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Mini review

Does fluoride exposure impact on the human microbiome?

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ABSTRACT

Fluoride is added to drinking water in some countries to prevent tooth decay (caries). There is no conclusive evidence that community water fluoridation (CWF) at WHO recommended concentrations for caries prevention has any harmful effects. However, research is ongoing regarding potential effects of ingested fluoride on human neurodevelopment and endocrine dysfunction. Simultaneously, research has emerged highlighting the significance of the human microbiome in gastrointestinal and immune health. In this review we evaluate the literature examining the effect of fluoride exposure on the human microbiome. Unfortunately, none of the studies retrieved examined the effects of ingested fluoridated water on the human microbiome. Animal studies generally examined acute fluoride toxicity following ingestion of fluoridated food and water and conclude that fluoride exposure can detrimentally perturb the normal microbiome. These data are difficult to extrapolate to physiologically relevant human exposure dose ranges and the significance to humans living in areas with CWF requires further investigation. Conversely, evidence suggests that the use of fluoride containing oral hygiene products may have beneficial effects on the oral microbiome regarding caries prevention. Overall, while fluoride exposure does appear to impact the human and animal microbiome, the long-term consequences of this requires further study.

1. Introduction

Fluoridation of public water supplies has been shown to significantly improve oral health by reducing the prevalence of dental caries in children and adults (Fawell et al., 2006; Rugg-Gunn and Do, 2012; Whelton et al., 2019). The World Health Organisation (WHO) recommends an optimal concentration of 1 mg/L NaF (maximum of 1.5 mg/L NaF) in public water supplies (also referred to as community water fluoridation or CWF) for maintaining good oral health (Fawell et al., 2006; Parnell et al., 2009; WHO, 2019). At these concentrations, fluoride ingestion through CWF is generally considered safe ((NAS), 2021; Parnell et al., 2009; WHO, 2019). However, chronic exposure to increased concentrations of fluoride, either from water supplies with naturally occurring fluoride content higher than that recommended by the WHO (endemic areas) or ingestion of fluoride containing dentifrices, can lead to dental fluorosis (Browne et al., 2005; Whelton et al., 2006, 2019). Although most fluoride containing oral hygiene products are intended for topical use only (i.e., applied in the mouth and then expectorated), some ingestion can occur and in children this may increase the risk of dental fluorosis (Whelton et al., 2019). Apart from the cosmetic impact, there is no convincing evidence that dental fluorosis effects overall health (Browne et al., 2005; Fawell et al., 2006; Whelton et al., 2019; WHO, 2019). However, ongoing research shows that long-term exposure of humans to water sources with fluoride concentrations above those recommended by the WHO can have negative health impacts. Skeletal fluorosis can occur in regions where ground water contains naturally high levels of fluoride ($>$ 4 mg/L) and can result in crippling damage to bones and joints (Kabir et al., 2019). At extremely high concentrations (~5 mg/Kg body weight (Guth et al., 2020)) fluoride can directly damage tissue and have severe toxic effects (Kabir et al., 2019). Due to concerns about fluoride toxicity, there has been intense research investigating whether public water fluoridation could have negative health impacts, including effects on neuronal development, cancer, diabetes and irritable bowel disease (Adkins and Brunst, 2021; Fawell et al., 2006; Fluegge, 2016; Follin-Arbelet and Moum, 2016; Guth et al., 2020; Lee et al., 2020; Miranda et al., 2021). Systematic reviews of the evidence have concluded that there is currently no convincing evidence for long-term health impacts of water fluoridation in humans at the recommended dosages for CWF (Agalakova and Nadei, 2020; Guth et al., 2020; Lambe et al., 2022; Miranda

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et al., 2021; (NAS), 2021; WHO, 2019).

2. Fluoride exposure and the microbiome

Dr. Joshua Lederberg coined the term "microbiome" to describe the ecosystem of symbiotic, and sometimes pathogenic microorganisms that reside in the human body. In the last 10 years, our knowledge of the role of the microbiome in human health has greatly expanded (Grice and Segre, 2012; Huttenhower et al., 2014; Lozupone et al., 2012). The GI tract is home to 10^{14} microorganisms, including bacteria, fungi and viruses. We now know that this microbiome protects us from exogenous disease-causing microbes, helps to develop our immune system, regulates metabolism and produces essential vitamins (Dave et al., 2012; Lozupone, 2018; Lyu and Hsu, 2018; Morgan et al., 2012). Development of this microbiome begins soon after birth and impaired development has been linked to atopic disease, obesity and IBD (Halfvarson et al., 2017; Kumbhare et al., 2019). In the oral cavity, maintaining a healthy microbiome is essential to prevent caries and periodontal disease (Rosier et al., 2017). There is a hypothesis that fluoride could potentially disturb the homoeostasis that exists between an individual and their microbiome resulting in unforeseen health impacts. The microbiome throughout the GI tract is exposed to fluoride following ingestion of fluoridated water or small amounts of dentifrice (Adkins and Brunst, 2021; Fawell et al., 2006) (Fig. 1). Fluoride intake values for the average adult (Fig. 1) can vary considerably due to location and type of climate, variations in diet, water consumption and oral hygiene practices (EFSA NDA Panel, 2013). As the first point of exposure, the oral cavity is likely to be exposed to the highest fluoride doses. Fluoride accumulation in dental plaque (a biofilm of bacteria and other microbes) in individuals using fluoride dentifrice has been well established, with levels ranging from 0.63 to 414 ng F/mg of plaque reported (Larsen et al., 2017). However, there is less published data on the levels of fluoride exposure in the mid and lower reaches of the GI tract. Fluoride absorption into the tissues increases as it descends the tract, with approximately 40% of fluoride being absorbed in the stomach as HF, with the remainder absorbed in the small intestine (Fawell et al., 2006). Approximately 10 % of ingested fluoride is excreted in faeces, indicating that all regions of the GI tract will receive some exposure (Buzalaf and Whitford, 2011; Johnston and Strobel, 2020). Although it is difficult to estimate average fluoride intake levels, individuals with a normal diet who are not exposed to water containing fluoride above the WHO recommended maximum limits (1.5 mg/L NaF) are not likely to exceed intake of > 120 μg/Kg/day (or >100 μg/Kg/day in children) and therefore are reported to be of low risk for severe to moderate fluorosis ((FSAI), 2018).

