# Toxicity of Dentifrices

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The regular use of adequate toothpaste with safe active ingredients possessing anti-bacterial, anti-inflammatory, anti-oxidant, and regenerative properties is one of the most effective strategies for oral healthcare. In addition to water, a typical toothpaste consists of a variety of components, among which three are of predominant importance, i.e., abrasive substances, fluoride, and detergents. These ingredients provide healthy teeth, but their environmental impact on living organisms are often not well-known.

Keywords: Danio rerio ; fluoride ; abrasive substances ; detergents

## 1. Fluoride

In the European Union, for example, the concentration of fluoride in toothpaste is typically limited to 0.145% (1450 ppm) for children older than 6 years and adult toothpaste, while toothpaste for children under 6 years of age usually has a lower concentration, around 0.1% (1000 ppm). This distinction is made to prevent potential overexposure to fluoride in younger children who might ingest toothpaste. In the United States, the Food and Drug Administration (FDA) has similar guidelines for fluoride concentration in toothpaste. Currently, the amount of toothpaste applied to the toothbrush is more important than the concentration of fluoride in the toothpaste. Modern recommendations are from 1000 ppm in children, and the amount of toothpaste is described as a little bit on the bristles, a grain of rice, or a pea [1].

In toothpaste, fluoride can be found in one of four forms: sodium fluoride (NaF), sodium monofluorophosphate (SMFP), stannous fluoride (SnF2), or amine fluoride (AmF). It can also be used as a combination of two active substances, e.g., NaF with SMFP and AmF with SnF2. The second combination is an essential one because AmF itself is unstable, which would severely limit its application. NaF reduces, more significantly than unstable AmF, the number of viable bacteria in the biofilm found on various oral surfaces after toothpaste application. Meanwhile, SnF2 shows significantly higher efficiency in eliminating live bacteria than NaF [2]. Moreover, toothpastes containing SnF2 are used to reduce dentine hypersensitivity to everyday irritating stimuli [3].

Fluoride is present in toothpaste in various concentrations. In the European Union market, the concentration of fluoride in products approved for trade in drugstores must not exceed 1500 ppm $F^-$. Higher concentrations are only available in pharmacies, and in the UK are only accessible with prescription. With a view to balancing the benefits of fluoride toothpaste and the risk of fluorosis, the European Academy of Paediatric Dentistry (EAPD) recommends limiting fluoride to a grain of rice-sized portion of toothpaste in children under 6 years of age and a toothpaste containing 1000 ppm $F^-$ up to 2 years of age [1].

Knowledge concerning the unquestionably desirable effects of fluoride on enamel development and its efficiency in aspects of caries prevention should be on a par with knowledge concerning fluoride toxicity. This problem has been commonly studied and described. Also, the biota in bodies of water is affected by deposited NaF, which is used as a pesticide and for industrial purposes. With the current use of *Danio rerio* in environmental toxicity studies, this model organism is a perfect tool to evaluate the adverse effects of fluoride in vertebrate developmental biology studies. It was shown that sodium fluoride exposure (18.599, 36.832 mg/L of fluoride for 30 and 60 days) significantly affects ovarian development, disrupts reproductive hormones, affects oogenesis, induces oxidative stress, and causes apoptosis through both external and internal pathways in the zebrafish ovary [4]. Moreover, fluoride can substantially inhibit the growth of zebrafish and specifically affect their reproductive system by impairing not only ovarian but also testicular structure, altering steroid hormone levels and expression of steroidogenic genes related to sex hormone synthesis in zebrafish [5]. In addition, fluoride significantly affected the secretion of thyroid hormones by altering the microstructure of the gland and changing the expression of genes that regulate their synthesis in male zebrafish [6]. Differential expression and activity of Nrf2 and other stress response genes were demonstrated in the liver of zebrafish after individual and combined exposure to the xenobiotics fluorine and arsenic [7]. Data clearly show that NaF exposure has significant effects on the induction of oxidative stress and alteration of gene expressions in the liver of female zebrafish [8]. It has been reported that reactive

oxygen species levels are raised along with increased malondialdehyde levels and reduced glutathione levels in the brain of zebrafish [9]. Moreover, it was found that zebrafish exposed to 15 ppm NaF for 30 and 90 days post-fertilization showed liver histopathology including hyperplasia, cytoplasmic degeneration, and nuclear fragmentation [10].

