, concentration or functionalisation (Niżnik et al., 2024). It is highly probable that AuNPs with a small diameter (below 5 nm) exert a more toxic effect compared to their larger counterparts (Adewale et al., 2019). Furthermore, the work of Chen et al. (2013) has suggested a possible link between gender and reaction to AuNPs, indicating that AuNPs could cause more severe liver damage in male mice than in females. Reports on the *in vivo* toxic effects of AuNPs in various forms and concentrations have mostly demonstrated accumulation in organs such as the liver, kidneys, brain, and spleen. They have also highlighted a potential impact on the processes such as DNA damage, apoptosis and acute inflammation in rodents and chicken broilers (Sani et al., 2021; Niżnik et al., 2024). However, it is worth emphasizing, that some studies have suggested that coating AuNPs with polymers or proteins may help prevent or mitigate the undesirable effects associated with these nanoparticles (Sabella et al., 2011).

There are few scientific reports examining the direct impact of AuNPs on the functioning of the thyroid gland (Table 3). However, AuNPs have been shown to affect functioning of the liver, an organ linked to the thyroid gland. In the work of Al-Bishri (2018), SNPs and AuNPs administered IP to male rats were analysed in terms of their effects on the parameters of liver and thyroid function. The animals showed altered activity of liver enzymes, a decrease in testosterone levels, and a change in thyroid hormone parameters, resulting in increased levels of THs and TSH in both study groups. Additionally, there are reports suggesting a possible impact of AuNPs on metamorphosis in frogs, where exposure to cetyltrimethylammonium bromide (CTAB) capped AuNPs shortened metamorphosis time by an average of 3 days (Fong et al., 2016). In another study, Weber et al. (2022) demonstrated the potential inhibitory effect of several organic and inorganic substances containing gold, including AuNPs, on human Dio1 using human liver microsomes.

Table 3. *In vivo* effects of gold nanoparticles (AuNPs) on the thyroid gland and thyroid-related genes and hormones

Research object	Route	Period	Effect	Source
Male rats	IP injection	21 days	Elevated serum TH and TSH levels	Al-Bishri (2018)
Frog tadpoles	Surrounding exposure	Up to 55 days	CTAB-capped AuNPs reduced time to metamorphosis by ~3 days	Fong et al. (2016)

IP – intraperitoneal; TH – thyroid hormone; TSH – thyroid-stimulating hormone; CTAB – cetyltrimethylammonium bromide

It was emphasised that some goldcontaining organic substances could inhibit selenoenzymes, including deiodinases. AuNPs with a diameter of 5 nm showed an inhibitory effect on human Dio1 in this study, indicating a possible adverse impact of AuNPs on health (Weber et al., 2022). However, due to the limited number of studies, the relationship between AuNPs and thyroid gland function requires further research.

Magnesium oxide nanoparticles

Magnesium is one of the most abundant elements found on Earth and plays a key role in optimal nerve transmission, catalysing numerous enzymatic reactions; it is also crucial for the synthesis of proteins, RNA and DNA (Kirkland et al., 2018). Magnesium oxide (MgO), one of the safe forms of this element, is widely used and recognised as a food additive by the European Union (EU) (Gelli et al., 2015; Yang et al., 2024). MgO NPs are characterised by their biocompatibility and biodegradability. Moreover, they possess antibacterial and antifungal properties, and can enhance mechanical strength, making them potentially useful in the fields of implant production, tissue engineering, cancer therapies, medical imaging, bioactive glass manufacturing or food packaging (Saberi et al., 2024, Yang et al., 2024). Potential safety of MgO NPs is further supported by their efficient metabolism and easy elimination from the body (Saberi et al., 2024). Nevertheless, given their current and prospective future applications, it is important to ensure that the use of MgO NPs does not pose health risks. While some studies have raised concerns about their safety, green-synthesised MgO NPs are considered a potentially safer alternative to commercially produced variants (Verma et al., 2020). Research suggests that MgO NPs exhibit cytotoxic effects primarily at higher doses rather than lower ones. Furthermore, they tend to show stronger cytotoxicity towards cancer cells than non-cancerous cells, which should increase their usefulness in medical applications (Majeed et al., 2018).