3. Antibacterial activity

Fluoride is known to have antibacterial and antifungal activity and in vitro has been shown to directly inhibit bacterial cell growth by inhibiting energy metabolism and glycolysis (Johnston and Strobel, 2020). This activity may be mediated by direct inhibition of enzymes such as enolase and by acidification of the cytoplasm. Considerable research has been carried out on the effects of fluoride on *S. mutans* due to the key role of this bacterium in caries development (recently reviewed by Liao et al. (2017)). Acid tolerance is key to the survival of this bacterium in acidic caries lesions (Marsh, 2003). Fluoride absorption by *S. mutans* (in the form of HF) has been shown to increase intracellular H^+ accumulation and inhibit H^+ extrusion by the plasma membrane ATPase, thereby diminishing acid tolerance and the ability to grow in acidic plaque (Belli et al., 1995; Marquis, 1990). Fluoride tolerance in bacteria has been reported which may be associated with mutations in the enolase gene or by the expression of fluoride efflux pumps encoded by the *crcB* and *eriC^F* genes (Liao et al., 2018, 2017). Despite the potent antibacterial activity of fluoride, studies which consider the effects of fluoride on the entire oral and gut microbiome communities have only recently begun to appear in the literature.

4. Impact of fluoride on the microbiome

The effect of fluoride on the human microbiome, through exposure to fluoride in dentifrice or fluoridated water, is under researched. A search of the published literature in the PubMed and Scopus databases with the terms “microbiome” or “microbiota” and “fluoride” retrieved 190 studies (date range 1986 to November 2022, Fig. 2). From this collection, we excluded studies that did not contain any significant microbiological analysis, studies that did not include fluoride in their analysis or those that focused solely on environmental, invertebrate or in vitro grown microbiomes (Fig. 2). Following this, 41 studies were included that examined the potential effects of fluoride on the human or animal microbiome (Table 1). None of the published studies have directly

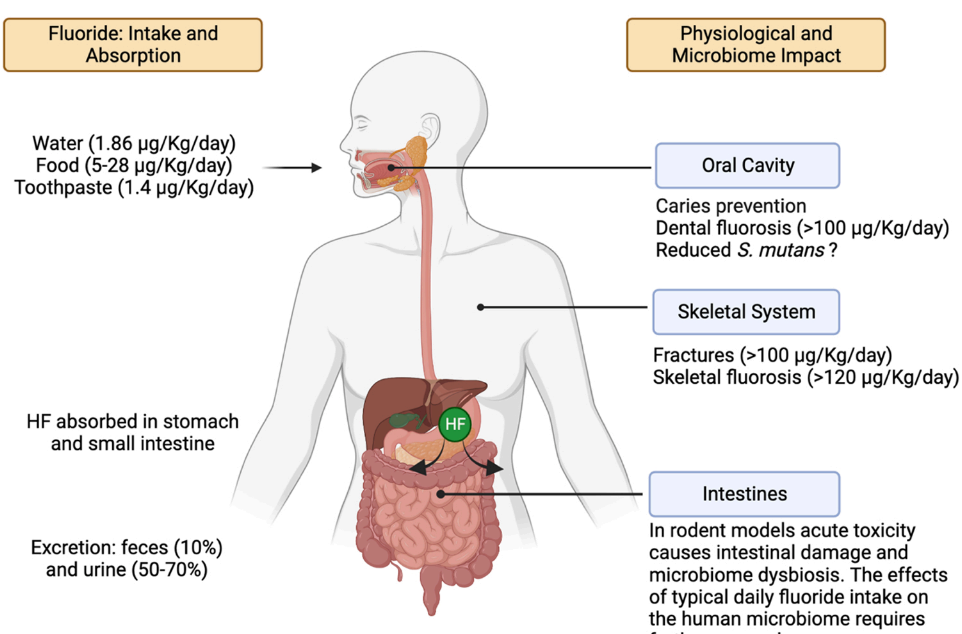

Fig. 1. Diagram outlining absorption and intake of fluoride in the human body. Ingested fluoride amounts (μg/Kg body weight/day) correspond to EU averages (Guth et al., 2020; (EFSA NDA Panel), 2013). Most ingested fluoride is converted to hydrogen fluoride (HF) in the stomach where it is absorbed into the bloodstream. Remaining fluoride in the gut is excreted in faeces, with the majority of the absorbed fluoride excreted in urine (Buzalaf and Whitford, 2011; Johnston and Strobel, 2020). Excessive fluoride consumption can result in high levels of absorbed fluoride being incorporated in teeth (fluorapatite) and bone which may cause dental or skeletal fluorosis. In children consumption of > 100 μg/Kg/day increases the risk for dental fluorosis (EFSA NDA Panel, 2013) and in adults, long-term consumption of > 120 μg/Kg/day can increase the risk of bone fractures or skeletal fluorosis (EFSA NDA Panel, 2013; WHO, 2019).