## 2. Abrasive Substances

Among all groups of toothpaste ingredients, abrasives and polishing agents are the most important, both in terms of function and quantity [10]. They constitute from 25 to 50% of the toothpaste content. They perform the basic function in the cleaning process, mechanically removing dental plaque and discoloration of external origin. They are also responsible for the texture of the paste [11].

The most popular abrasives are calcium carbonate, calcium and magnesium hydroxides, silicon oxide, hydroxyapatite, or polymethacrylate. However, the effectiveness of these compounds depends not on their chemical composition, but rather on the shape and size of the grains contained in the preparation. A spherical shape is considered to be optimal. In turn, the grain size should not exceed 10 micrometers [12].

The properties of kinds of toothpaste approved for sale on the European market are laid down in the standard ISO 11609 [13] according to it, the optimal RDA (Relative Dentin Abrasion) value for toothpastes for everyday use is assumed to be in the range of 30–70. Nowadays, in the selection of toothpaste composition and production, a tendency to reduce abrasiveness without losing cleaning efficiency is noticeable. This may be mainly due to the increased use of high-performance abrasives such as hydrated silica [14]. Calcium carbonate nanoparticles ($CaCO_3$-NPs) are promising materials for various industrial applications. It is necessary to understand their toxicological profile in biological systems as human and environmental exposure to $CaCO_3$-NPs increases along with global manufacturing production. By analyzing the cytotoxicity of $CaCO_3$-NPs on two cell lines (NIH 3T3 and MCF7), calcium carbonate nanoparticles were shown to be safe in vitro as they did not cause cell mortality or genotoxicity. In addition, zebrafish treated with $CaCO_3$-NPs developed without any abnormality, confirming the safety and biocompatibility of this nanomaterial [15].

Abrasive substances in toothpastes approved for sale on the European market have certain size and shape standards. Currently, nanoparticles (NPs) are the most commonly used. One of them is a nanoparticle of calcium carbonate. Calcium carbonate nanoparticles were shown to be safe in vitro as they did not cause cell mortality or genotoxicity. Nanotechnology investigates materials at the nanoscale level (0.1–100 nm in diameter). There are many commercially available nanoproducts such as silver, silicon, titanium, zinc, and gold. They are used in a variety of applications and released to the environment. Titanium dioxide ($TiO_2$) is one of the most commonly used NPs. The doses ($TiO_2$) specified in the standards were safe for zebrafish, but significantly excessive doses showed autophagy and cell necrosis. Studies on $TiO_2$ molecules have shown that a larger dimension than nano causes developmental abnormalities. Different nanoparticles such as the rare earth oxide, iron oxide, gold, silica, and carbon induce autophagy depending upon molecule size and dispersion. Currently, toothpastes have less abrasion, which does not affect the quality of cleaning.

Among various types of nanoparticles, silica nanoparticles (Si-NPs) have become popular as nanostructuring, drug delivery, and optical imaging agents. Si-NPs are highly stable and could bioaccumulate in the environment. Although toxicity studies of Si-NPs to human and mammalian cells have been reported, their effects on aquatic biota, especially fish, have not been significantly studied. Results from the studies on the effect of nanoparticles on zebrafish are generally consistent with similar studies on human and mouse cells that have been reported so far. Thus, fish cell lines could be valuable for screening emerging contaminants in aquatic environments including NPs through rapid high-throughput cytotoxicity bioassays [16].

There are many commercial nanoproducts such as silver, silicon, titanium, and gold. They have various applications and are commonly found in the environment. Titanium dioxide ($TiO_2$) is one of the most commonly used NPs. $TiO_2$-NPs are used in plant production and medicine, as well as the production of food, toothpaste, sunscreens, cosmetics, and in wastewater treatment. Studies on the effects of this compound at higher doses on zebrafish showed autophagy and necrosis in Sertoli cells, which consequently negatively affected the spermatogenic cells and testicular morphology of zebrafish [17].