These NPs may potentially exhibit dose-dependent toxicity to rat lungs, as they were shown to affect certain tissue damage markers, such as alkaline phosphatase or lactate dehydrogenase activity (Gelli et al., 2015). They have also been demonstrated to increase shrimp mortality and pose risk to zebrafish embryos by increasing their mortality and decreasing heartbeat and hatching rates in a dose-dependent manner (Anjana et al., 2020; Verma et al., 2020). Mangalampalli et al. (2018) reported that NPs (<60 nm) and microparticles of MgO administered to rats led to Mg accumulation in numerous tissues, genetic material damage, increased chromosome aberrations and alterations in various morphological parameters such as platelet, red and white blood cell counts, haemoglobin levels or haematocrit percentage. Additionally, the activity of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were also affected. However, these changes occurred mainly due to the use of relatively high doses of MgO NPs.

Mg is also involved in thyroid function, particularly in the conversion of the inactive hormone T4 into its active T3 form. It is also crucial for thyroid iodine utilisation and may play a role in iodine transport (Moncayo and Moncayo, 2014; Zhou et al., 2022). A significant reduction in serum Mg levels, known as hypomagnesemia, is associated with many abnormalities, including altered thyroid activity (Pham et al., 2014). This can result in increased thyroid volume, or anomalies in TH levels (Zhou et al., 2022). Moreover, studies have shown that serum Mg levels are reduced in patients with thyroid cancer, whereas excessively high Mg doses could increase thyroid activity (Zhou et al., 2022). Other studies have reported possible effects of MgO NPs on the thyroid, although this effect appeared to be less toxic compared to other previously discussed NPs (Table 4). MgO NPs have been shown to influence the levels of THs both after IP and oral administration. Moreover, MgO has been demonstrated to exert positive effects against diabetes mellitus (DM) (Shaukat et al., 2022). Disorders of the thyroid gland are frequently associated with diabetes,

Table 4. *In vivo* effects of magnesium oxide nanoparticles (MgO NPs) on the thyroid gland and thyroid-related genes and hormones

Research object	Route	Period	Effect	Source
Male rats	Oral administration	30 days	Improved serum TH and TSH levels after diabetes induction	Shaukat et al. (2022)
Female rats	IP injection	14 days 28 days	Decreased serum TH and increased TSH levels	Obaid et al. (2022)
Female rats	Oral administration	30 days	MgO/ZnO core/shell NPs decreased serum T3 and increased T4 and TSH levels	Mohammed et al. (2022)

IP – intraperitoneal; TH – thyroid hormone; TSH – thyroid-stimulating hormone; T3 – triiodothyronine; T4 – thyroxine; ZnO – zinc oxide

particularly type 1 diabetes, although this can also occur in type 2 diabetes (Hage et al., 2011). In rats, chronic supplementation with MgO NPs may have a stabilising effect on the TH and TSH levels, which often fluctuate in DM patients. Additionally, MgO NPs may improve other disturbed parameters in DM patients, such as mean serum insulin, glucagon or glucose levels (Shaukat et al., 2022).

A possible negative effect of MgO NPs on thyroid function in healthy animals was observed in a study on rats, where low doses of MgO NPs were administered via IP injections. Reduced TH and elevated TSH levels were observed with both daily injections administered either for 14 or 28 days. It is likely that MgO NPs probably influenced the levels of T3 and T4, leading to a compensatory rise in TSH, producing effects akin to hypothyroidism. This may indicate a detrimental impact of MgO NPs on the functioning of the thyroid gland in healthy individuals (Obaid et al., 2022).

One approach that can modify the properties of NPs, and thus possibly reducing their toxicity, is the synthesis of core/shell structures (Ghosh Chaudhuri and Paria, 2012). Mohammed et al. (2022) created a structure with a core of MgO NPs and a ZnO NPs shell. This type of nanomaterial has been shown to affect thyroid parameters in rats when administrated orally. While only a few undesirable effects were observed – such as alterations in blood parameters, hair loss, and growth impairment – these could be linked to changes in thyroid function. In addition, these NPs reduced T3 levels, with the effect becoming more pronounced with increasing NP doses. Moreover, they were shown to increase T4 levels, even when applied at relatively small doses, as well as TSH levels, which could be an indirect effect of altering TH levels (Mohammed et al., 2022). It is possible that the MgO/ZnO core/shell NP structure exerted fewer toxic effects on the thyroid compared to ZnO NPs. However, the available information on the impact of MgO NPs on the thyroid gland are still insufficient to fully determine their toxicity and to compare it with that of MgO/ZnO core/shell NPs.

