Neurotoxic effect of active ingredients in sunscreen products, a contemporary review

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Abstract

Sunscreen application is the main strategy used to prevent the maladies inflicted by ultraviolet (UV) radiation. Despite the continuously increasing frequency of sunscreen use worldwide, the prevalence of certain sun exposure-related pathologies, mainly malignant melanoma, is also on the rise. In the past century, a variety of protective agents against UV exposure have been developed. Physical filters scatter and reflect UV rays and chemical filters absorb those rays. Alongside the evidence for increasing levels of these agents in the environment, which leads to indirect exposure of wildlife and humans, recent studies suggest a toxicological nature for some of these agents. Reviews on the role of these agents in developmental and endocrine impairments (both pathology and related mechanisms) are based on both animal and human studies, yet information regarding the potential neurotoxicity of these agents is scant. In this review, data regarding the neurotoxicity of several organic filters: octyl methoxycinnamate, benzophenone-3 and –4, 4-methylbenzylidene camphor, 3-benzylidene camphor and octocrylene, and two allowed inorganic filters: zinc oxide and titanium dioxide, is presented and discussed. Taken together, this review advocates revisiting the current safety and regulation of specific sunscreens and investing in alternative UV protection technologies.

Keywords:
Neurotoxicity
Sunscreen
Zinc oxide
Titanium dioxide
Octyl methoxycinnamate
Benzophenone-3
4-Methylbenzylidene camphor
Octocrylene

1. Introduction

Sunscreen application is the main strategy used to prevent the maladies inflicted by the sun since the 1930s. Unfortunately, although global use of sunscreen is continuously on the rise, so is the prevalence of malignant melanoma – a cancer type which is mainly caused by sun exposure [1–4]. There are several types of electromagnetic radiation emitted by the sun. One type – ultraviolet (UV) radiation – is composed of three wavelengths: UVA rays, which range at 320–400 nm and are not absorbed by the ozone layer, UVB rays, which range 290–320 nm and are partially absorbed by the ozone layer, and UVC rays, which are stopped by the ozone layer. The detrimental effects of exposure to UVA and UVB rays, which can cross the epidermis, have been reviewed and it was concluded that such exposure leads to reactive oxygen species (ROS) generation, DNA/protein/lipid damage, activation of various signal transduction pathways, compromised skin defense systems, altered growth, differentiation, senescence and tissue degradation, to name a few [5–7]. Two kinds of UV filters are currently being used in sunscreens for minimization of these adverse effects: organic (chemical) filters, e.g. octyl methoxycinnamate (OMC), benzophenone-3 (BP-3) or octocrylene (Table 1), which absorb light in the UV range, and inorganic (physical) filters, zinc oxide (ZnO) and titanium dioxide (TiO2), which scatter and reflect UV rays. Sunscreens are usually comprised of more than one of these UV filters: organic, inorganic or a mixture of both types, which gives broad-spectrum of protection. Beyond its debatable efficiency, questions regarding the main ingredients of different sunscreens are being raised in recent years, mainly about the prevalence of these ingredients in the environment and about their potential toxicity.

1.1. Human exposure and detrimental effects

Many factors might influence human exposure to UV filters: geographic location, season, lifestyle, gender or occupation, which means it can be highly individualized. For instance, a study in Australia showed 56% of people apply sunscreens at least 5 days per week, and 27% of
people use it less frequently — 2 or fewer days per week [8] and a study in
Denmark showed 65% of the sunbathers used one or more sunscreens
[9].

Dermal exposure is the most relevant entry route of chemicals re-
lated to sunscreen use, however considering a common human behavior
related to sunscreen application, e.g. eating and drinking with sunsc-
reen applied on hands and lips, gastrointestinal or pulmonary exposure
should also be considered [10]. The typically recommended mode of
application (2 mg/cm²) [13] implies a single dose of sunscreen
product may be as large as 40 g, assuming application on the total body
surface (2 m² for an average adult male), which for an average adult
male weighting 78 kg and a typical concentration of about 10% of ac-
tive ingredient in a commercial product, means maximum exposure
around 50 mg/kg body weight (bw) [14]. Simple calculation suggests
that with a maximum skin penetration up to 5% for some organic
filters [15], the total amount of compound absorbed from a single application
might be up to 200 mg, or 2.56 mg/kg bw, assuming an average bw of
78 kg for adult males. However, with application frequently thinner than
recommended, partial body cover and different properties of
compounds, these doses are usually much lower. For instance, a study
on Australian population showed that the median daily amount of
sunscreen applied was 1.5 g/day (range, 0–7.4 g/day) and the median
quantity of sunscreen applied was 0.79 mg/cm² [8], whereas sunbather
in Denmark applied on average 0.5 mg/cm² [9], in both cases it was
less than half the amount needed to achieve the labeled sun protection
factor.

Levels of UV filters found in human samples are usually low. In one
epidemiological study, 2517 urine samples from United States (US)
general population were analyzed for the presence of benzophenone-3
(BP-3), as part of the 2003–2004 National Health and Nutrition
Examination Survey [16]; BP-3 was detected in 97% of the samples, with
mean concentration of 22.9 ng/ml and 95th percentile concentration
of 1040 ng/ml. In another study, investigating correlation between couples’ presence of urinary benzophenone-type UV filters and
sex ratio of their offspring, the mean concentrations of these com-
pounds ranged from 0.05 ng/ml to 8.65 ng/ml, with BP-3 as the most
predominant among the study population (samples collected between
2005 and 2009 in Michigan and Texas) [17]. Interestingly, about nine
times higher than previously reported levels of BP-3 (up to 13000 ng/
ml, average around 200 ng/ml) were found in urine samples collected
in 2007–2009 from Californian females, which is probably a result of
specific demographics [18].

The experimental studies confirm substantial absorption and dis-
tribution of organic filters, whereas inorganic filters seem to penetrate
the human skin in a minimal degree. When adults applied a sunscreen
formulation containing 10% of BP-3, 4-methylbenzylidene camphor (4-
MBC) and octyl methoxycinnamate (OMC) on a daily basis (2 mg/cm²)
for a week, the mean urine concentrations for these ingredients were
60, 5, 5 ng/ml for females and 140, 7, 8 ng/ml for males, respectively
[19]. At the same time, maximum plasma concentrations for these in-
gredients, reached 3–4 h after application, were 200, 20, 10 ng/ml for
females and 300, 20, 20 ng/ml for males, respectively. Similar findings
were reported following a 4 day exposure to these ingredients, which
were detectable in the plasma of human males and females merely 2 h
following application [20]. More data on human skin penetration and
distribution of various UV filters, both organic and inorganic, can be
found in recent reviews [21,22,15].

Of importance, some UV filters were also found in human milk
samples. In a cohort study between 2004 and 2006, 54 human milk
samples were analyzed; UV filters were detectable in 46 samples and
levels were positively correlated with the reported usage of UV filter
products [23]. Concentrations of ethylhexyl methoxy cinnamate
(EHMC), octocrylene (OC), 4-MBC, homosalate (HMS) and BP-3 ranged
2.10–134.95 ng/g lipid, with EHMC and OC being most prevalent (42
and 36 positive samples, respectively) and an average of 7 positive
samples for the other three [23]. In other study, levels of BP-3 in ma-
ternal urinary samples taken in gestational weeks 6–30 were positively
 correlated with the overall weight and head circumference of the baby
[24]. These reports rise concerns about potential prenatal exposure and
developmental toxicity of UV filters.

Besides intentional sunscreen application, additional routes might
intensify human contact, namely occupational and environmental ex-
posure. Workplace contact may be a source of substantial exposure to
sunscreens, especially inorganic filters — nanoparticles (NPs) of ZnO

| Table 1                          |
|----------------------------------|
| Organic UV filters.              |

| UVB filters                                      | United States adopted name (USAN) | Other names             |
|-------------------------------------------------|-----------------------------------|-------------------------|
| 4-methylbenzylidene camphor*                    | Enzacamene                        |                         |
| Homosalate                                      | Homosalate                        |                         |
| Isomeryl-p-methoxy cinnamate                   | Amiloxate                         |                         |
| Octyl dimethyl PABA                             | Padimate O                        | OD-PABA                 |
| Octyl methoxycinnamate                         | Octinoxate                        | 2-ethylhexyl 4-methoxy cinnamate |
| Octyl salicylate                                | Octisalate                        | 2-ethylhexyl salicylate  |
| p-aminobenzoic acid                            | p-aminobenzoic acid               | 4-aminobenzoic acid, PABA|
| Tretihamalane                                   | Trolamine salicylate              |                         |

| UVA filters                                     |                                   |                         |
|-------------------------------------------------|-----------------------------------|-------------------------|
| Diodinphenyl dibenzimidazole tetrasulfonate     | Binidsulizole disodium            |                         |
| Butyl methoxy benzoxymethane                    | Avobenzone                        |                         |
| Methyl anthranilate                              | Meralidate                        |                         |
| Terephthalylidine dicamphor sulfonic acid       | Eramsule                          | Mesoryl SX              |

| UVB-UVA filters                                 |                                   |                         |
|-------------------------------------------------|-----------------------------------|-------------------------|
| Benzophenone-3                                  | Oxybenzone                        | 2-hydroxy-4-methoxybenzophene|
| Benzophenone-4                                  | Sulisobenzone                     |                         |
| Benzophenone-8                                  | Dioxybenzone                      |                         |
| 3-Benzylidene camphor*                          | Belotrizinol                      | Mesoryl SD              |
| Bis-ethylhexoxyphenol methoxy phenyl triazine   | Cinoxate                          | Tinosorb S              |
| Cinoxate                                        | Cinoxate                          |                         |
| Drometizole trisiloxane*                        | Mesoryl XL                        |                         |
| Methylene bis-benzotrianolyl Tetramethylbutylphenol | Biostrizole                     | Tinosorb M              |
| Octocrylene                                     | Octisalate                        | 2-ethylhexyl 2-cyano-3,3-diphenylacrylate|
| Pheny benzimidazole sulfonic acid               | Ensilizole                        |                         |