## 3. Detergents

Sodium lauryl sulfate, often abbreviated as SLS, is present in most toothpaste as well as shampoos, scalp cosmetics, hair dyes, bleaches, shower gels, cleansers, make-up bases, liquid soaps, washing powders, oils, and bath salts. Although SLS can be extracted from coconuts, it is produced by chemical synthesis for use in industry [18].

SLS is the sodium salt of sodium dodecyl sulfuric acid and is classified in the cosmetic ingredient database as a denaturant, a surfactant detergent, an emulsifier, and a foaming agent [18]. The function of detergents in toothpaste is to lower the surface tension, and thus facilitate the removal of dental plaque. They also show a slight antibacterial effect and have an inhibiting effect on plaque build-up. In normal use, they have no clinically significant effect on hard tissues but may have an irritating effect on soft tissues. This in turn may lead to the exacerbation of ongoing periodontal diseases, as well as influence the formation and development of gingival recession and recurring ulceration [19].

Detergents may affect soft tissues in different ways. For instance, SLS, the anionic sodium dodecyl sulfate (SDS) or Betaine amphoteric surfactants, can cause necrosis of epithelial cells. In contrast, the non-ionic surfactant (Pluronic™) increases epithelial cell viability. At the same time, detergents may increase the activity of inflammatory factors such as TNF, IL-1β, and IL-8, which are known factors related to the persistence of periodontal inflammation. It should be noted, however, that studies on the effect of detergents on soft tissues were conducted in vitro, so they did not take into account the protective effect of saliva. Moreover, it should not be forgotten that the above-mentioned agents contained in toothpastes co-exist with other substances, which may limit their harmful effects. For example, triclosan, often found in toothpastes, has anti-inflammatory effects and may mitigate the irritating effects of SLS [11][19].

Currently, surfactants are widely distributed in the environment as organic pollutants, and their toxicity has attracted a lot of attention. Ref. [20] assessed the effect of SDS, cationic surfactant-dodecyldimethylbenzylammonium chloride (1227), and non-ionic surfactant-polyoxyethylene fatty alcohol (AEO) on the behavior of zebrafish larvae. Five behavioral parameters were recorded using a larval rest/wake assay, including rest total, number of rest bouts, rest bout length, total activity, and waking activity. The results revealed that 1227 and AEO at 1 µg/mL affected larval locomotor activity, and that SDS had no significant impact on larval behavior [20]. In addition, the toxicity assay of three surfactants on developing zebrafish embryos was also performed. All three surfactants induced concentration-dependent shorter body length compared to SDS and 1227. Furthermore, in situ hybridization showed dependent responses. Exposure to AEO resulted in smaller head size and smaller eye size, and the smaller head size could be associated with reduced EGR2 expression. Altered ntl expression showed that developmental retardation is due to inhibited cell migration and growth. These findings provide references for ecotoxicological evaluations of different types of surfactants and play a warning role in the use of surfactants [20].

## 4. Antibacterial Agents

In the oral cavity, like other areas of the gastrointestinal tract, there is a natural microflora, the presence of which gives the host several beneficial properties. However, in the absence of proper oral hygiene, dental plaque (biofilm) can build up beyond what is consistent with oral health. This shifts the balance of dominant bacteria away from those related to health. Such shifts may predispose the site to dental caries, gingivitis, or periodontal disease [21]. Possible strategies for maintaining the stability and beneficial properties of the natural oral microflora include improving oral hygiene, for example, by using products containing safe antimicrobial, anti-inflammatory, and antioxidant substances.