Titanium dioxide nanoparticles

Titanium is classified as a transition metal, occurring naturally as an oxide. Its most common form is titanium oxide (TiO_2), also known as titania. This compound has many applications, including in dental implants, sunscreen creams, and the pharmaceutical industry (Kim et al., 2019). It is known due to its pigmenting properties and is widely used as a white colorant in plastics and paints (Ziental et al., 2020). Additionally, it is used as a food additive (E171) for aesthetic purposes. TiO_2 NPs are among the most extensively applied NPs in the world (Cornu et al., 2022). The significant presence of TiO_2 NPs in sunscreens and toothpastes contributes to their release into the environment, including sewage, thereby affecting aquatic organisms (Luo et al., 2020). TiO_2 exists in three primary polymorphic forms: anastase, rutile and brookite, exhibiting different characteristics, including potential toxicity (Luo et al., 2020; Ziental et al., 2020; Cornu et al., 2022). The use of TiO_2 NPs as a food additive has become increasingly controversial in recent years due to concerns about their safety. The European Food Safety Authority (EFSA), has raised concerns about its potential to cause genotoxicity, leading to the additive's withdrawal from the EU market (Younes et al., 2021). Children are particularly vulnerable to the possible risks associated with E171, as it is commonly found in foods they favour, such as sweets, cakes or candies (Cornu et al., 2022). There is a substantial body of evidence from *in vivo* and *in vitro* studies, emphasising the potential harmful effects of TiO_2 NPs on bodily functions (Cornu et al., 2022). Prolonged exposure to TiO_2 NPs may pose a threat to human skin, although studies suggest that this nanomaterial probably does not penetrate the deeper layers of the skin (Luo et al., 2020; Ziental et al., 2020). Additionally, the form of these NPs used in sunscreen creams is usually coated with silica and alumina, which can modify its toxic properties (Luo et al., 2020). Nonetheless, there are works questioning the safety of TiO_2 NPs, indicating potential adverse effects on the functioning of nervous system, liver, kidneys and many other organs (Kirkland et al., 2022; Zhang et al., 2023b).

The safety of TiO_2 NPs has also been examined in terms of the proper functioning of the thyroid gland and its closely associated organs. TiO_2 NPs have demonstrated hepatotoxicity, with the severity of this effect potentially influenced by the dosage and route of administration (Kirkland et al., 2022). Additionally, given their ability to cross the blood-brain barrier, TiO_2 NPs may also affect the brain, causing neurotoxic effects (Zhang et al., 2023b). The neurotoxicity of these NPs raises the question of their possible influence on numerous hormonal regulations controlled by the brain, including the functioning of the HPT axis. Although the effects of TiO_2 NPs on thyroid functions have still not been thoroughly investigated, there seems to be more interest in this topic compared to some previously discussed NPs (Table 5). However, most of the results are derived primarily from studies on rodent

Table 5. *In vivo* effects of TiO_2 NPs on the thyroid gland and thyroid-related genes and hormones

Research object	Route	Period	Effect	Source
Male rats	IP injection	1 week 2 weeks 4 weeks	Decreased serum TH and increased TSH levels. Decreased thyroid gland weight	Luaibi et al. (2023)
Male and female rats	Oral administration	28 days	Increased thyroid gland weight	Akagi et al. (2023)
Male and female rats	Oral administration	5 days	Decreased serum T3 levels and changes in follicle shape (males), changes in follicular epithelium (males, females)	Tassinari et al. (2014)
Female mice	Oral administration (gavage installation)	30 days	Increased serum fT3, fT4 and TSH levels	Hong and Wang (2018)
Zebrafish larvae	Surrounding exposure	Until 7th day post fertilization	Increased effect of applied compounds on thyroid and related genes	Wang et al. (2014)
Zebrafish larvae	Surrounding exposure	Until 6th day post fertilization		Miao et al. (2015)
Zebrafish	Surrounding exposure	4 months		Guo et al. (2019)
Zebrafish larvae	Surrounding exposure	Until 6th day post fertilization		Lei et al. (2020)

IP – intraperitoneal; TH – thyroid hormone; TSH – thyroid-stimulating hormone; T3 – triiodothyronine; fT3 – free triiodothyronine; fT4 – free thyroxine

thyroid glands and aquatic organisms, which limits a comprehensive understanding of their potential harmfulness.