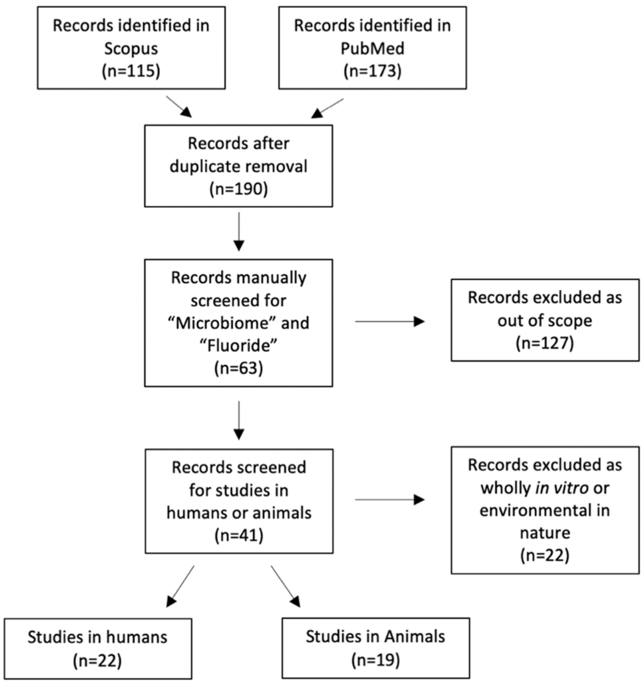

Fig. 2. Flow diagram outlining document search strategy. The Scopus and PubMed databases were searched (November 2022) for the terms "fluoride" and "microbiome" or "microbiota". Studies that did not analyse the relationship between fluoride and microbes were excluded as out of scope. Studies that were wholly in vitro, environmental or relating to invertebrate hosts were also excluded resulting in 41 publications that examined the impact of fluoride on the human or animal microbiome.

examined the effects of ingested fluoridated water on the human microbiome. The following sections describe recent research that has been carried out with human participants and/or using animal models of fluoride exposure.

4.1. Studies in humans

Most studies in humans have focused on the impact of fluoride dentifrices, varnishes and mouthwashes on the oral microbiome. The effects of ingestion of these formulations on the gut microbiome have not been investigated. Most studies report limited or no effects on the oral microbiome, with some studies suggesting beneficial effects. Widyarman et al. carried out a small study of 10 children with caries examining the impact of amorphous calcium phosphate fluoride treatment on the oral microbiome (Widyarman et al., 2021). Fluoride treatment was carried out weekly for a period of one month. Changes in plaque ecology were identified post-treatment and these changes were characterised as not detrimental and possibly health promoting (Widyarman et al., 2021). Silver diamine fluoride (SDF), a compound which combines the antibacterial properties of silver with fluoride, has been assessed in several small studies for its impact on the oral microbiome during caries treatment (Mei et al., 2020; Paul et al., 2021; Ruff et al., 2021). These studies report relatively minor changes to the plaque microbiome in children treated with SDF (Mei et al., 2020). Stannous and fluoride ion (F/Sn)-containing products have also been investigated in several small studies (Anderson et al., 2020; Benjasupattananan et al., 2005; He et al., 2021; Kruse et al., 2021; Loveren et al., 2009; Reilly et al., 2014). Kruse et al. compared two groups (n = 16) over 36 months, with one group using a stannous and fluoride ion (F/Sn)-containing toothpaste and mouth rinse and the second group that used ion free preparations (Kruse et al., 2021). The F/Sn treated group showed small changes in microbiome composition and these were considered harmless. In a second study, Anderson et al. compared the microbiomes of two groups over 36 months (Anderson et al., 2020). One group (n = 16) used a stannous and fluoride ion (F/Sn)-containing toothpaste and mouth rinse and the second used ion free preparations (n = 22). The genus *Prevotella* was found in higher abundance in controls whereas the "health-associated taxa" *Neisseria* and *Granulicatella* were found more abundantly in the fluoride treated group. In general, stannous and fluoride ion (F/Sn)-containing toothpastes may reduce the levels of cariogenic bacteria, but larger studies may be required to confirm these findings (Benjasupattananan et al., 2005; Loveren et al., 2009).

In a larger study, Koopman et al. investigated the effects of using fluoride mouthwash in adolescents undergoing orthodontic therapy (n = 91) (Koopman et al., 2015). The impact of fluoride treatment on the composition of supragingival plaque in terms of composition and species richness were described by the authors as 'trivial'. Giersten et al. also reported limited impact of a fluoride containing mouthwash on the oral microbiota after 4 weeks of use (Giertsen et al., 1999). As discussed previously, fluoride may directly impact on the growth of cariogenic *S. mutans* in the oral cavity. The potential oral health benefits of this activity was directly examined by Badjatia et al. in group of children (n = 42) who were divided in two groups, one of whom received fluoride varnish (Badjatia et al., 2017). Oral *S. mutans* levels were determined by culture at 3 and 6 months in both groups. The group that received fluoride varnish treatment exhibited significantly reduced *S. mutans* levels (> 3-fold lower counts) at the end of the study. Similarly, Bizhang et al. reported that the use of fluoride varnish as part of periodontal maintenance therapy resulted in significantly reduced levels of *S. mutans* and lactobacilli (Bizhang et al., 2007). Conversely, Reilly et al. reported that a single application of 5% sodium fluoride varnish had no significant impact on overall plaque microbiome structure (Reilly et al., 2016). Gedam et al. also reported that use of a NaF mouthwash in children could significantly reduce *S. mutans* counts and was similar in efficiency to a chlorhexidine mouthwash (Gedam and Katre, 2022). Another streptococcal species with a protective role against caries is *S. dentasani*. *S. dentasani* was recently shown to inhibit the growth of *S. mutans* and may have potential as a caries protective, probiotic organism. However, a single study examining the abundance of *S. dentasani* in Colombian children (n = 100) showed that use of fluoride containing products was associated with a significant reduction in the levels of this organisms (Angarita-Díaz et al., 2019).