The presence of distinct microbes in the periodontal environment, e.g., Aggregatibacter actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Tannerella forsythensis (T.f.), Treponema denticola (T.d, Porphyromonas endodontalis (P.e.), Fusobacterium nucleatum (F.n.), and Prevotella intermedia (P.i.). Nonnenmacher C. et al. [22]; Lee HJ. et al. [23] has been associated with increased levels of host-produced pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and interleukin 17A (IL17A) [24]. It has become common knowledge that infection-induced chronic inflammation is closely associated with an imbalance of reactive oxygen/nitrogen species and antioxidant defense, so-called oxidative stress [25][26].

Antimicrobial oral hygiene products include chlorhexidine, fluorides [27][28], xylitol [29][30], triclosan [28], and their combinations [31]. These compounds show antibacterial, anti-caries, and anti-inflammatory activity in vivo. However, their toxicity may be underestimated.

The most common chemical antiseptic in toothpaste is triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol), which is still widely used not only in personal care products such as soaps, toothpaste, and deodorants but also in cleaning (detergents, disinfectants) and plastic products [32][33]. Therefore, triclosan can be a significant contaminant in the aquatic environment, even though it is rapidly degraded by photodegradation [34].

Once triclosan levels are detected in various human tissues such as adipose tissue, brain, and liver [35][36], studies on its long-term effects on human health have been undertaken [37]. Toxic effects of triclosan have been also extensively evaluated using zebrafish as an animal model [38][39][40][41]. Triclosan's mechanisms of toxicity encompass a range of

effects on zebrafish, including endocrine disruption, oxidative stress, microbiota imbalance, altered behavior, and developmental and reproductive effects. Understanding these mechanisms is crucial for assessing the potential harm of triclosan on zebrafish populations and broader aquatic ecosystems. It can act as an endocrine disruptor by binding to hormone receptors, particularly those associated with thyroid hormones. In zebrafish, disruptions in thyroid hormone signaling can lead to developmental abnormalities, hinder growth, and impact the timing of metamorphosis [42]. Triclosan can also induce oxidative stress within cells by generating reactive oxygen species (ROS), which are harmful molecules that can damage cell structures and DNA. In zebrafish, oxidative stress can result in cellular dysfunction, inflammation, and even cell death. This oxidative damage can affect various physiological processes, including organ function and tissue integrity [38]. Triclosan's antimicrobial properties can extend beyond their intended use, affecting not only pathogenic bacteria but also beneficial microbial communities in aquatic environments. In zebrafish, exposure to triclosan can disturb the gut microbiota, which plays a vital role in digestion, nutrient absorption, and overall health. Imbalances in the microbiota can lead to various health issues, including impaired growth and weakened immunity [43]. Studies suggest that triclosan exposure can influence behavior and neurological function in aquatic organisms. In zebrafish, exposure to triclosan has been linked to alterations in swimming behavior, impaired neural development, and changes in neurotransmitter levels. These effects can impact zebrafish survival, predator–prey interactions, and overall ecosystem dynamics [44][45]. Disruption of hormone signaling can have significant consequences for reproductive and developmental processes in zebrafish. Exposure to triclosan has been associated with delayed hatching, altered embryonic development, and reduced fertility. These effects can impact zebrafish populations and have cascading effects on aquatic ecosystems [46]. There are more than 70 papers from the last 10 years concerning the evaluation of triclosan activity in the zebrafish model. It was revealed that triclosan disrupts the early stages of zebrafish by interfering with many developmental processes such as cartilage development, organogenesis, breeding, and changes in biomarker levels [47][48]. Furthermore, triclosan leads to craniofacial morphosis in zebrafish [49], and acute triclosan exposure induces subtle cardiotoxicity in developing fish [50]. Triclosan decreased zebrafish hatching rate and led to a series of malformations, such as cardiovascular malformation [48]. Additionally, otolith formation and eye and body pigmentation were disturbed along with growth restriction and pericardial edema [51]. Ninety-six-hour LC50 studies performed in zebrafish embryos and adults showed lethal concentrations of 0.42 and 0.34 mg/L, respectively [40]. Also, foraging efficiency was decreased [41].