* Not approved by the Food and Drug Administration, used in other parts of the world.
and TiO2, which are frequently manufactured and stored as nanopowder. One study reported the presence of ZnO NPs in the work environment in an industrial scale plant in Japan. Electron microscopy analysis revealed the presence of a large number of submicron and micro-sized aggregated ZnO structures (concentrations not showed) [25]. Occupational exposures to TiO2 NPs have been reported more frequently and was summarized in a recent review, demonstrating that the respirable TiO2 concentration in the workers’ breathing zone might reach 150 μg/m³ [26]. Experimental studies suggest that with insufficient protection, inhalation of nanoparticles aerosol might result in pulmonary and systemic alterations. A single 10–30 min inhalation of a high dose (20–42 mg/m³) of ZnO NPs aerosol increased levels of the inflammatory cytokines interleukin (IL)-6, IL-8, and tumor necrosis factor α (TNF-α) in bronchoalveolar lavage fluid within 3 h after exposure in humans Kuschnier et al., 1997. However, chronic, low-concentration exposure is more likely in workplaces, its exposure in humans Kuschner et al., 1997. However, chronic, low-concentration exposure in workplaces, its effects are poorly known. Occupational exposure to sunscreen NPs was discussed more extensively in [12].

Environmental exposure is another way UV filters reach humans. Swimming in or drinking contaminated water might increase the contact, and thus absorption (through dermal and oral route) of these compounds. Recent data reviews indicate that the highest UV filter concentrations were found in rivers, reaching 0.3 mg/l for the benzo-phenone derivatives (e.g. BP-3), whereas ng to μg/l range were detected in lake and sea water. Moreover, lower levels (few ng/l) of organic UV filters were found in tap and groundwater [27–29,31]. Organic UV filters accumulate in wastewater treatment plants (WWTPs) up to mg/l concentrations, and since conventional WWTPs are not able to remove them, they are consequently released into rivers, lakes and oceans Ramos et al., 2016. Swimming pools are sinks for UV filters and its chlorine byproducts, at the μg/l range, or higher. Analysis by Sharifan et al. suggested that small, urban swimming pools might contain significantly higher, than in natural waters, levels of UV filters: 2.85, 1.9, 1.78 and 0.95 g/l, respectively of EHMC, OC, 4-MBC and BP-3, which question their safety for using them people, especially children [30].

Due to the widespread application of these compounds in many daily-use products and growing awareness of the risk associated with the sun exposure, the market of UV filters increases every year. Thus, increasing usage, persistent input and accumulation in environment is becoming an issue of great concern because of threat to human health, but also to the environment. UV filters were found to be ubiquitous in many aquatic systems and aquatic biota. Occurrence and impact (including toxicity) of UV filters on environment have been reviewed extensively elsewhere [27–29,31]. Aquatic organisms are frequently studied and UV filters were found at ng/g range in many of them, especially in fish and mussels, but also crustaceans, mammals and aquatic birds [28]. In a study of the presence of several UV filters in Swiss lakes and rivers (which receive input from waste water treatment plants and recreational activity), and the fish which live inside them, water concentrations of BP-3, 4-MBC, EHMC and OC ranged 2–35 ng/l, while lower limit of detection (LOD) in fish for those compounds was 3–60 ng/g, with concentrations reaching as high as 166 ng/g for 4-MBC [11]. Later study showed even higher levels of BP-3, 4-MBC and EHMC (range 6–68 ng/l) in Swiss river, moreover substantial amount of EHMC was found in fish (up to 337 ng/g) and in corromants (up to 701 ng/g), suggesting food-chain accumulation [10]. Many ecotoxicological studies addressed the potential damage of sunscreens and their components and in vitro experiments suggested that UV filters might be toxic for some aquatic microorganisms. UV filters were detected in nearshore waters around Majorca Island at variable concentrations: 53.6–577.5 ng/l for BP-3, 51.4–113.4 ng/l for 4-MBC, 6.9–37.6 μg/l for Ti, 1.0–3.3 μg/l for Zn, and various popular sunscreen formulations were shown to affect negatively the growth of local phytoplankton Chaetoceros gracilis, however at concentrations much higher than those detected in natural waters (EC50: 45–218 mg/l after 72 h treatment) Tovar-Sanchez et al., 2013. This is a typical observation. For instance, EC50 values of selected organic filters (e.g. BP-3, BP-4, EHM C, 4-MBC) in standardized toxicity assays on three aquatic species, Daphnia magna, Raphidocelis subcapitata and Vibrio fischeri, were in the mg/l range for all the species, which suggest minimal risk for these organisms in their natural ecosystems [32]. However, like many researchers suggest, toxic effects of chronic, low-dose exposures cannot be ruled out and require further investigations [32]. Moreover, with increased usage and lack of efficient removal, environmental contamination will probably increase in the future. Recent report on coral bleaching showed that environmental contamination with BP-3 already poses a hazard to coral reefs. The levels of BP-3 detected in coral reefs in the U.S. Virgin Islands (75–1400 μg/l) and Hawaii (0.8–19.2 μg/l) might lead to death of several local coral species with LC50: 8–340 µg/l and LC20: 0.062–8 µg/l (4 h exposure) [33].

Therefore, while sunscreens have been effective in protecting against a variety of UV-related pathologies, such as sunburns, actinic keratoses, squamous cell carcinomas and melanomas [34], growing popularity and thus, possibility for exposure questions their safety in environment and human health. Available data imply, that sunscreen compounds might block vitamin D synthesis or act as endocrine disruptor and lead to developmental toxicity. The effects of sunscreen on cutaneous synthesis of vitamin D induced by sunlight have been a subject of debate for recent years, however the newest analysis suggests, that normal usage of sunscreen by adults do not decrease cutaneous synthesis of vitamin D [35]. The endocrine disruptive and developmental toxicity of many organic UV filters in experimental models is well established, these filters seem to be associated with altered estrogen, androgen and progestrone activity, reproductive and developmental toxicity and impaired functioning of the thyroid, liver or kidneys, reviewed elsewhere [36,37,38,39]. Since many of UV filters were shown to cross the blood-brain barrier (BBB), the risk for neurotoxicity also occurs. In this review, the potential neurototoxicological effects of exposure to sunscreen have been discussed, as literature regarding the neurotoxicity of both organic and inorganic UV filters is presented.

2. Organic filters

Organic or chemical filters are the most popular and widely used in sunscreens and other cosmetic products. Data from 2003 indicate that over 80% sunscreen products contained OMC, 60% contained BP-3, and 20% contained octocrylene (OC) or HMS, whereas inorganic filters were present in around 20% of products [39]. Organic filters can be classified by the type of ultraviolet (UV) radiation they absorb, namely UBV, UVA or UBV-UVa filters (Table 1). As mentioned previously, the main route of human exposure is dermal absorption, however other routes and environmental exposure should be also considered. The last is particularly true for organic filters, which, due to their high lipophilicity could bioaccumulate in aquatic organism and reach humans through the food chain. Thus, they also are emergent as an environmental pollutant [40]. Chemical UV filters are easily absorbed by the skin and reach the systemic circulation, and accumulate in various tissues, as adipose tissue, liver and the brain [41–44]. Their lipophilicity permits them to readily cross the BBB, nonetheless, the effect of organic UV filters in the central nervous system (CNS) has been yet to fully addressed. However, there is a wide range of in vitro and in vivo studies of the toxic effects of UV filters as endocrine disruptors. And since it is known that other chemicals classified as endocrine disruptors can impair neuronal transmission, synaptic plasticity and produce neurotoxic effects [45], chemical filters might potentially produce similar effect. The documented neurotoxic effects of organic UV filters have been described below and summarized in Table 2.

2.1. Octyl methoxycinnamate

Octyl methoxycinnamate (OMC) is a UBV filter also known as
ocutnicotin and 2-ethylhexyl 4-methoxy cinnamate. This compound is approved as a cosmetic ingredient in US and in European Union (EU) in concentrations of 7.5–10% [1].

Dermal penetration of OMC has been measured in vitro, with values ranging from 0.2% to 4.5% of the applied dose, depending on the experimental conditions, however systemic absorption seems to be much lower. In humans, when a cream containing 10% OMC was applied to the entire body (40 g), OMC was absorbed through the skin and is detectable in blood (maximum concentrations 10 ng/ml in females and 20 ng/ml in males) and in urine (5 ng/ml in females and 8 ng/ml in males). Taking the highest detectable concentration (20 ng/ml) and assuming 4.7l of blood, the systemic absorption represents only 0.002% of the applied dose [19].