Luaibi et al. (2023) observed a decrease in thyroid gland weight after administration of TiO_2 NPs to rats. Conversely, Akagi et al. (2023) reported opposing results, although it is important to note that these differences were observed at specific doses that varied between males and females. Other studies conducted in rats showed that TiO_2 NPs injected IP or administered orally, lowered T3 levels in male rats, while the same effect was not observed after oral administration in females, further confirming that the action of TiO_2 NPs may be genderspecific (Tassinari et al., 2014; Luaibi et al., 2023). Additionally, changes in the thyroid follicular epithelium and follicle shape have been observed (Tassinari et al., 2014). In a study by Luaibi et al. (2023), TiO_2 NPs also exhibited a decreasing effect on T4 levels in rats. In contrast, research by Hong and Wang (2018) demonstrated that TiO_2 NPs increased fT3 and fT4 levels in female mice. Moreover, TiO_2 NPs were found to modulate TSH levels in rodents (Hong and Wang, 2018; Luaibi et al., 2023).

Co-exposure to TiO_2NPs and certain pollutants may exacerbate their negative effect on the thyroid gland in some aquatic organisms. Studies have demonstrated that compounds such as pentachlorophenol, bisphenol A, lead, and BDE-209 not only influenced TH levels, but also affected the expression of genes related to the proper functioning of the HPT axis in zebrafish, such as *dio2*, *tg*, *tsh*β or *ttr*. The addition of TiO_2 NPs often enhanced or completely altered the effects of these pollutants (Wang et al., 2014; Miao et al., 2015; Guo et al., 2019; Lei et al., 2020). This phenomenon was likely due to the large surface area of NPs. When present in water, TiO_2 NPs can absorb some pollutants and facilitate their entry into organisms in greater quantities than would occur in the absence of the NPs. Consistently, some other studies reported no effect on TH levels or the expression of *dio2*, *tg*, *tshβ* and *ttr* mRNA in zebrafish larvae when exposed solely to TiO_2 NPs (Wang et al., 2014; Miao et al., 2015). Similar results were obtained in tailfin biopsies from *Rana catesbeiana*, where these NPs did not affect *thra* and *thrb* transcript levels. This suggest that TiO_2 NPs may not significantly affect the thyroid gland of aquatic organisms when present in an unpolluted environment (Hammond et al., 2013).

While it is highly probable that TiO_2 NPs disrupt the functioning of the thyroid gland in rodents, they may not directly impair this organ in aquatic organisms at environmentally relevant doses. However, due to their physicochemical properties, TiO_2 NPs may enhance the absorption and thus concentration of pollutants in animals such as zebrafish, thereby contributing to endocrine disruption of the thyroid gland, making them potentially hazardous water contaminants.

Mechanism of action of metal NPs on the thyroid gland

The effects of metal NPs on the thyroid gland lead to various changes in this organ and its regulation. Most research indicates that NPs may primarily accumulate in organs such as the liver, spleen,

kidneys, brain, lungs or heart (Mangalampalli et al., 2018; Fujihara and Nishimoto, 2024; Niżnik et al., 2024), while accumulation in the thyroid gland is rarely mentioned. Possibly, NPs influence this organ indirectly, as a result of accumulation in organs involved in its hormonal regulation, expression of specific genes and protein changes. Some authors propose that the mechanism by which metal NPs affect the thyroid gland and other organs involves the increased ROS production and oxidative stress activation (Carew et al., 2015; Sakr and Steenkamp, 2021).