Additionally, chronic triclosan exposure may cause biological genotoxicity, hepatotoxicity, immunotoxicity, neurotoxicity, and cardiotoxicity, as well as impairment of lipid metabolism [49][52]. Triclosan increased levels of cholinesterase, lactate dehydrogenase, and glutathione S-Transferase in zebrafish larvae but not adult fish. Furthermore, it was reported that triclosan impaired lipid metabolism homeostasis in zebrafish by enhancing the mRNA expression of lipid b-oxidation genes [40].

In behavioral studies, triclosan reduced swimming distance and increased freezing duration in 5 dpf zebrafish. Also, the anxiety level was augmented, which was suggested to result from decreased acetylcholinesterase (AChE) activity [44]. Decrease in acetylcholinesteras activity, together with the influence on myelin basic protein (MBP) and synapsin IIa (syn2a) genes after 4 days of treatment of triclosan, also resulted in motor neuron innervations in skeletal muscles and reduced touch-evoked escape response in zebrafish larvae [45]. Neurotoxic effects of triclosan may also result from an increase in oxidative stress processes, which has been demonstrated in the gill and ovary of zebrafish [53].

Chlorhexidine has been commonly used in dental practice as an antiseptic agent since 1970. It is a highly bactericidal and bacteriostatic compound, and it has a stronger effect on Gram-positive bacteria than on Gram-negative bacteria *Enterobacteria*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, as well as different species of *Actinomyces* and *Streptococcus*, including *Streptococcus mutans*, which is considered the main etiological agent of dental caries. Some Pseudomonas and Proteus strains, acid-fast bacilli, and bacterial spores are resistant to it. The antibacterial effect is related to the damage of the bacterial cell wall (increased permeability). Chlorhexidine binds to dental plaque and the oral mucosa and is gradually released, protecting against bacteria for a long time (8–12 h). It is also completely safe, although it can sometimes cause local hypersensitivity [54].

Although chlorhexidine is one of the most used biocides in the world, its toxicity to aquatic organisms is poorly understood. Only Jesus and co-workers evaluated its effects on zebrafish embryos [55]. The revealed toxicity of chlorhexidine on zebrafish after 96 h of incubation showed EC50 of 804.0 µg/L, whereas the 15 min EC50 is 1694.0 µg/L. Furthermore, early hatching as well as developmental abnormalities were observed. Moreover, among enzymatic biomarkers, cholinesterase activity was increased in chlorhexidine solutions at a range of concentrations of 80–900 µg/L. Only the highest concentration increased catalase without influence on glutathione-S-transferase and lactate dehydrogenase activities [55].

## 5. Whitening and Flavoring Agents

Teeth whitening is the most popular cosmetic dental procedure. It comes as no surprise, then, that whitening toothpaste is a popular choice for whitening teeth at home. This market need is fully understood and addressed by most toothpaste brands, which offer teeth-whitening product lines. Therefore, the whitening properties of toothpaste can be considered important and desired, whether playing a major or supportive role in daily oral care routine or not. Teeth-whitening ingredients cover different abrasives and bleaching agents, also of herbal origin, which remove and prevent extrinsic stains [56]. Additionally, most ingredients used in toothpaste, especially fluoride and abrasives, are characterized by unpleasant tastes, which are covered by various flavoring agents. Flavoring agents cover non-sugar sweeteners (i.e., sorbitol, glycerol) or refreshing ingredients (i.e., menthol, eucalyptus), which give a cooling and refreshing effect [57]. Though toothpaste flavors are not used to induce any therapeutic effects, those of herbal origin possess additional bioactive properties which may be of help in keeping teeth and gums healthy. On the other hand, flavors are also responsible for most allergy-related adverse reactions to toothpaste [58]. Hence, flavoring agents, even if they do not play a significant part in maintaining oral hygiene, play a great role in consumer choice and acceptance. The following part discusses literature data on toxic effects on zebrafish of the two most studied compounds of whitening and flavoring agents, i.e., hydrogen peroxide and glycerol, respectively.