Several studies indicated that OMC acts as an endocrine disruptor due to the ability to interfere with endocrine system at different levels [47,59,60]. In vitro and in vivo studies in rodents have shown that OMC have estrogen activity [61,62]. In humans OMC exposure has minor, but statistically significant effects on the levels of testosterone and estradiol [19]. Moreover, some studies suggested that OMC can interact with the hypothalamo-pituitary-thyroid (HPT) axis [63]. Ovario-rectum rats exposed to 57.5 mg/20 g body weight of OMC applied via food presented a decrease in thyroxine (T4) levels without changes in triiodothyronine (T3) levels when administered orally (10–1000 mg/kg/day) for 5 consecutive days [47]. Experiments with rats showed that OMC (0.263 μM) decreases the hypothalamic release of GnRH and decreased GABA release in males. Decreased Asp and Glu production in females. Decreased hypothalamic release of LH/FSH. Decreased GABA release in males, decreased Asp and Glu levels in females. [43,45,46]
neurotransmitters aspartate (Asp) and glutamate (Glu), but not gamma-aminobutyric acid (GABA) in females, whereas in males decreased Glu and increased GABA release [48]. Similar results were found in hypothalamus isolated from immature rats (pre-pubertal and peri-pubertal males and females) [49]. These results indicate that OMC disrupts the normal neuroendocrine mechanism in a sex-dependent manner. Moreover, a study of offspring of dams treated with OMC (500–1000 mg/kg/day) showed sex-dependent behavioral changes, namely decreased motor activity in females, but not in males, and improved spatial learning in males, suggesting that OMC can affect neurodevelopment, however the doses used in these experiments were extremely high, not relevant to possible human exposure [46]. Corroborating these observations, recent studies in neuroblastoma cell line (SH-SY5Y) demonstrated that exposure to high concentrations of OMC (0.01–100 μM) decreased cell viability and increased apoptosis, however effective concentrations were not observed in vivo [50].

2.2. Benzophenone-3

Benzophenone-3 (BP-3, oxybenzone) is a common organic filter used in sunscreens and other personal care products (nail polish, lotions, lipsticks) in a maximum allowed concentration of 6% in US. It is used as broad-spectrum UV filter due to absorption of both UBV and short UVA rays [1].

BP-3 applied topically in human can cross the skin by direct penetration through the intercellular laminae of the stratum corneum (SC) or by passive diffusion by high-concentration gradient and then reach the blood [64]. When 25 volunteers applied a commercially available sunscreen containing 4% BP-3 for 5 days, their urine samples showed that approximately 4% of BP-3 is absorbed into the system [65]. BP-3 was detected in more than 80% of urine samples of healthy Danish children and adolescents (median concentration 0.92 ng/ml) [66]. Repeated (4 days) topical applications (2 mg/cm² of sunscreen formulation) of BP-3 resulted in urine levels up to 81 ng/ml and plasma levels up to 18 ng/ml [20]. When orally administrated to rats, 4-MBC reaches the liver where it is metabolized to 3-(4-carboxybenzylidene) camphor and 3-(4-carboxybenzylidene) hydroxycamphor [71]. 4-MBC exhibits a toxic activity as estrogenic endocrine disruptor [62,59,68]. Moreover, in vivo studies suggest that 4-MBC affected the thyroid axis [63].

Several studies described the effects of 4-MBC on developing neuroendocrine system. Rats exposed to 4-MBC (7–47 mg/kg) in diet before mating, during pregnancy and lactation, and in the offspring until adulthood, showed a region- and sex-dependent alteration in the oestrogenic genes in the brain [55,54,53]. For instance, the expression of progesterone receptor (PR) was decreased in the ventromedial hypothalamic area of 4-MBC-treated females, but not in males [55]. In addition, females showed impaired prospective and a non-receptive sexual behavior after 4-MBC exposure [55]. Female sexual behavior was significantly impaired at the lowest doses studied 7 mg/kg/day, which resulted in rat milk concentration of 208.6 ng/g lipid, which is over 10 times higher than value (19 ng/g lipid) found in human milk [55]. Subcutaneous administration of high dosages (up to 500 mg/kg/day) during pregnancy and lactation altered the hypothalamic secretion of excitatory amino acids Glu and Asp in male offspring. These neurotransmitters play a role as stimulators of gonadal axis, thus the observed changes are consistent with alterations in sexual development of male offspring, affecting pre-pubertal stage, but stimulating the peri-pubertal stage [56]. In addition, 4-MBC has been reported to have an acetylcholinesterase (AChE) inhibitory activity. Zebrafish embryos exposed to 15 μM 4-MBC for 3 days showed abnormal axial curvature and exhibited impaired motility. 4-MBC also impaired muscle development and axon pathfinding [57]; however, the dose used in the study was significantly higher than those detected in environmental aquatic media. Inhibition of AChE activity was also observed in mammalian Neuro-2a cells exposed to 10 and 100 μM for 45 min, indicating a possible mechanism for the 4-MBC-induced muscular and neuronal defects [57]. 4-MBC (up to 100 μM) has been shown recently to decrease cell viability and induce apoptosis in neuroblastoma cell line (SH-SYSY), suggesting possible neurotoxic effects, however again, effective concentrations were not observed in vivo [50].

2.3. Benzophenone-4

Benzophenone-4 (BP-4, sulisobenzone) is frequently used as UV absorber at concentration up to 10% [1].

BP-4 was found in human placenta (0.25–5.41 ng/g), suggesting efficient skin penetration and accumulation, which may lead to exposure of human embryos and fetuses [69]. BP-4, like BP-3 is a benzophenone derivative, yet its potency as an estrogenic disruptor has not been well defined.

In zebrafish, adult males exposed to high concentrations (3000 μg/l) of BP-4 for 14 days displayed estrogenic activity by up-regulation of estrogenic-related genes: vitellogen 1 (vgt1), vitellogen 3 (vgt3) and the cyp19b in the brain, however lower dosages did not induce changes. In contrast, in the liver, some of these genes (vgt1, vgt3) were down-regulated [52]. No other effects in the nervous system were reported.

2.4. 4-methylbenzylidene camphor

4-methylbenzylidene camphor (4-MBC) or enzacamene is an organic camphor derivative used as a UVB filter in sunscreen and other cosmetic products. Although the compound is not approved by the Food and Drug Administration (FDA), other countries allow it usage at maximum concentration of 4% [1].

4-MBC is a high lipophilic component which can be absorbed through the skin and was found in human tissues, including placenta [70]. Repeated (4 days) topical applications (2 mg/cm² of sunscreen formulation) of 4-MBC resulted in urine levels up to 4 ng/ml and plasma levels up to 18 ng/ml [20]. When orally administrated to rats, 4-MBC reaches the liver where it is metabolized to 3-(4-carboxybenzylidene) camphor and 3-(4-carboxybenzylidene) hydroxycamphor [71]. 4-MBC exhibits a toxic activity as estrogenic endocrine disruptor [62,59,68]. Moreover, in vivo studies suggest that 4-MBC affected the thyroid axis [63].

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2.5. 3-benzylidene camphor

3-benzylidene camphor (3-BC) is a lipophilic compound closely related to 4-MBC. It is used in sunscreen products in EU, at a maximal concentration of 2% [1].

After topical application to rats for 6 days (60–540 mg/kg/day) 3-
Octocrylene (OC) is an ester belonging to the cinnamates family and is present in sunscreen and daily care cosmetic products at a maximal concentration of 10%. It can absorb UBV and high energy components of UVA radiation [74]. To date, there are several studies on its accumulation and toxicity, especially in aquatic organisms [75–77]. Zebrafish embryos and adult male exposed to environmentally relevant concentrations of OC in water (22–925 µg/l) absorbed and accumulated this compound. Moreover, the microarray analysis from adult zebrafish male exposed to OC (383 µg/l) showed major impairments in the expression of 628 genes in the brain regulating mainly developmental processes and 136 genes in the liver, responsible mainly for metabolism [58].

2.6. Octocrylene

Inorganic (physical) ingredients used in modern sunscreens include metal oxide particles, typically titanium dioxide (TiO₂) and zinc oxide (ZnO), which occurs typically at 5–10% concentration (maximum allowed is 25%). While chemical filters still dominate in sunscreen products, the usage of physical compounds is constantly growing. One of the reasons is that they have a higher spectrum of protection - TiO₂ is very effective in absorbing UBV, while ZnO absorbs mainly the UVA range, and the combination of both particles provides a broad UV protection. Other advantages of physical filters are lack of skin sensitization and limited skin penetration [78]. However, these mineral filters, when in normal pigment size range (200–400 nm for ZnO, 150–300 nm for TiO₂) have poor particle dispersion, which makes them difficult to apply; they also reflect and scatter light, which result in undesirable visible white film on the skin. With nanotechnology, these materials can be reduced to nanoparticles (NPs) (<100 nm) which are easier to apply and are transparent on the skin [12]. Nevertheless, with micromanization some properties are changed - they may be more bioactive and easier penetrate the skin and other tissues, leading to concerns about their safety use. Moreover, part of the absorbed UV radiation can generate free radicals on the surface of metal oxides in the presence of water and this photocatalytic activity increases with decreasing NPs size. NP-induced cyto- and genotoxicity has been associated with increased photocatalytic activity, leading to increased production of free radicals [79]. Despite increased awareness of nanomaterials toxicity, the nanoneurotoxicity is a relatively new field with numerous data gaps awaiting improvements. One of the main reasons for this is the lack of reliable methods for NPs detection and quantification. Only estimates and predictions about NPs concentration in natural environments are available, and they suggest that TiO₂ might be present in the range 0.7–24.5 µg/l, whereas ZnO might reach higher levels, up to 76 µg/l [80]. Analogously, NPs accumulation and physiological concentrations are difficult to assess; thus, most studies report changes in Zn and Ti ion levels only. This also raises questions regarding the relevance of predominantly high-dose exposures used in toxicological studies. To date, most studies attesting to neurotoxic effect of NPs have been carried out in acutely high concentration exposure scenarios, and their relevance to “real-life” exposure scenarios needs to be further assessed.