The combination of fluoride and arginine in dentifrice has also been shown to reduce the levels of *S. mutans* in the oral cavity in a small group of individuals (n = 15) with active caries (Zheng et al., 2017). A more comprehensive analysis of the impact of fluoride dentifrice (1450 ppm) and a combined fluoride/arginine dentifrice was recently carried out by Carda-Diéguez et al. who studied 53 caries active and caries free individuals over 6 months of product use (Carda-Diéguez et al., 2022). The approach involved analysis of plaque composition (metagenomics) and transcriptional activity (meta transcriptomics) following three months use of the fluoride dentifrice. Following this period reduced levels of *Streptococcus* and *Veillonella* species was observed. *Veillonella* species generally thrive in acidic plaque environments and these results strongly indicate a reduction in acidogenicity of the biofilm. Unexpectedly, periodontal pathogens *Prevotella* and *Porphyromonas* species were increased and were more transcriptionally active after 3 months, which may again be related to the reduced acidity of the plaque environment.

Few studies have examined the impact of fluoride ingestion through CWF or other supplements (e.g., salt) on the microbiome. One small study of the plaque microbiome in 56 individuals described by Wolff et al. found a specific biofilm community in adults who took fluoridated salt or tablets in childhood (Wolff et al., 2019). Evidence for a long-term impact of fluoride consumption in childhood would require further investigation. The only study to examine the potential impacts of excessive fluoride exposure through drinking water was carried out by Wang et al. (Wang et al., 2021). This study was carried out in Guizhou province, China, an area with endemic dental fluorosis due to fluoride contamination of ground water. This analysis investigated oral

Table 1
Studies examining the impact of fluoride on human or animal microbiomes.