Glycerol, apart from covering bitter taste, also improves texture and prevents the loss of water and subsequent hardening of toothpaste [57]. It is one of the most common hydrophilic solvents, a humectant with cryoprotectant properties and a low level of toxicity, frequently used in pharmaceutical formulation and biomedical studies including sperm cryopreservation. However, glycerol (5–15%) has been shown to reduce by more than a half the motility of zebrafish sperm within 15 min of incubation, indicating a lack of suitability as a cryoprotectant in zebrafish [59]. Moreover, no zebrafish oocyte exposed for 30 min to 10% glycerol retained the ability to mature and subsequently be fertilized (0% survival). Altogether, it shows that approximately 10% glycerol possesses an inhibitory effect on both zebrafish male and female fertility potential, therefore significantly reducing the reproductive success of zebrafish [60]. Glycerol-induced toxicity has been also intensively studied in zebrafish larvae. Embryotoxic effects of glycerol are concentration-dependent with a strong correlation to embryo stage/larvae age and exposure time. Thus, 0.5% glycerol is a maximal concentration without an effect on embryo survival, when applied to four-cell-stage embryos (1 hpf) with subsequent 48 h exposure [61]. When incubation time is shortened to 24 h, the maximal tolerated concentration reaches 1.5%, and embryos treated within the first 24 hpf with 2.5% glycerol display multiple abnormalities including anterior–posterior axis truncation, u-shaped somites, and cardia bifida [62]. Moreover, embryos subjected to 5% glycerol between 36 and 48 h show a survival rate at the level of ~60% [61]. Accordingly, the older larvae are, the less vulnerable to higher concentrations of glycerol. Larvae at 5–7 dpf remain morphologically unaffected at the concentration of 2.5% after 24 h exposure. However, this concentration affected blood circulation and impaired motility, expressed as the lack of touch response [62]. Finally, adult zebrafish exposed for 10 days to a low concentration of glycerol (0.1%) showed decreased aggressive behavior and disturbed ability to interact with conspecifics [63]. Altogether, the data presented above demonstrate that a compound recognized as well-tolerated and non-toxic for humans may exhibit abundant toxic effects in fish; therefore, it should be considered as pollutive to the water environment.

Hydrogen peroxide ($H_2O_2$) is a common whitening agent which removes extrinsic stains, thereby lightening tooth color [56]. $H_2O_2$ concentrations in water systems may range from nanomolar to micromolar and originate from natural bioactivities of aquatic ecosystems, as well as pollution sources [64]. It is a potent oxidant, one of the ROS molecules, with a strong potential for toxicity in humans and animals. Unsurprisingly, $H_2O_2$ causes a significant lethality in zebrafish. Exposure to 1 mg/mL $H_2O_2$ led to embryo death within 32 h with yolk abnormalities and the tail deformed, while the remaining embryos had delayed development and tail deformation [65]. Moreover, 4 hpf zebrafish larvae exposed to 5 mM $H_2O_2$ for up to 96 hpf showed a high mortality rate, a significant increase in ROS production, and cardiotoxicity expressed as pericardial edema [66]. Finally, exposure to 1 mM $H_2O_2$ of zebrafish from 4 hpf to 96 hpf caused an increase in mortality rate (over 60%) and oxidative damage (loss of SOD and CAT activity), as well as a decrease in hatching rate and heart rate, accompanied by body malformations such as yolk sac edema and bent spine [67]. The reason that $H_2O_2$ has high toxicity is inevitably linked to its oxidative potential; this oxidant has been widely used in toxicological studies to induce reactive oxygen species (ROS) generation and cytotoxicity in the zebrafish model [68][69][70][71][72][73]. Interestingly, the impact of low concentrations of $H_2O_2$ on zebrafish behaviors has also been of scientific interest. Yoon H. et al. [64] studied changes in the behavior of zebrafish after short-term exposure to low concentrations of $H_2O_2$. It has been shown that the safe $H_2O_2$ concentration for both larval and adult zebrafish is 10 nM. Meanwhile, 100 nM $H_2O_2$ affected color preference in 5 dpf larval zebrafish, as well as decreased average velocity, average acceleration, active time, and total distance moved in larvae and adult fish [64].

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