3. Zinc oxide

Zinc oxide nanoparticles (ZnO NPs) are used not only in sunscreens, but also in pigments (UV-absorbers, paintings) and electronic equipment (thin film transistors, semi-conductors, liquid crystal displays, light-emitting diodes) due to their exceptional optoelectronic, piezoelectric, ferromagnetic and optical properties. Moreover, their anti-septic activity makes them potentially useful in treatment of bacteria-related infections or diseases [81]. As their commercial utilization has increased, wider application raises the potential risk of human exposure [82].

3.1. ZnO NPs absorption and transport across the BBB

Several in vitro and in vivo studies evaluated the fate and toxicity of ZnO NPs from different exposures: dermal, gastrointestinal or pulmonary. Dermal absorption is a major route of ZnO NPs exposure from sunscreen application. Most studies demonstrated that ZnO NPs did not penetrate into deeper layers of the skin (SC) [83,84,22,85,86]. However, some data indicated that ZnO NPs penetrated the skin to a limited extent. A small increase of zinc ions (Zn²⁺) in the blood and urine was observed in humans exposed to ZnO NPs-containing sunscreen products for five constitutive days via healthy skin [87]. Human skin in vitro was shown to absorb 0.34% of ZnO NP after 72 h [88]. In general, penetration ability of NPs increases when the skin barrier is damaged, pursuant to sunburn, skin disease or physical damage. ZnO NPs were found to better penetrate tape-striped, lesioned or wounded, rather than healthy human skin [85,89]. Moderate skin sunburn increased the penetration of ZnO NPs in pigs, however transdermal absorption was not detected [90]. In vitro studies reported similar findings, only a limited number of ZnO NPs were found on the outer surface of the SC, and no particles were observed in the deeper SC layers [83,84]. Generally, the risk of ZnO NPs exposure from dermal absorption is rather low, however, considering a common human behavior related to sunscreen application, e.g. eating and drinking with sunscreen applied on hands and lips, gastrointestinal or pulmonary exposure should also be considered, moreover, as mentioned previously, the occupational exposure might be of high concern for some people [12].

Inhalation might be specifically associated with increased brain exposure, since the olfactory nerves can directly transport particles into the brain. In fact, Kao et al. observed the translocation of ZnO NPs into the brain following nasal administration (6 h airborne exposure) in a Sprague Dawley rats [91]. In healthy human adults inhaling 500 µg/m³ of ZnO NPs for 2 h, the results were below the threshold for acute systemic effects on the respiratory, hematologic, and cardiovascular endpoints [92]. Other studies have shown that various NPs can enter the brain across the BBB [81,93]; however, a limited number of studies address this issue for ZnO NPs. The BBB was found to be intact in rats after repeated oral administrations of ZnO NPs for 28 days (500 mg/kg) [94], however the presence of ZnO NPs in the rat brain was observed after oral administration for 21 days (500 mg/kg) [95]. Moreover, Yeh et al. (2012) showed increased 65Zn accumulation in the mouse brain up to 10 days after single-dose (120 g) intravenous injection of small (10 nm) 65ZnO NPs [96]. In adult mice, neuronal NPs localization was observed for several days after single oral (gavage) administration of 3 mg of fluorescent ZnO NP. Decreased fluorescent signal over time is consistent with biodegradation or elimination of NPs from the brain [97]. Additional studies are needed to investigate the brain penetration capacity of ZnO NPs.

Other reviews discuss absorption, distribution, metabolism and excretion of ZnO NPs in humans and experimental models more extensively [12,79,82]. To date, data available indicate that ZnO NPs can be absorbed via different routes and distributed to a range of organs, including the brain and placenta. Distribution depends on the size of ZnO NPs, the dose, time and route of exposure. The fate of ZnO NPs
Table 3
Neurotoxic effects of ZnO NPs.

| Compound                      | Exposure model                        | Experimental design                                      | Effect                                                                                     | Reference |
|-------------------------------|---------------------------------------|----------------------------------------------------------|-------------------------------------------------------------------------------------------|-----------|
| ZnO NPs                       | Wistar rats                           | Intraperitoneal injection bimonthly, 8 weeks             | Attenuated spatial cognition capability, enhanced long-term potentiation.                   | [99]      |
|                               | Wistar rats                           | Intravenous injection single dose                        | Increased brain Zn concentrations; no changes in neurotransmitter levels, locomotor activity, exploratory behavior or spatial working memory. | [100]     |
|                               | Wistar rats, male                     | Intraperitoneal injection, 10 days                       | Decreased iron and calcium, but not Zn, sodium and potassium levels in rat brain homogenates; unchanged emotional behavior. | [101]     |
|                               | Wistar rats, male                     | Oral (gavage)                                           | Elevated TNF-α, IL-1β, IL-6, CRP, MDA, decreased GSH and SOD levels, CAT, and GPx activity. | [102]     |
|                               | Sprague-Dawley rats                   | Oral                                                     | Increased Zn levels in the brain of male rats.                                            | [103]     |
|                               | CS7BL/6J mice, male                   | Intraperitoneal injection 3 times per week, 4 weeks     | Impaired learning and memory abilities, suppression of cAMP/CREB signaling pathway.         | [104]     |
|                               | Swiss albino mice, male               | Intraperitoneal injection every other day, 8 times      | Improved behavioral and cognitive impairment in mice with depressive-like behaviors.         | [105]     |
|                               | Swiss albino mice, male               | Oral                                                     | Elevated ROS levels, altered antioxidant system, increased DA and NE levels, presence of ZnO NPs in neurons. | [95]      |
|                               | ICR mice, pregnant female             | Subcutaneous at GD 5, 8, 11, 14, 17                      | Changed DA, 5-HT and their metabolites levels in a 6-week old offspring.                   | [106]     |
|                               | Cyprinus carpio                       | Waterborne                                              | Changed CAT, SOD, GPx activity, GSH levels and lipid peroxidation.                         | [107]     |
|                               | Prochilodus lineatus                  | Waterborne                                              | Increased protein oxidative damage, decreased AChE activity.                               | [108]     |
|                               | Apis mellifera carnica                | Oral (food)                                             | Decreased brain weight and increased brain AChE and GST activity.                          | [109]     |
|                               | Isolated rat neurons                 | 0.8 mg/ml                                               | Increased the opening number of sodium channels, delayed rectifier potassium channels, enhanced excitability of neurons. | [110]     |
|                               | Rat primary neurons                   | 1 mg/ml                                                 | Concentration-dependent cytotoxicity, disrupted cell membranes, DNA damage.               | [111]     |
|                               | Mouse neural stem cells               | 1–100 μg/ml                                             | Concentration-dependent decrease in cell viability; apoptosis, necrosis, release of zinc ions. | [112]     |
|                               | RCS96 rat Schwann cells               | 3–24 ppm                                                | Concentration- and time-dependent decrease in cell viability; apoptosis and necrosis, G2/M phase cell cycle arrest, release of Zn ions. | [113]     |
|                               | Human olfactory neurosphere-derived cells | 6–48 h                                             | Decreased cell viability, activation of numerous pathways associated with stress, inflammation and apoptosis. | [114]     |
|                               | RCG-5 rat retinal ganglion cells      | 4–72 h                                                  | Decreased expression and activity of the plasma membrane calcium ATPase, disrupted intracellular calcium homeostasis, increased ROS production. | [115]     |
|                               | RCG-5 rat retinal ganglion cells      | 2.5–10 μg/ml                                            | Decreased cell viability; activation of numerous pathways associated with stress, inflammation and apoptosis. | [116]     |
|                               | PC12 rat pheochromocytoma and SH-SYSY human neuroblastoma | 6–72 h | Decreased expression and activity of the plasma membrane calcium ATPase, disrupted intracellular calcium homeostasis, increased ROS production. | [117]     |
|                               | SH-SYSY human neuroblastoma          | 5–30 mg/ml                                              | Decreased mitochondrial membrane potential, increased ROS production, increased caspase-12. | [118]     |
|                               | SH-SYSY human neuroblastoma          | 5–30 mg/ml                                              | Decreased cell viability, mitochondrial impairment, internalization of ZnO NPs in membrane-bound vesicles. | [91]      |
|                               | U87 human brain tumor                 | 1–200 μg/ml                                             | Concentration-dependent cytotoxicity e.g. increased formation of micronuclei.               | [119]     |
|                               | Rat primary astrocytes                | 6, 12, 24 h                                             | Reduced cell viability, increased LDH release, stimulated ROS generation, caspase-3 activation, decreased MMP, phosphorylated JNK, ERK, p38 MAPK. | [120]     |
|                               | C6 glia cells                         | 3, 6, 24 h                                              | Time- and concentration-dependent cytotoxicity, apoptosis and increased ROS production.     | [121]     |
|                               | A172, U87, LN2308, LN18, LN229 glioma cell lines and normal human astrocytes | 1, 5, 10 mmol/l | Concentration-dependent cytotoxicity and ROS generation in glioma lines, but not in normal human astrocytes. | [122]     |
|                               | N9 mouse microglial cell line         | 5–60 min; 1–24 h                                        | Increased intracellular calcium and ROS levels, decreased intracellular ATP level, upregulated apoptosis markers. | [123]     |

(continued on next page)
remains unclear; most data suggest that ZnO NPs decompose in medium or in cells and release Zn$^{2+}$ which are responsible for toxic effects. However, this issue, together with the risk of long-term exposure and absorption via healthy vs. damaged skin remain to be established.