ROS are products generated during the oxidative metabolism of cells. The term ROS is broad and encompasses both free radical molecules and non-radicals (Averill-Bates, 2024). Under normal conditions, they are neutralised by a variety of antioxidants, such as superoxide dismutase, glutathione dismutase, glutathione peroxidase, catalase, vitamin C, vitamin E and glutathione. However, an excess of ROS may be harmful when the body is unable to adequately neutralize them. They can interfere with the body functions at the cellular and tissue levels (Yang and Lian, 2020; Wypych et al., 2024). Such disruptions may initiate effects such as a genotoxicity, carcinogenicity or inflammation (Dayem et al., 2017).

NPs can induce toxic effects by triggering inflammation, oxidative stress, genotoxicity or alterations in enzyme activity, similar to the effects caused by ROS (Hou et al., 2018; Sakr and Steenkamp, 2021). Mitochondria are among the most significant sources of intracellular ROS, and their activity can be influenced by the presence of metal NPs (Figure 2) (Sharma et al., 2011; Wang et al., 2014; Dayem et al., 2017; El-Kady et al., 2023). Although the precise mechanisms by which NPs affect ROS production remain unclear, they may vary depending on the type of nanomaterial used (Dayem et al., 2017). For example, SNPs, can impair mitochondrial processes after accumulating in the cytoplasm by affecting the respiratory chain electron transport, which results in increased ROS production (Kulak et al., 2018). Data indicate that many metal NPs can induce toxic ROS-mediated effects, as evidenced by alterations in the activity and mRNA expression of ROS-related enzymes such as catalase or peroxidases (Carew et al., 2015; Kulak et al., 2018; Sruthi et al., 2018). However, NPs typically disrupt the endocrine system, which may be associated with altered cellular functionality caused by excessive amounts of ROS. These general changes may also help explain the differing effects observed between adult and developing individuals, as well as between individuals of different sexes, due to different functioning of their endocrine systems.

The effects of metal NPs include alterations in the mass of the thyroid gland, histological changes, and disrupted production of THs, and TSH (Sulaiman et al., 2018; Sakr and Steenkamp, 2021). Moreover, some studies have provided evidence for the influence of metal NPs on the regulation of transcription of genes related to thyroid function and the HPT axis (Miao et al., 2015; de Oliveira et al., 2020). Collectively, these findings suggest that metal NPs affect thyroid function, however, likely in an indirect manner, by inducing ROS production (Carew et al., 2015; Parang and Davood, 2019; Sakr and Steenkamp, 2021). Nevertheless, the precise mechanisms of these interactions require further investigation.

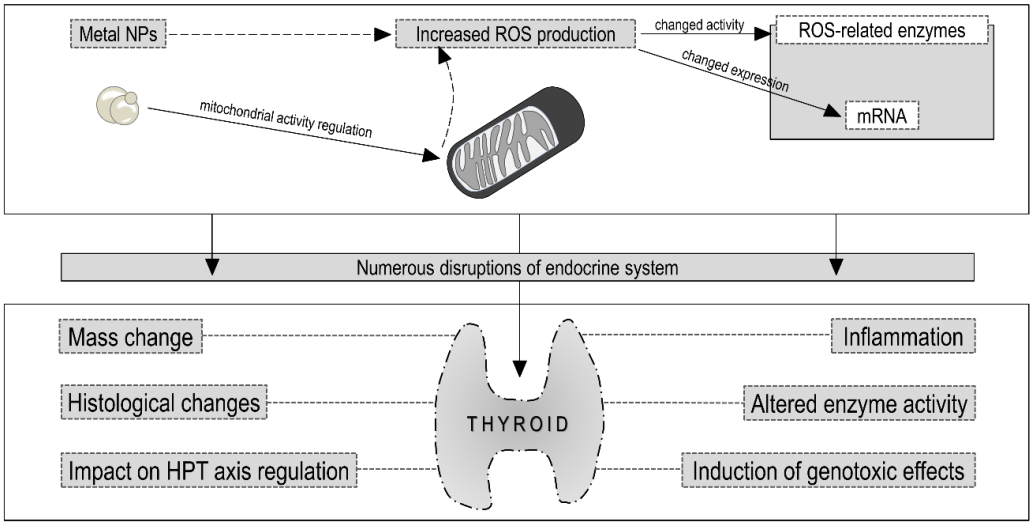

Figure 2. Diagram showing the mechanism linking reactive oxygen species (ROS) activity with the toxic effects of metal nanoparticles (NPs)
HPT axis – hypothalamic-pituitary-thyroid axis