Human Studies	Year	Description
Carda-Diéguez et al., 2022. Functional changes in the oral microbiome after use of fluoride and arginine containing dentifrices: a metagenomic and metatranscriptomic study. Microbiome 10, 159	2022	Metagenomic and Metatranscriptomic analyses of human dental plaque to evaluate the effect of brushing with fluoride (Fl) and Fl+Arginine containing dentifrices. *Veillonella* and *Streptococcus* were reduced but unexpectedly, periodontal pathogens *Prevotella* and *Porphyromonas* species were increased.
Gedam and Katre, 2022. Efficacy of Probiotic, Chlorhexidine, and Sodium Fluoride Mouthrinses on mutans streptococci in 8- to 12-Year-Old Children: A Crossover Randomised Trial. Lifestyle Genom 15, 35–44	2022	Use of a NaF mouthwash in children could significantly reduce *S. mutans* counts and was similar in efficiency to a chlorhexidine mouthwash.
Chen et al., 2021. The beneficial or detrimental fluoride to gut microbiota depends on its dosages. Ecotox Environ Safe 209, 111732	2021	This is an *ex-vivo* study of human faeces grown in a laboratory fermenter. Addition of low fluoride concentrations (1 and 2 mg/L) had limited effects on the faecal microbiome. Higher concentrations (10 and 15 mg/L) had effects that could be considered detrimental with a reduction in some beneficial microbes.
He et al., 2021. A randomised, controlled comparison of a stannous-containing dentifrice for reducing gingival bleeding and balancing the oral microbiome relative to a positive control. Am J Dent 34, 222–227.	2021	A randomised, controlled, double-blind clinical study (43 test & 43 control participants). The experimental stannous-containing sodium fluoride dentifrice significantly reduced gingival bleeding and promoted a shift in the oral microbiome towards those genera associated with oral health.
Kruse et al., 2021. Long-term use of oral hygiene products containing stannous and fluoride ions: effect on viable salivary bacteria. Antibiotics 10, 481.	2021	This study compared the microbiomes of two groups (n = 16) over 36 months. One group using a stannous and fluoride ion (F/Sn)-containing toothpaste and mouth rinse and the second that used ion free preparations. Culture based techniques were used to characterise oral populations. The F/Sn treated group showed small changes in microbiome composition and these were considered harmless.
Paul et al., 2021. Microbial population shift and metabolic characterization of silver diamine fluoride treatment failure on dental caries. PLoS One 16, e0242396.	2021	The study describes the microbial profiles of children (n = 20) who continued to develop new carious lesions following treatment with silver diamine fluoride. *Leptotrichia* and *Granulicatella* were enriched in non-responders along with the highest abundance of phosphotransferase systems.
Ruff et al., 2021. Predicting Treatment Nonresponse in Hispanic/Latino Children Receiving Silver Diamine Fluoride for Caries Arrest: A Pilot Study Using Machine Learning. Frontiers Oral Heal 2, 695759.	2021	This study analysed the oral microbiome in children (n = 20) to develop a predictive model of silver diamine fluoride treatment non-response. Results indicated that two bacteria, *Prevotella pallens* and *Veillonella dentocariosa*, may be useful in predicting treatment nonresponse
Wang et al., 2021. Structural changes in the oral microbiome of the adolescent patients with moderate or severe dental fluorosis. Sci Rep-uk 11, 2897.	2021	This analysis investigates microbiome differences in 42 individuals, categorised into Healthy (N = 9), Mild (N = 14) and Moderate/Severe (N = 19) fluorosis groups. The study demonstrates a significant shift in the oral microbiome of the M/S group, with increased Firmicutes and reduced Bacteroidetes. The significance of this shift is unknown and due to the small sample size can be considered preliminary.
Widyarman et al., 2021. Casein phosphopeptide–amorphous calcium phosphate fluoride treatment enriches the symbiotic dental plaque microbiome in children. J Dent 106, 103582.	2021	A small study of 10 children with caries examining the impact of amorphous calcium phosphate fluoride treatment on the microbiome. Fluoride treatment was carried out weekly for a period of one month. Changes in plaque ecology were identified post-treatment and these changes were characterised as not detrimental and possibly health promoting.
Anderson et al., 2020. Influence of the long-term use of oral hygiene products containing stannous ions on the salivary microbiome – a randomised controlled trial. Sci Rep-uk 10, 9546.	2020	This study compared the microbiomes of two groups over 36 months, one group (n = 16) using a stannous and fluoride ion (F/Sn)-containing toothpaste and mouth rinse and the second that used ion free preparations (n = 22). 16 S sequencing analysis was performed. The genus *Prevotella* was found in higher abundance in controls whereas *Neisseria* and *Granulicatella*, "health-associated taxa", were found more abundantly in the fluoride treated group.
Mei et al., 2020. Effect of silver diamine fluoride on plaque microbiome in children. J Dent 102, 103479.	2020	A small study characterising the impact of silver diamine fluoride application in 14 children with caries. No overall microbiome changes were observed in children treated with SDF with arrested caries.
Angarita-Díaz et al., 2019. Presence of *Streptococcus dentisani* in the dental plaque of children from different Colombian cities. Clin Exp Dent Res 5, 184–190	2019	The use of fluoride containing products was associated with a significant reduction in the levels of *Streptococcus dentisani.*
Wolff et al., 2019. Amplicon-based microbiome study highlights the loss of diversity and the establishment of a set of species in patients with dentin caries. PLoS One 14, e0219714.	2019	This study found a specific biofilm community in adults (n = 56) who took fluoridated salt or tablets in childhood.
Badjatia et al., 2017. Effects of fluoride varnish on streptococcus mutans count in saliva. Int J Clin Paediatric Dent 10, 62–66.	2017	A group of children (n = 42) were divided in two groups and one group received fluoride varnish. Oral *S. mutans* levels were determined by culture at 3 and 6 months. The group that received fluoride varnish treatment exhibited significantly reduced *S. mutans* levels (> 3-fold lower counts) at the end of the study.
Zheng et al., 2017. Ecological Effect of Arginine on Oral Microbiota. Sci Rep-uk 7, 7206.	2017	A combination of fluoride and arginine in dentifrice was shown to reduce the levels of *S. mutans* in the oral cavity in a small group of individuals (n = 15) with active caries.
Reilly et al., 2016. Short-term effects of povidone iodine and sodium fluoride therapy on plaque levels and microbiome diversity. Oral Dis 22, 155–161.	2016	Reports that a single application of 5% sodium fluoride varnish in children (n = 12) had no significant impact on overall plaque microbiome structure.
Koopman et al., 2015. The effect of fixed orthodontic appliances and fluoride mouthwash on the oral microbiome of adolescents – a randomised controlled clinical trial. PLoS One 10, e0137318.	2015	The effects of using fluoride mouthwash were investigated in adolescents undergoing orthodontic therapy (n = 91). The impact of fluoride treatment on the composition of supragingival plaque in terms of composition and species richness were described by the authors as 'trivial'.
Reilly et al., 2014. Biofilm community diversity after exposure to 0·4% stannous fluoride gels. J Appl Microbiol 117, 1798–1809.	2014	Concludes that the immediate benefits of using 0·4% SnF_2 gels in children may be strictly from fluoride ions inhibiting tooth demineralization rather than changes to the microbiome.
van Loveren et al., 2009. Effect of various rinsing protocols after use of amine fluoride/stannous fluoride toothpaste on the bacterial composition of dental plaque. Caries Res 43, 462–647.	2009	The results from 30 participants indicate that using the AmF/SnF_2 toothpaste and rinse combination could result in lower levels of cariogenic bacteria in plaque.
	2007	

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Table 1 (*continued*)

Bizhang et al., 2007. Microbiota of exposed root surfaces after fluoride, chlorhexidine, and periodontal maintenance therapy: a 3–year evaluation. J Periodontol 78, 1580–1589.		This study reports that the use of fluoride varnish as part of periodontal maintenance therapy (n = 33 participants) resulted in significantly reduced levels of *S. mutans* and lactobacilli.
Benjasupattananan et al., 2005. Effect of a stannous fluoride dentifrice on the sulcular microbiota: a prospective cohort study in subjects with various levels of periodontal inflammation. Oral Health Prev Dent 3, 263–72.	2005	Use of stannous and fluoride ion (F/Sn)-containing toothpastes in 37 adults results in a shift towards a gingival health associated microbiota in patients with mild gingivitis and caries-prone patients
Giertsen et al., 1999. Effects of mouth rinses with xylitol and fluoride on dental plaque and saliva. Caries Res 33, 23–31.	1999	Reports limited impact of a fluoride containing mouthwash in small groups (n = 10) on the oral microbiota after 4 weeks of use