### 3.1.2. Neurotoxic effects in vivo

Although increasing number of studies aimed to investigate the potential toxicity of ZnO NPs in different cell types and animal systems [98,82,12], little is known about their neurotoxic effects (Table 3), especially in vivo. ZnO NPs exposure was shown to induce neurobehavioral changes in experimental animals. Impaired learning and memory abilities, and hippocampal pathological changes were demonstrated in old (18 months) mice following ZnO NPs exposure (intraperitoneally, i.p., 5.6 mg/kg, three times per week for four weeks) [104]. The spatial learning and memory ability was attenuated in ZnO NPs-treated (i.p. 4 mg/kg, biweekly for 8 weeks) Wistar rats. The exposed animals exhibited prolonged latency and swimming anxiety in Morris water maze (MWM), and enhanced long-term potentiation (LTP), but not sufficient depotentiation in the dentate gyrus (DG) region of the hippocampus [99]. ZnO NPs administered i.p. for several days ameliorated the behavioral and cognitive impairment in young Swiss male mice with depressive-like behaviors, suggesting that they may affect neuronal synaptic plasticity [105]. Subcutaneous administration of ZnO NPs in pregnant ICR mice at gestation day (GD) 5, 8, 11, 14 and 17 (100 μg/day) affected dopamine (DA), 5-hydroxytryptamine (5-HT) and their metabolites levels in a 6-week old offspring [106]. This observation questions the safety of ZnO NPs exposure during pregnancy, potential transfer through placenta and the effect on developing brain. In contrast, single intravenous injection of ZnO NPs (25 mg/kg) did not affect locomotor activity, exploratory behavior, spatial working memory or neurotransmitter: norepinephrine (NE), epinephrine (EPI), DA, and 5-HT levels in adult male Wistar rats 14 days after injection, despite the plasma and brain Zn$^{2+}$ levels increased in treated group [100]. Sub-acute ZnO NPs treatment (25 mg/kg, 10 days) resulted in minimal effect on emotional behavior (e.g. unaffected anxious index), but showed alteration in trace elements homeostasis in rat brain homogenates: decreased levels of iron (Fe$^{2+}$) and calcium (Ca$^{2+}$), while Zn$^{2+}$, sodium (Na$^{+}$) and potassium (K$^{+}$) concentrations remained unchanged [101].

Discrupted ion homeostasis is an important pathomechanisms of neurotoxicity, and ZnO NPs might affect it. Long-term (13 weeks) oral ZnO NPs administration (134.2, 268.4, 536.8 mg/kg/day), resulted in detection of slightly, but significantly higher Zn$^{2+}$ levels in the brain of male rats (but not in female) [103]. In isolated rat hippocampal CA3 pyramidal neurons the ZnO NPs solution (1 mg/ml) was shown to enhance the current amplitudes of $I_{h}$ and $I_k$ by increasing the opening number of sodium channels, delaying rectifier potassium channels, and enhancing the excitability of neurons, leading to intracellular Na$^{+}$ accumulation and K$^{+}$ efflux. These might disturb the ionic homeostasis and the physiological functions of neurons [110].

Oxidative stress and disrupted antioxidant system is another effect observed in brains of ZnO NPs-treated animals. Oral ZnO NPs (500 mg/kg) administration for 21 consecutive days resulted in elevated ROS levels and altered antioxidants: glutathione (GSH) levels, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase activity (GST), in both the brain and liver of male Swiss albino mice [95]. Combined with increased DA and NE levels in the cerebral cortex, these results suggest a neurotoxic potential for ZnO NPs [95]. Changes in CAT, SOD, GPx activity, GSH levels and lipid peroxidation was also observed in the brain and other organs of juvenile carp (Cyprinus carpio) exposed to waterborne ZnO NPs (0.5, 5, 50 mg/l) for 1, 3, 7, 10 and 14 days [107]. Exposure to environmentally relevant concentrations of ZnO NPs (7, 70, 700 μg/ml) for 5 and 30 days led to increased protein oxidative damage in the brain and gills, but not in the liver, and decreased AChE activity in the brain and muscle of Prochilodus lineatus juvenile fish [108]. Honey bees (Apis mellifera carnica) exposed to ZnO NPs (0.8 mg Zn/ml) in food for 10 days showed decreased brain weight and increased brain AChE and GST activity [109]. Week-long oral administration of ZnO NPs (600 mg/kg) to male Wistar rats resulted in decreased brain CAT, GPx, and GR activities, decreased GSH and SOD levels, but elevated malondialdehyde (MDA) level and inflammatory markers: TNF-α, IL-1β, IL-6, C-reactive protein (CRP).

The neurotoxic effects were partially reversed by the antioxidant and anti-inflammatory compound, hesperidin [102]. The pro-oxidant and pro-inflammatory effect of ZnO NPs was also observed in the serum and the brain of mice injected with ZnO NPs (i.p., 5.6 mg/kg) three times per week for four weeks [104]. In this study the suppression of cAMP/CREB signaling pathway was also identified: the contents of hippocampal cyclic adenosine monophosphate (cAMP), cAMP response element binding protein (CREB), phosphorylated CREB and synapsin I, were decreased in ZnO NPs-treated mice in an age-dependent manner [104].

### 3.1.3. Neurotoxic effects in vitro

The neurotoxic effect of ZnO NPs in vitro has been also evaluated, demonstrating oxidative stress- and apoptosis-related cytotoxicity. Deng et al. [112] have demonstrated that ZnO NPs impaired viability of neural stem cells (NSCs) in a concentration-, but not size-dependent manner. Twenty-four-hour exposure of concentrations higher than 12 ppm induced apoptosis and necrosis in the NSCs. Authors suggested that observed changes might result from the Zn$^{2+}$ dissolved in solution, increased lactate dehydrogenase (LDH) release, stimulate ROS generation, and elicit caspase-3 activation in a concentration- and time-dependent manner [121]. Apoptosis was shown by nuclear condensation and poly(ADP-ribose) polymerase-1 (PARP) cleavage. ZnO NPs stimulated the phosphorylation of c-Jun N-terminal kinase (JNK), extra-cellular signal-related kinase (ERK), and p38 mitogen-activated protein kinase (p38 MAPK). A decrease in mitochondrial membrane potential (MMP) and increase in the expression of Bax/Bcl-2 ratio was also observed after treatment with ZnCl$_2$ [112]. In primary rat astrocytes ZnO NPs exposure (4, 8, 12 μg/ml for 6–24 h) was found to reduce cell viability, increased lactate dehydrogenase (LDH) release, stimulate ROS generation, and elicit caspase-3 activation in a concentration- and time-dependent manner [121]. Apoptosis was shown by nuclear condensation and poly(ADP-ribose) polymerase-1 (PARP) cleavage. ZnO NPs stimulated the phosphorylation of c-Jun N-terminal kinase (JNK), extra-cellular signal-related kinase (ERK), and p38 mitogen-activated protein kinase (p38 MAPK). A decrease in mitochondrial membrane potential (MMP) and increase in the expression of Bax/Bcl-2 ratio was also observed, suggesting mitochondria-mediated apoptosis [121]. ZnO NPs (1–100 μg/ml, 24 h) induced concentration-dependent cytotoxicity, disrupted cell membranes and DNA damage in rat primary neuronal cells, human fibroblasts and AS49 cells, but not in HepG2 cells and human skin keratinocytes [111]. A time- and concentration-dependent cytotoxicity characterized by apoptosis and increased ROS production.