Future perspective and limitation of the studies

Currently, organisms are increasingly exposed to simultaneous contact with different NPs, as well as with various other chemical compounds. Some studies have demonstrated that metal NPs can increase the accumulation of certain chemical compounds that have a negative effect on the thyroid gland (Wang et al., 2014; Miao et al., 2015; Guo et al., 2019; Lei et al., 2020). This underlines the need for research on the simultaneous impact of several NPs on organisms, but also the influence of different chemicals in combination with NPs. Moreover, the potential impact of NPs on various organs should be considered. In addition to the organs most frequently affected by NP accumulation, others, such as the thyroid gland, may also be affected by the potential negative effects of NPs (Tassinari et al., 2014; Luaibi et al., 2023). Further research will allow a more precise identification of the mechanisms responsible for the potential adverse effects following exposure to NPs. The current lack of sufficient data on the impact of NPs on organisms results in a limited number of legal regulations regarding their use. Consequently, the classification of their safety is based on incomplete data, potentially compromising their effectiveness. Additionally, as NPs become more prevalent in daily life, education and awareness of their potential risks are essential to prevent their overuse.

A more comprehensive understanding of the effects of NPs on living organisms will require studies across a wider range of species. The current number of species used to study the impact of metal NPs on thyroid function is limited. Most published data refer primarily to rodents, chickens, zebrafish and frogs. However, even within these groups, the number of studies remains low, restricting the ability to determine the actual effects of metal NPs on the thyroid. Furthermore, factors such as differences in sex, species, exposure duration, dosage, particle size, and methods of NP application and synthesis make it challenging to compare the results from different studies and draw definitive conclusions. While this methodological diversity is valuable for gathering varied insights into the effects of specific NPs, the limited number of studies investigating their impact on the thyroid gland complicates the assessment of potential negative effects

Nevertheless, data on the effects of NPs on living organisms are still relatively new. It is possible that the forthcoming years will bring further data on the potential effects of metal NPs in a broader range of animals and organs. This expanding body of research may ultimately uncover the mechanisms underlying NP toxicity and offer a deeper understanding of their biological impact.

Summary

This review focused on demonstrating the possible harmful impact of metal nanoparticles (NPs) on thyroid gland function. Current evidence suggests that not all metal NPs exert an equal effect on the thyroid gland. There are many studies highlighting the possible adverse influence of silver NPs on this organ. On the other hand, the modulatory impact of ZnO NPs on the thyroid gland seems to be less pronounced, likely due to the efficient and rapid removal of excess Zn from the body. Many researchers have also emphasised that AuNPs exert adverse effects mainly when their size is quite small, particularly below 5 nm in diameter. Additionally, MgO NPs are considered less toxic than many other NPs, while TiO_2 NPs remain controversial, especially when applied as a food additive. However, it is worth noting that studies have shown potential modulatory effects of all the discussed metal NPs on various organs, including the thyroid gland. Additionally, their possible harmful impact on this organ may be linked to their large surface area, which allows them to absorb pollutants, and disturb thyroid function. Although the number of studies examining the relationship between metal NPs and the thyroid gland has been significantly increasing in recent years, it remains insufficient for precise comparisons due to variations in research protocols used. Nevertheless, the scientific evidence collected by the researchers seems to indicate that metal NPs may disrupt the endocrine system of different species, including thyroid function, underscoring the importance of continuing research in this area, also using other animal models.

The controversy surrounding the topic of metal NPs does not necessarily imply the need to abandon their application. Factors such as the frequency of exposure and the method of administration play a key role in determining their toxicity. Even when adverse effects of metal NPs are confirmed, various methods, such as coating NPs, can alter their physicochemical properties, thereby potentially reducing their toxicity. Another important aspect is the dose of NPs, especially for those that tend to accumulate in tissues and are eliminated more slowly. Moreover, simultaneous exposure to multiple NPs may alter the perception of a safe dose, due to increased

activation of similar mechanisms responsible for inducing the toxic effect, highlighting the need for further research in this area.

Conflict of interest

The Authors declare that there is no conflict of interest.

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