Animal Studies	**Year**	**Description**
Chen et al. Fluoride induced leaky gut and bloom of *Erysipelatoclostridium ramosum* mediate the exacerbation of obesity in high-fat-diet fed mice. *J Adv Res, In Press*	2022	A faecal microbiota transplantation from fluoride-treated mice was sufficient to induce obesity. The authors propose that the fluoride-induced bloom of *Erysipelatoclostridium ramosum* was responsible for exacerbation of obesity.
Komuroglu et al., 2022. Metagenomic analysis of intestinal microbiota in florated rats. Biol Trace Elem Res 200, 3275–3283.	2022	Reports a dramatic loss of Firmicutes (Lactobacilli) in the gut microbiome of rats treated with 100 ml/L NaF in drinking water.
Zhu et al., 2022. Fluoride exposure cause colon microbiota dysbiosis by destroyed microenvironment and disturbed antimicrobial peptides expression in colon. Environ Pollut 292, 118381.	2022	Describes microbiome changes in the gut of mice exposed to 25–100 mg F/L and associates these changes with increased expression of inflammatory cytokines and antimicrobial proteins
Zhong et al., 2022. Effect of fluoride in drinking water on faecal microbial community in rats. Biol Trace Elem Res 1–9.	2022	Five groups with five rats (0, 25, 50, 100 & 150 mg/L NaF treatment) were analysed for dental fluorosis and microbiome changes after 12 weeks. Fluorosis severity and fluoride concentration in faeces increased with treatment. Decreased *Clostridium* sensu stricto*, Roseburia, Turicibacter*, and *Paenalcaligenes* were associated with the grade of fluorosis. The high fluoride concentrations used and the nature of the model suggest that more research is needed.
Dionizio et al., 2021. Intestinal changes associated with fluoride exposure in rats: Integrative morphological, proteomic and microbiome analyses. Chemosphere 273, 129607.	2021	Male rats ingested water with 0, 10, or 50 mgF/L for thirty days. The treatment groups exhibited inflamed ileum morphology and changes in the microbiome, most notably reduced Clostridia.
Li et al., 2021. Environmental fluoride exposure disrupts the intestinal structure and gut microbial composition in ducks. Chemosphere 277, 130222.	2021	Ducklings (n = 7) were exposed to NaF in feed (750 mg/kg) for 28 days and compared to a fluoride free control group (n = 7). Impaired intestinal barrier was observed along with changes in gut microbiome composition. The high fluoride concentrations used and the nature of the model suggest that more research is needed.
Liu et al., 2021. Co-exposure to fluoride and arsenic disrupts intestinal flora balance and induces testicular autophagy in offspring rats. Ecotox Environ Safe 222, 112506.	2021	Four groups or rats were treated for 10 days (control group, 100 mg/L NaF, 50 mg/L $NaAsO_2$ and combined 100 mg/L NaF + 50 mg/L NaAsO2). Alterations in testicular morphology and the faecal microbiome were observed.
Xin et al., 2021a. Probiotic alleviate fluoride-induced memory impairment by reconstructing gut microbiota in mice. Ecotox Environ Safe 215, 112108.	2021	The gut microbiomes of fluoride and fluoride + probiotic treated mice were analysed. Fluoride treatment resulted in reduction in Firmicutes, including *Lactobacillus* spp, and treatment with *Lactobacillus johnsonii* BS15 was protective against the detrimental effects of fluoride treatment on the gut microbiome.
Xin et al., 2021b. Preventive effects of *Lactobacillus johnsonii* on the renal injury of mice induced by high fluoride exposure: Insights from colonic microbiota and co-occurrence network analysis. Ecotoxicol Environ Safe 228, 113006.	2021	*L. johnsonii* BS15 protected the kidney tissue from renal damage induced by high fluoride exposure. Colonic microbiome structure and diversity was significantly altered by fluoride exposure and probiotic treatment.
Yan et al., 2021. Co-exposure to inorganic arsenic and fluoride prominently disrupts gut microbiota equilibrium and induces adverse cardiovascular effects in offspring rats. Sci Total Environ 767, 144924.	2021	Rats were exposed to Arsenic (50 mg/L), Fluoride (100 mg/L) or a combination of both *in utero* and postnatal. All treatments significantly altered the gut microbiome and induced cardiovascular defects. The high concentrations of fluoride and the nature of the model mean that these finding require further investigation.
Fu et al., 2020. Fluoride-induced alteration in the diversity and composition of bacterial microbiota in mice colon. Biol Trace Elem Res 196, 537–544.	2020	Mice were treated with 100 mg/L NaF for 60 days (n = 9) and their microbiome was compared to control mice (n = 9). Fluoride increased microbiome richness and resulted in decreased Firmicutes and increased Bacteroidetes. The high fluoride concentrations used and the nature of the model suggest that more research is needed.
Miao et al., 2020. Dietary high sodium fluoride impairs digestion and absorption ability, mucosal immunity, and alters cecum microbial community of laying hens. Animals 10, 179.	2020	Hens (n = 288) were distributed in to three experimental groups (control [31.19 mg/kg NaF], low-F [431.38 mg/kg NaF] and high-F [1237.16 mg/kg NaF]). These treatments induced severe damage to the intestinal mucosa and the gut microbiome, however the high fluoride concentrations used and the nature of the model suggest that more research is needed.
Qiu et al., 2022. Gut microbiota perturbations and neurodevelopmental impacts in offspring rats concurrently exposure to inorganic arsenic and fluoride. *Environ Int* **140**, 105763	2020	Rats were exposed to Arsenic (50 mg/L), Fluoride (100 mg/L) or a combination of both *in utero* and postnatal. lts Concurrent As and F^- exposure led to more prominent effects on neurodevelopment and gut microbiome composition.
Sun et al., 2020. Probiotic *Lactobacillus johnsonii* BS15 prevents memory dysfunction induced by chronic high-fluorine intake through modulating intestinal environment and improving gut development. Probiotics Antimicrob Proteins 12, 1420–1438.	2020	Fluoride-induced memory dysfunction in mice could be partly offset by supplementing the treatment with *Lactobacillus johnsonii* BS15.
Wang et al., 2020. Fluoride-induced rectal barrier damage and microflora disorder in mice. Environ Sci Pollut Res Int 27, 7596–7607.	2020	This study indicated that excessive fluoride damages the intestinal structure, disturbs the intestinal micro-ecology and causes intestinal microflora disorder in mice.
Xin et al., 2020. *Lactobacillus johnsonii* BS15 improves intestinal environment against fluoride-induced memory impairment in mice—a study based on the gut–brain axis hypothesis. Peerj 8, e10125.	2020	Fluoride treated mice exhibited behavioural defects (100 mg NaF/L) and exhibited neuronal defects and inflammatory responses. These effects could be partly offset by supplementing the treatment with *Lactobacillus johnsonii* BS15. The authors conclude that this is due to the improved intestinal barrier function induced by the probiotic strain.
Liu et al., 2019. Intestinal barrier damage involved in intestinal microflora changes in fluoride-induced mice. Chemosphere 234, 409–418.	2019	Three groups of 24 mice were analysed (50 mg/L fluoride, 100 mg /L Fluoride and a control group). Fluoride induced changes in cecum morphology. The microbiome of the high fluoride group was compared to the control group and significant reduction in Firmicutes and increased Bacteroidetes was observed. The high concentrations used suggest that further research is necessary.
Yasuda et al., 2017. Fluoride depletes acidogenic taxa in oral but not gut microbial communities in mice. mSystems 2, e00047–17.	2017	A well carried out study in mice using relevant concentrations of fluoride in drinking water (4 ppm or 4 ppm + gavage). Fluoride treated groups exhibited reduction in acidogenic bacteria in the oral cavity, which could be caries-protective. However no