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Table 3 (continued)

| Compound | Exposure model | Experimental design | Effect | Reference |
|----------|----------------|---------------------|--------|-----------|
| BV-2 mouse microglia cell line | 2–24 h | Increased cytotoxicity; activated PINK1/parkin-mediated mitophagy. | | [125] |
was also observed in ZnO NPs-treated (5–80 μg/ml) C6 glial cells [122]. ZnO NPs exposure (1–100 μg/ml) resulted in increased intracellular Ca\(^{2+}\) and ROS levels, decreased intracellular ATP level and upregulated apoptosis markers in mouse microglial cell line [124]. ZnO NPs (1, 5, 10 mmol/L, 24 h) evoked cytotoxicity in the human glioma cell lines (A172, U87, LN2308, LN18, LN229), but not in normal human astrocytes. Cytotoxicity observed in the glioma cells was related to increased ROS generation, and N-acetyl-l-cysteine (NAC) treatment decreased the cytotoxic effect of the ZnO NPs in these cells [123]. ZnO NP-induced cytotoxicity was also observed in microglia (BV-2 cells) exposed to 10 μg/ml ZnO NPs for 2–24 h [125]. ZnO NPs induced parkin protein translocation from the cytoplasm to the mitochondria, implying the involvement of mitophagy in ZnO NPs-induced toxicity [125].

The neurotoxicity of ZnO with four different hierarchical architecture: monodispersed spherical NPs (35 nm), hollow ZnO microspheres (2.7 mm), and larger, prism- and flower-like structures, was evaluated in RSC96 rat Schwann cells [113]. Cells were treated with ZnO at doses 4, 8, 40, 80, 400 μg/ml for 6, 12, 24, and 48 h. ZnO NPs and microspheres displayed significant cytotoxic effects on Schwann cells in concentration- and time-dependent manners, whereas no or low cytotoxic effect was observed when the cells were treated with the prism-like and flower-like ZnO. Cell apoptosis and G2/M cell cycle arrest were observed when RSC96 Schwann cells were exposed to ZnO nanoparticles and microspheres at a dose of 80 μg/ml for 12 h. The time-dependent increase of Zn\(^{2+}\) concentration in the culture media suggests that the cytotoxic effects were associated with the decomposition of ZnO hierarchical architecture and the subsequent release of Zn\(^{2+}\), and not exclusively to the nanoparticulated fraction [113]. Neurotoxicity of ZnO NPs was examined in rat pheochromocytoma PC12 and human neuroblastoma SH-SY5Y cells, showing significant cell loss after 24 h treatment at concentration of 0.1 mM in PC12 cells and 1 mM in SH-SY5Y cells [91]. Moreover, when the PC12 cells were treated with 1 mM (81.4 μg/ml) ZnO NPs for 10 min, the endocytosis of ZnO NPs was observed and increased cellular Zn\(^{2+}\) levels indicated that ZnO NPs may be converted to Zn\(^{2+}\) in endosomes, and then be mobilized into the cytoplasm, leading to Zn\(^{2+}\) dyshomeostasis [91]. The cytotoxic and genotoxic effects of ZnO NPs in SH-SY5Y cells under different exposure conditions were also investigated by Valdiglesias et al. [119]. Despite the results showed that ZnO NPs (10–80 μg/ml) do not enter the neuronal cells, their presence in the medium induced decreased in cell viability, apoptosis, cell cycle alterations, and genotoxicity, including micromolecule production, H2AX (H2A histone family, member X) phosphorylation and DNA damage (primary and oxidative) in a concentration- and time-dependent manner. Unlike in previously described studies, free Zn\(^{2+}\) released from the ZnO NPs was not responsible for the viability decrease [119]. Exposure of SH-SY5Y cells to ZnO NPs (10, 15, 20, 25, 30 mg/ml) resulted in neurotoxicity, as confirmed by LDH activity assay, mitochondria toxicity test (MTT) and Muse™ cell viability assay. Allopurinol, NAC and α-tocopherol protected from ZnO NP-induced cytotoxicity. Electron microscopy revealed typical necrotic characteristics, such as swelling or loss of cell organelles and rupture of the cytosolic or nuclear membrane at 12 h and 24 h after ZnO NPs exposure. Apoptotic changes (annexin V and caspase-3/7 activities) were evident at 12 h and 24 h, but not 6 h after exposure to 15 mg/ml ZnO NPs. PI3 kinase (PI3 K) and p-Akt (protein kinase B) activities induced by ZnO NPs were significantly decreased by esculetin (anti-oxidant) or LY294002 (PI3 K inhibitor). Esculetin reduced the production of ROS and the depletion of antioxidant enzymes induced by ZnO NPs. ZnO NPs induced apoptosis via the PI3 K/Akt/caspase-3/7 pathway and necrosis by lipoxigenase (LOX)-mediated ROS production [118]. Treatment with ZnO NPs (1–200 μg/ml) induced cytotoxicity (e.g. increased formation of micromolecule) in the human brain tumor U87 cells in a concentration-dependent manner, but did not affect normal human HEK cells [120]. Different types of ZnO NPs (coated < 200 nm and uncoated < 30 nm) (10–80 μg/ml, incubated for 2, 6 or 24 h) induced cytotoxicity in human olfactory neurosphere-derived (hONS) cells via mechanisms associated with cell stress, inflammation and apoptosis [114]. Changes in cytokines IL-6 and IL-8 secretion, increase in caspase-3/7 activity, and phosphorylation of key proteins involved in signaling pathways: MAPK/ERK (pMEK, pERK, pJNK, p–cJUN, p-p38), Akt (pAkt, pBAD) and NF-kB (pNF-kB, p1-kB) has been demonstrated. Microarray RNA analysis revealed that short-term (2 h) exposure to ZnO NPs activated pathways involved in cellular stress responses (e.g. upregulation of Nrf2-mediated oxidative stress response pathway), whereas longer (6 h) exposure affected pathways more related to cell injury and repair. Of note, the cellular response was dependent on NPs surface coatings [114]. ZnO NPs (2.5–10 μg/ml) induced cytotoxicity in rat retinal ganglion cells (RGC-5) and inhibited cell proliferation in a time- and concentration-dependent manner. Moreover, ZnO NPs treatment led to cell cycle arrest of S and G2/M phases, ROS production and increased level of caspase-12 and decreased levels of bcl-2 and caspase-9 [115]. Further, the same group showed that ZnO NPs decreased the MMP in RGC-5 cells [116], ZnO NPs (2.5–10 μg/ml) have been shown to decrease the expression and activity of the plasma membrane calcium ATPase, increase intracellular Ca\(^{2+}\) level and disrupt the intracellular calcium homeostasis which might trigger mitochondrial dysfunction, ROS production and cell death [117].

3.2. Titanium dioxide

Titanium dioxide (TiO\(_2\)) is widely used as a white pigment in paint, ink, plastic, and paper and as food additive, while the nanosized TiO\(_2\) is also used for its photocatalytic activity in self-cleaning materials and for its UV absorption capacity in sunscreen. Moreover, TiO\(_2\) is included in the list of inactive ingredients by the FDA, considering it safe to be used in dental paste, oral capsules, suspensions, tablets, dermal preparations and non-parenteral medicines. TiO\(_2\) particles are believed to possess low toxicity and thus are widely used in biomedical applications for their excellent biocompatibility. The range of light that is scattered as well as other properties of TiO\(_2\) depend on the particle size. It naturally exists in three crystal structures: anatase (tetragonal), rutile (tetragonal), and brookite (orthorhombic). Anatase and rutile TiO\(_2\) both have a tetragonal structure, while the TiO\(_2\) octahedron of anatase TiO\(_2\) is distorted to be larger than that of the rutile phase [126]. When the size of TiO\(_2\) is diminished to nanoscale (diameter < 100 nm), the bioactivity and physicochemical properties of nanosized TiO\(_2\) are significantly different from the properties of their bulk analogue [127,128]. Nanoparticles of TiO\(_2\) (TiO\(_2\) NPs) are allowed as sunscreen additives in concentrations of up to 25% [1]. The increased use of nanosized materials has led to an increased burden of TiO\(_2\) NPs in aquatic environments. It is, however, unclear how high levels might occur in environment and if they are harmful to organisms [129]. Analogous to ZnO NPs, the increased demand for products containing TiO\(_2\) is met by increased occupational exposure. Apart from the NIOSH 2011 current intelligence bulletin, to date, no occupational or environmental exposure limits for TiO\(_2\) NPs have been set by any other regulatory agency. The number of workers currently exposed to TiO\(_2\) dust is not available.

Often when a product is so attractive to industry, the understanding about its risk assessments is insufficient and lags behind their rapid advancement and widespread applications [130]. In the case of TiO\(_2\) NPs, it is not yet clear how they are transported into or out of the brain, how they accumulate or what kind of behavioral or cognitive dysfunction they may cause, however the evidence summarized in this (Table 4) and other review articles [130,128,79,149–154] may indicate that their toxic potential remains to be fully elucidated

3.2.1. TiO\(_2\) absorption and transport across the BBB

Dermal absorption is the most relevant entry route of chemicals related to sunscreen use. Several studies have analyzed TiO\(_2\) penetration into intact or damaged skin using different models. On the whole,
dopamine; DOPAC: 3,4-dihydrophenylacetic acid; GC

Abbreviations

N-MPG: N-(2-mercaptopropionyl)glycine; NPs: nanoparticles; PD: Parkinson disease; P-gp: P-glycoprotein; ROS: reactive oxygen species; Ser: serine; SOD2: superoxide dismutase 2; TCA: tricarboxylic acid cycle; TNF-α: tumor necrosis factor α; Thr: threonine; Ti: titanium; TiO2: titanium dioxide.

Table 4

| Compound                     | Exposure model          | Experimental design                                      | Effect                                                                 | Reference |
|------------------------------|-------------------------|---------------------------------------------------------|----------------------------------------------------------------------|-----------|
| TiO2 NPs                     | Mice                    | Intratracheal instillations once per week for 4 weeks   | Inflammatory cell aggregation and neuron necrosis. Ti level in the brain 3 days after a single instillation was upregulated by 100%. | [131]     |
| Wistar rats, male            | Intratracheal           | 0.1, 1.0, 10.0 mg/kg                                     | Ti accumulation in the brain and dose-dependent injury. TiO2 NPs with diameter of 200 nm did not cause significant alterations in the brain. | [132]     |
| BBB model based on rat primary endothelial cells (BECs) and astrocytes | Acute exposure: 24 h, 0–500 μg/ml                | Reduced expression of P-gp, claudin 5, caveolin-1, and caveolin-2 associated with BBB integrity. | [133]     |
| Fisher F344 rats, male        | Intravenous single dose | 1 mg/kg                                                 | Upregulation of tight junction proteins, modulation of P-gp mRNA expression and persistent brain inflammation markers: IL-1β, IP-10, GFAP and CXCL1. No Ti accumulation in the brain after 24 | [134]     |
| Mice                         | Intranasal              | 90 days, 2.5, 5.0, 10 mg/kg                             | Ti accumulation in the brain. Oxidative stress, high levels of lipid, protein, and DNA peroxidation, overpolarization of glial cells, tissue necrosis, hippocampal cell apoptosis. Microarray showed significant alterations of 249 genes expression. | [135]     |
| Mice, female                 | Intranasal instillation every other day for 2, 10, 20, 30 days | 500 μg                                                  | Ti accumulation in hippocampus after 30 days of rutile exposure. Irregular arrangement and loss of neurons, morphological changes and oxidative damage in hippocampus. Increased TNF-α and IL-1β levels. | [136]     |
| Mice, female                 | Intranasal instillation every other day for 2, 10, 20, 30 days | 500 μg                                                  | Imbalance of monoaminergic neurotransmitters, increased NE and 5-HT, while levels of DA, DOPAC, HVA and 5-HIAA were decreased. | [137]     |
| Wistar rats, male            | Intragastrical          | 60 days, 50, 100, 200 mg/kg                             | Downregulated AChE activity. Increased plasmatic brain IL-6. Increased GFAP expression. | [138]     |
| Zebrafish embryos            | 96 hpf                  | 0.1, 1, 10 μg/ml                                        | Hatching time was decreased, with increase in malformation rate. Increased plasmatic brain IL-6. | [139]     |
| Caenorhabditis elegans       | 24 h                    | 7.7, 38.5 μg/ml                                         | GC-MS-based metabolomics perturbations mainly occurred in TCA cycle, glyoxylate, tricarboxylate, inositol phosphate, Gly, Ser, Thr, Gln, and Glu metabolism. | [140]     |
| Caenorhabditis elegans       | 96 h                    | 1–100 μg/l                                             | Light exposure induced the production of ROS and increased toxicity from a median effect concentration of more than 100 mg/l to 53 mg/l. | [141]     |
| D384 glial cell line and SH-SYSY human neuroblastoma | 24 h                    | 15, 31 μg/ml                                            | Concentration- and time-dependent alterations of mitochondrial function, cell membrane damage, inhibition of cell proliferation. Effects dependent on TiO2 size. Neuronal cells were more sensitive than glial cells. | [142]     |
| U373 human glial cells and C6 rat glial cells | 24–96 h                 | 2.4–40 μg/cm²                                           | Morphological changes associated with depolymerization of F-actin, apoptotic cell death. | [143]     |
| U373 human glial cells and C6 rat glial cells | 2–24 h                  | 20 μg/cm²                                               | Increased expression of antioxidant enzymes: GPx, CAT, SOD2, lipid peroxidation and mitochondrial depolarization. | [144]     |
| PC12 rat pheochromocytoma    | 6–48 h                  | 1–100 μg/ml for                                       | Apoptosis prevented by a ROS scavenger, N-MPG. | [145]     |
| Co-culture of PC12 cells with primary rat microglia | 24–48 h                 | 0.25–0.5 mg/ml                                         | Supernatant from TiO2 NPs treated microglia caused significant cytotoxicity in PC12 cells. | [146]     |
| PC12 cell line               | 24 h                    | 1–125 μg/ml                                            | Decreased cell viability, mitochondrial impairment and decreased DA levels. | [139]     |
| BV2 microgial cells          | 6, 18 h                 | 2.5–120 ppm                                            | Release of ROS, mitochondrial hyperpolarization. | [147]     |
| BV2 microgial cells, N27 neurons, primary cultures of rat striatum | 2, 6, 24, 48 h           | 2.5–120 ppm                                            | Microglia generated ROS damages neurons in complex primary cultures. No cytotoxicity in isolated N27 neurons. | [148]     |

Abbreviations: 5-HIAA: 5-hydroxyindole; 5-HT: 5-hydroxytryptamine; AchE: acetylcholine esterase; BBB: blood-brain barrier; CAT: catalase; CXCL1: chemokine C-X-C motif ligand 1; DA: dopamine; DOPAC: 3,4-dihydrophenylacetic acid; GC-MS: gas chromatography mass spectrometry; GPx: glutathione peroxidase; HVA: homovanillic acid; hpf: hours post fertilization; IL-1β: interleukin-1β; IL-6: interleukin-6; IP-10: interferon gamma-induced protein 10; NE: norepinephrine; N-MPG: N-(2-mercaptopropionyl)glycine; NPs: nanoparticles; PD: Parkinson’s disease; P-gp: P-glycoprotein; ROS: reactive oxygen species; Ser: serine; SOD2: superoxide dismutase 2; TCA: tricarboxylic acid cycle; TNF-α: tumor necrosis factor α; Thr: threonine; Ti: titanium; TiO2: titanium dioxide.

studies demonstrated that TiO2 NPs cannot permeate intact and damaged skin and can be found only in the stratum corneum and epidermis, without reaching the brain or peripheral organs [155–158]. Furthermore, low cytotoxicity observed in human HaCaT keratinocytes, suggests a low toxic potential of these nano-compounds at the skin level. These results can be explained by the great stability and low ionizing capacity of these particles and are in accordance with several studies in the literature [159–161]. However, studies simulating real-world scenarios on sunburned skin, with UV exposure in long-term chronic exposure conditions need to be conducted to assure the safety of TiO2 in sunscreen.

Long-term intake of TiO2 NPs at low doses was assayed in rats. Animals received 1 or 2 mg/kg TiO2 suspension per day for 5 consecutive days. On the sixth day their gut tissue was analyzed for TiO2 content and possible adverse effects. A sex-specific effect on villus cells proliferation was observed in male rats, indicating a potential role for the endocrine system in this process. Oxidative stress in intestinal cells was transient and decreased after 24 h [162].

NPs have the ability to cross the BBB. While this may be desirable for drug-delivery systems [163], it can also pose a risk of unwanted accumulation of potentially harmful chemicals in the brain. In an in vivo study by Li et al. (2010), mice were chronically exposed to TiO2 NPs of
3 nm diameter (4 mg/kg) via intratracheal instillations. After 4 weeks, inflammatory cell aggregation and neuron necrosis were present. The amount of Ti in the brain was measured by inductively coupled plasma mass spectrometry (ICP-MS) 3 days after a single instillation of 4 mg/kg TiO2 and found to be upregulated by 100% (120 ng/g Ti in controls, compared to 240 ng/g in treated animals) [131].

3.1.2. TiO2 neurotoxic effects in vivo

The diameter of TiO2 NPs seems to be important for its carriage. Rats were treated with TiO2 NPs (0.1, 1, 10 mg/kg) suspension of different diameters (10, 20, and 200 nm) through intratracheal instillation. Seventy-two hours later, TiO2 NPs with diameters of 10 and 20 nm were both transported into the brain, inducing dose-dependent alteration in pro-inflammatory markers (TNF-α, IL-1β and IL-10). However, TiO2 NPs with diameter of 200 nm did not cause significant alterations in the brain [132]. In a BBB model based rat primary endothelial cells (BECs) and astrocytes, TiO2 NPs (acute exposure for 24 h with 0−500 μg/ml or chronic exposure for 5 days with 0−100 μg/ml) could not only pass through the BBB but also disrupt its integrity by reducing the expression of P-glycoprotein (P-gp), claudin 5, caveolin-1, and caveolin-2, which are associated with the BBB integrity [133].

The effects of TiO2 NPs on the brain may not occur by a direct interaction between the chemical and the BBB. [134] described the in vivo uptake and clearance of TiO2 NPs by BECs and demonstrated a Ti burden in the liver, spleen and lungs up to a year after intravenous (i.v.) administration of TiO2 NPs (1 mg/kg) to rats, with a very low clearance rate. At this dose, the authors did not observe Ti accumulation in the brain, however upregulation of tight junction proteins, modulation of P-gp mRNA expression and persistent brain inflammation markers such as IL-1β, IP-10 (interferon gamma-induced protein 10), GFAP (glial fibrillary acidic protein) and CXCL1 (chemokine C-X-C motif ligand 1) were observed. The authors suggested that TiO2 NPs can exert an indirect effect on the CNS that seems dependent on circulating biomarkers potentially released by organs accumulating Ti [134].