(*continued on next page*)

Table 1 (*continued*)

		impact on the gut microbiome was observed at these concentrations. The authors conclude that most fluoride may be absorbed in the upper GI-tract.
Luo et al., 2016. Dietary high fluorine alters intestinal microbiota in broiler chickens. Biol Trace Elem Res 173, 483–491.	2016	Chickens exposed to high levels of fluoride in their diet (400–1200 mg/kg) exhibited changes in the gut microbiome. The high fluoride concentrations used and the nature of the model suggest that more research is needed.

microbiome differences in 42 children (12–14 years), categorised into healthy (N = 9), mild (N = 14) and moderate/severe (N = 19) dental fluorosis groups. The study demonstrates a significant shift in the oral microbiome of the moderate/severe group, with increased Firmicutes and reduced Bacteroidetes. The significance of this shift in relation to caries prevention or general health is unknown. It must be noted that the sample size examined here is extremely small, however this study does present a preliminary indication that excessive fluoride exposure in children may impact on the oral microbiome (Wang et al., 2021).

Studies in humans have not considered the impact of ingested fluoride on the gut microbiome. The only study to address this question was published by Chen *at al.* who carried out an *ex-vivo* study of human faeces, involving culture of faecal samples in a laboratory fermenter (Chen et al., 2021). Addition of low fluoride concentrations (1 and 2 mg/L) had limited effects on the faecal microbiome, and may even promote "health-associated" taxa including *Faecalibacterium* and *Lactobacillus*. Higher concentrations (10 and 15 mg/L) had effects that could be considered detrimental with a reduction in some beneficial microbes (Chen et al., 2021).

4.2. Animal models

A variety of animal models have been used to examine the effects of fluoride ingestion, largely involving mice, rats and hens. Although animals are a useful tool to study the impact of fluoride on human health, extrapolation of the findings to humans can be problematic due to differences in fluoride susceptibility, intestinal barrier function and metabolic rates. It has been estimated that typical human fluoride ingestion of 1–2 mg/day could be modelled in rodent studies using drinking water containing 9–29 mg/L fluoride National Toxicology Program (NTP), 2016. However, many of the studies listed here examine the impact of acute fluoride toxicity on the microbiome, which may occur in some endemic areas, rather than long-term exposure to lower concentrations. An exception to this was a recent study by Yasuda et al. who investigated fluoride ingestion in mice using low concentrations of fluoride in drinking water (4 mg/L), with and without gavage supplementation to represent exposure to fluoride in dentifrice (Yasuda et al., 2017) Fluoride treated groups exhibited reduction in acidogenic bacteria in the oral cavity, which the authors suggest could be caries-protective. However, no impact on the faecal microbiome was observed at these concentrations. The authors concluded that fluoride may be absorbed in the upper and mid GI-tract with little impact on the stool microbiome (Yasuda et al., 2017). Many other studies in mice and rats examine the impact of acute toxicity which is often associated with damage to the integrity of the intestinal barrier (Wang et al., 2020), or use fluoride in combination with other toxic metals (Qui et al., 2022). Dionizio et al. showed that male rats that ingested water with 50 mg/L fluoride for thirty days exhibited inflamed ileum morphology and changes in the gut microbiome, most notably reduced Clostridia (Dionizio et al., 2021). Liu et al. exposed groups of 24 mice to even higher concentrations (50–100 mg/L Fluoride) and noted extensive changes in cecum morphology (Liu et al., 2019). The gut microbiome of the high fluoride group exhibited significant reduction in Firmicutes (Lactobacilli) and increased Bacteroidetes compared to controls. Fu et al. treated mice with drinking water containing 100 mg/L NaF for 60 days (n = 9) and their gut microbiome was compared to control mice (n = 9) (Fu et al., 2020). Fluoride increased microbiome richness and resulted in decreased Firmicutes and increased Bacteroidetes. Komoroglu et al. also reported a dramatic loss of Firmicutes (Lactobacilli) in rats treated with 100 mg/L NaF in drinking water (Komuroglu et al., 2022). Zhu et al. also described microbiome changes in the gut of mice exposed to 25–100 mg F/L and associated these changes with increased expression of inflammatory cytokines and antimicrobial proteins (Zhu et al., 2022). Whilst the majority of these studies focus on the upper extrapolated likely dose range of systemic fluoride exposure in humans, they do appear to indicate that fluoride effects on the gut versus the oral microbiome may be substantially different. Research is needed in order to determine whether daily exposure to fluoride at concentrations recommended for community water fluoridation could elicit similar effects on the gut microbiome in humans.