Brain levels of 0.05−0.15 μg/ml were detected after intranasal administration of 2.5−10 mg/kg TiO2 NPs for 90 consecutive days in association with oxidative stress, high levels of lipid, protein, and DNA peroxidation, overpolarization of glial cells, tissue necrosis, hippocampal cell apoptosis in mice. Microarray showed significant alterations of 249 genes expression involved in oxidative stress, apoptosis, memory and learning, brain development, lipid metabolism, DNA repair, signal transduction, immune response and response to stimulus in the brain-injured mice. Some of these genes may be potential biomarkers of brain toxicity caused by TiO2 NPs exposure [135].

Female mice were intranasally instilled with 500 μg of two types of well-characterized TiO2 NPs (i.e. 80 nm, rutile or 155 nm, anatase) every other day for 2, 10, 20 or 30 days. High Ti accumulation (ranging from 0.13 to 0.3 μg/ml) was more pronounced in hippocampus after 30 days of rutile exposure, compared to other brain regions (cerebellum, olfactory bulb or cortex). Histological analysis revealed irregular arrangement and loss of neurons, morphological changes and oxidative damage in the hippocampus. Increased TNF-α and IL-1β levels were also observed [136]. Translocated TiO2 NPs (500 μg) caused imbalance of monoaminergic neurotransmitters, with significantly increased NE and 5-HT levels, while levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic (HVA), and 5-hydroxyindole acetic acid (5-HIAA) were decreased [137].

Acetylcholinesterase activity was evaluated in plasma and brain of rats after 60 days intragastric treatment with anatase TiO2 NPs (50, 100, 200 mg/kg). Plasmatic AChE activity was decreased with the increasing TiO2 NPs doses. The higher doses of TiO2 NPs caused a significant decrease in the AChE activity in the brain. These effects were accompanied by IL-6 increase in the brain and plasma and increased levels of GFAP in cerebral cortex, suggesting neuroinflammation [138]. Cognitive function may have also been compromised in this model, but behavioral experiments are lacking. Studies that describe the specific proteins that carry TiO2 to and/or from the brain are lacking.

In zebrafish larvae exposed to environmentally relevant concentrations (1−10 μg/ml) of TiO2 NPs induced Parkinson’s disease (PD)-like symptoms, with locomotor alteration, reduced DA, Lewy bodies formation and alterations in mRNA levels of pink1, parkin and a-syn, that were significantly increased in a dose-dependent manner. The authors observed TiO2 accumulation in brain and oxidative stress, with cell death in hypothalamus. To further investigate TiO2 effects on DAgergic cells, the authors exposed PCl2 cells to 1−125 μg/ml TiO2 NPs for 24 h. Cell viability was decreased at the higher dose and similarly to zebrafish, DA levels were decreased. This study suggests a role for TiO2 exposure in the development of PD [139].

Caenorhabditis elegans (C. elegans) is an excellent biological model organism for environmental risk assessment. Gas chromatography mass spectrometry (GC–MS)-based metabolomics approach was used to understand the toxicity of sub-lethal concentrations (7.7 and 38.5 μg/ml) of TiO2 NPs (< 25 nm). Most of the significant perturbations occurred in organic acids (citrict, lactic, fumaric, succinic and malic acids) and amino acids. Differential marker metabolites identified from the metabolomic analysis suggested that the disturbances, mainly occurred in metabolism of: glyoxylate, inositol phosphate, tricarboxylate, glycine (Gly), serine (Ser) threonine (Thr) glutamine (Gln) and Glu [140]. Toxicity of bulk-scale (~160 nm) and nanoscale (21 nm) TiO2 was tested under dark and light conditions. Light exposure induced the production of ROS by nanoscale TiO2 and increased toxicity of the nanomaterial from a median effect concentration of more than 100 mg/l to 53 mg/l. The observation that light increased the toxicity of the highly photoactive nanoscale TiO2 suggests that ROS play a role in the photoactivated toxicity of the nanomaterial. No evidence of intracellular oxidative stress was found. Because TiO2 accumulated in worm intestines, as observed by microscopy, the authors suggested that ROS were formed extracellularly in the apical surface of the worms’ intestinal cells [141].

3.2.3. TiO2 neurotoxic effects in vitro

In vitro human cell models may represent a valid instrument to investigate TiO2 NPs effects on CNS and to determine their underlying mechanistic processes, providing information about doses of exposure. [142] demonstrated concentration- and time-dependent alterations of the mitochondrial function on D384 (glial cell line) and SH-SY5Y (neuronal cell line) cells starting at the dose of 31 and 15 μg/ml TiO2 (15−69 nm in diameter, anatase isoform), respectively, after 24 h exposure. Neuronal cells were more sensitive than glial cells. These effects were more pronounced in cells exposed to NPs compared to TiO2 bulk, where with the latter effects appeared only at the highest doses (125 and 250 μg/ml) after 24 and 48 h, similarly in both cerebral cell lines. Cell membrane damage was present in both cell lines starting at 125 μg/ml after 24 h exposure and also dependent on TiO2 size. TiO2 NPs were potent inhibitors of cell proliferation in human CNS cells after prolonged exposure (up to 10 days) at doses ranging from 0.1 to 1.5 μg/ml [142].

TiO2 NPs induced apoptosis in both human (U373) and rat (C6) glial cells at 96 h of treatment, evidenced by active caspase-3 starting at 5 μg/cm2. At this concentration, DNA fragmentation assessed with the TUNEL assay was observed in U373 cells, but not in C6 cells. Morphological changes associated with depolymerization of F-actin were found, accompanied by apoptotic cell death [143]. In a similar protocol of exposure, TiO2 NPs induced oxidative stress in U373 and C6 glial cells, mediating changes in the cellular redox state, which was correlated with increase in antioxidant enzyme expression (GPx, cata-lase and SOD2) and lipoperoxidation. Mitochondrial depolarization was also observed. These effects occurred within 24 h exposure to 20 μg/cm2 TiO2 NPs [144]. Oxidative stress was also present in rat PC12 cells exposed to TiO2 NPs 50 μg/ml for 24 h (P25 type, 21 nm in average size) and N-(2-mercaptopyrrolyl)-glycine (N-MPG), a kind of ROS scavenger, prevented apoptosis in this model [145], indicating that
oxidative stress is an important factor in TiO₂ NPs-induced neurotoxicity.

P25 (an uncoated photo-active, largely anatase form of nanosize TiO₂, not used in sunscreen) stimulates ROS in BV2 microglia at 2.5–120 ppm, starting at 5 min exposure [147] and was later found to be nontoxic to isolated N27 neurons. However, P25 rapidly damages neurons at low concentrations (5 ppm, 6 h) in complex brain cultures of supernatant from TiO₂ NPs-treated (0.25–2.5 μg/ml) microglia caused significant cytotoxicity in PC12 cells. The authors suggested that TiO₂ NPs stimulated microglia produced inflammatory factors, which caused PC12 cells cytotoxicity [146].

Recent studies report endoplasmic reticulum stress (ER stress) as a common response to NPs related toxicity. The ER stress also known as unfolded protein response (UPR) refers to an important cellular self-protection mechanism, which can be activated to counteract the cell situation of stress (overloading proteins or direct ER damage). ER stress was observed in human epidermal keratinocytes (HEKn) and human umbilical vein endothelial cells (HUVEC) exposed to up to 20 μg/cm² for 16–24 h [164], [165]. Analogous to neural cells, oxidative stress was also observed in different cell types, demonstrating that TiO₂ can affect a wide range of tissues. For example, the lung is a primary target of NPs exposure, especially in occupational settings. In the case of TiO₂ inhalation, nanoscale particles may deposit in the lung interstitium and vascular endothelial cells [171]. Several excellent reports are available on TiO₂ effects on peripheral tissues, such as skin [167], kidney [168], liver [169], lung [170] and vascular endothelial cells [171].

4. Conclusions and future perspectives

Although some UV-related pathologies could be prevented by applying sunscreen, the efficiency and safety of these products is questionable. As the use of sunscreen is continuously increasing worldwide, so do the levels of environmental accumulation and human, and wildlife exposure. Whether concentration resulted from daily use and/or environmental contact possesses a realistic hazard to humans and other organisms is still unknown. Numerous studies raised concerns about the association between exposure to substances commonly found in sunscreens and endocrine and developmental impairments. In this review, the potential neurotoxicity of such substances is presented and the question of cost-benefit is raised regarding large scale use of sunscreen in its current composition. Although most studies reviewed in this paper reported adverse neurotoxic effects of UV filters at concentrations substantially higher than those observed in environment and human tissues, these studies should not be disregarded, as they afford potential pathomechanisms which might occur in other conditions or sensitive populations. It is noteworthy, that gene x environment interactions vis-à-vis toxicity of sunscreen components has yet to be studied. Unfortunately, the effects of repeated, long-term and low-dose exposures to single compounds and mixtures of various UV filters is also poorly studied. More studies are needed to evaluate the realistic hazard of contemporary sunscreens. Furthermore, it is also timely and meritorious to advance studies on alternative, safer and more effective UV filters.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgment

This work has been supported by the National Institutes of Health [grant numbers NIEHS R01ES07331, NIEHS R01ES10563 and NIEHS R01ES020852].

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