Zhong et al. employed a more nuanced approach to model dental fluorosis by exposing rats to fluoride concentrations that more closely represent total daily exposure levels in humans Zhong et al., 2022. For this study, groups of rats (n = 5) were exposed to increasing fluoride concentrations in drinking water (0, 25, 50, 100 & 150 mg/L NaF treatment) over 12 weeks. Fluorosis severity and fluoride concentration in faeces increased with treatment. Decreased *Clostridium, Roseburia, Turicibacter*, and *Paenalcaligenes* in the gut microbiome were associated with the grade of fluorosis. Although this study suggests that dental fluorosis could be accompanied by changes to the microbiome, further research is needed to determine if these results can be extrapolated to fluorosis in humans. Some investigators have questioned whether probiotics could protect from the toxic effect of fluoride. Xin et al. showed that fluoride treatment resulted in a reduction in Firmicutes, including *Lactobacillus* spp., and that treatment with *Lactobacillus johnsonii* BS15 was protective against the detrimental effects of fluoride treatment on the gut microbiome of mice (Xin et al., 2020). Similarly, Xin et al. described behavioural defects in fluoride treated mice (100 mg NaF/L) which exhibited neuronal defects and inflammatory responses (Xin et al., 2021a). These effects could be partly offset by supplementing the treatment with *Lactobacillus johnsonii* BS15. Similar results in mice were reported by Sun et al. (2020). The authors conclude that the protective effects are due to improved intestinal barrier functions induced by the probiotic strain (Sun et al., 2020; Xin et al., 2021a,b).

A combination of arsenic and fluoride can be found in drinking water in some endemic areas and this combination has been shown to be exceptionally toxic to the gut microbiome. Rats exposed to Arsenic (50 mg/L), Fluoride (100 mg/L) or a combination of both exhibited significantly altered the gut microbiome and induced cardiovascular defects (Yan et al., 2021) and neurodevelopmental defects (Qui et al., 2020). Similarly, Lui et al. exposed groups of rats to NaF (100 mg/L), $NaAsO_2$ (50 mg/L) or a combination of both and observed changes in testicular morphology and severe disturbance of the faecal microbiome (Liu et al., 2021).

Hens (Luo et al., 2016; Miao et al., 2020) and ducks (Li et al., 2021) have also been used as models of fluoride toxicity. These studies generally involved high concentrations of fluoride (400–1200 mg/kg) and induced severe damage to the intestinal mucosa and the gut microbiome.

5. Conclusion

Microbiome research is still in its early days and studies investigating the impact of fluoride on the human microbiome have only begun to appear in the literature. Studies of the oral microbiome to date suggest that application of topical fluorides has a minor impact on the oral microbiome and these alterations may be beneficial. Exposure to fluorides in drinking water at concentrations that induce dental fluorosis may have a more severe impact on the oral microbiome, but larger studies are required to confirm what are currently only preliminary findings. Animal studies, while suggesting more significant effects on oral and gut microbiomes are difficult to evaluate due to differences in gut barrier structure and fluoride metabolism in rodents. Although most rodent studies appear to focus on the effects of acute toxicity, some have demonstrated impacts on the microbiome using fluoride exposure levels associated with dental fluorosis (Yasuda et al., 2017; Zhong et al., 2022).

In conclusion, there is a need for large population-based studies to assess the impact of fluorides and CWF on the oral and gut microbiome in children and adults. It should be noted that investigators might choose to examine the impact at low intakes, for example corresponding to the CWF levels, or alternatively might focus on the populations where drinking water is contaminated by naturally occurring high levels of fluoride. In either case, if a change in the microbiome is observed, follow-up studies will be required to investigate whether the fluoride-associated microbiome change directly impacts human health. The lack of such studies in the literature is likely due to the difficulties in identifying matched fluoride exposed and non-exposed populations, in addition to the inherent difficulties in accurately determining fluoride exposure from diet, drinking water and tooth brushing.

Some data suggests that fluorides may induce changes to the oral microbiome that are beneficial, such as reducing the levels of cariogenic *S. mutans*, however the impact on the gut microbiome in humans is largely unexplored. High concentrations of fluoride are likely to impact on the structure of the oral and gut microbiomes and further research is needed to determine if this impacts on overall health. It is worth noting that despite the growing evidence for the positive effects of fluoride on the oral microbiome, evidence for the negative impact of excess sugar intake on the oral microbiome is overwhelming and public health initiatives that tackle excess sugar consumption are likely to be a more effective method to improve the health of the oral microbiome and reduce the incidence of dental caries (Marsh, 2003; Pitts et al., 2017; Sheiham and James, 2014; Shanshan et al. , 2023).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Authors G.P.M., L. Z., B.D., M.H. and T.M. served as members of the Irish Expert Body on Fluorides and Health but write in an academic capacity and subject matter expertise (www.fluoridesandhealth.ie).

Data availability

No data was used for the research described in the article.

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