

# The effect of community water fluoridation on dental caries in children and young people in England

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## Title

The effect of community water fluoridation on dental caries in children and young people in England; an ecological study.

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## **Abstract**

### Background

The protective effect of community water fluoridation (CWF) against dental caries may be modified by secular changes in health behaviour. We aimed to determine the contemporary association between fluoride in public water supplies (PWS) and dental caries indicators and inequalities in England.

### Methods

We estimated exposure to CWF and PWS fluoride concentrations from national monitoring data, using Geographic Information Systems and water supply boundaries, categorising mean period exposure into  $<0.1$ ,  $0.1-0.2$ ,  $0.2-0.4$ ,  $0.4-0.7$ , and  $\geq 0.7$  mg/l. We used area-level health outcome and confounder data in multivariable regression models to determine the association between fluoride and caries outcomes and calculated preventive fractions using these coefficients.

### Results

The odds of caries and of severe caries in five-year-olds fell with increasing fluoride concentration in all SES quintiles ( $p < 0.001$  to  $p = 0.003$ ). There was a negative trend between increasing fluoride concentration and dental extractions ( $p < 0.001$ ). Compared to PWS with  $<0.2$  mg/l, CWF prevented 17% (95% CI 5%-27%) to 28% (95% CI 24%-32%) of caries (high-low SES) and 56% (95% CI 25-74%) of dental extractions. The association between fluoride concentration and caries prevalence/severity varied by socioeconomic status (SES) ( $p < 0.001$ ).

### Conclusions

Exposure to fluoride in PWS appears highly protective against dental caries and reduces oral health inequalities.

## Background

Dental caries, also known as dental decay or tooth decay, is a largely preventable disease. Despite reductions in prevalence in England since the 1970s, it remains a significant public health problem in England, affecting a quarter of five-year-olds, and is a common cause of child hospital admission, including for extractions under general anaesthetic (1-3). Sizeable inequalities in caries prevalence still exist, with better dental health seen in affluent compared to deprived communities (3).

Fluoride is naturally present in drinking water and some foods in varying amounts. Community water fluoridation (CWF) schemes adjust fluoride concentrations in public water supplies (PWS) to reduce levels of dental caries in the populations they serve. Approximately 10% of the population of England are now served by a CWF scheme with a target concentration of 1mg/L(4). Fluoride has also been included in toothpaste and products for professional application such as gels and varnishes.

Though the protective effect of CWF on caries is established (5), secular changes in diet and exposure to fluorides such as from toothpaste may also affect caries risk (6), requiring contemporary estimates of effectiveness. Additionally, the evidence for the effect on dental health inequalities is less certain than that for overall impact.

We aimed to determine the contemporary association between fluoride concentration in public water supplies in England and dental caries indicators to assess whether the addition of fluoride to public water supplies in England remains an effective and equitable public health intervention.

## Methods

### *Exposure Assessment*

Exposure indicators were estimated using PWS fluoride concentration obtained from routine fluoride monitoring data from 2005-2015 submitted from water companies to the Drinking Water Inspectorate (DWI), the water quality regulator for public water supplies in England. Water companies in England have a duty to monitor the fluoride concentration of public water supplies in the WSZs they supply (7, 8).

Drinking water is an important source in human total fluoride intakes(9, 10), and elevated fluoride water concentrations correlate with human biomarkers of exposure(11-14), as does living in an area served by a CWF scheme(14). WSZ boundaries demarking areas within CWF schemes have therefore been used as an efficient method for estimating population exposure to fluoride in public water supplies(14, 15). Detailed descriptions of the method we used to assign fluoride concentration exposure data to populations are available elsewhere(16) (see also the technical appendix). Briefly, we used Geographic Information Systems (GIS) point-in-polygon (PIP) methods to assign the population-weighted centroid of each Lower layer Super Output Area (LSOA) (the smallest geographical unit of analysis) to a WSZ shape file (supplied by the DWI), for each year of available WSZ data (2005-2015). The linked LSOA-WSZ pairs were then merged with the DWI fluoride concentration and fluoridation scheme flagging dataset, using a unique identifier. If required, arithmetic mean period fluoride concentrations for the exposure period of

interest were then aggregated from LSOA to higher geographic levels, weighted by the exposed population. We further sought to match exposure data to the dental outcomes; for caries in 5-year-old children 2014/15 mean fluoride concentration were calculated for calendar years 2009-2015; for hospital admission (extraction) fluoride concentrations were calculated for 2007-15. Fluoride concentration in water supply, regardless of source, was grouped into the following *a priori* defined categories: 0.0- $<0.1\text{mg/l}$ ,  $0.1- <0.2\text{mg/l}$ ,  $0.2- <0.4\text{mg/l}$ ,  $0.4- <0.7\text{mg/l}$ ,  $\geq 0.7\text{mg/l}$ .

### *Covariate and outcome data*

Dental caries data were obtained from the 2014/15 survey of five-year-old children undertaken for the National Dental Epidemiology Programme for England(1) (NDEP); the outcome measures selected were prevalence (percentage with at least one decayed, missing or filled primary tooth -  $\%d_{3\text{mft}} > 0$ ) and severity (mean number of decayed missing or filled primary teeth -  $d_{3\text{mft}}$ ). Aggregate counts of affected children were extracted at LSOA level from the survey dataset.

Counts of hospital admission of children and young people, aged 0-19 years, for extraction of one or more primary or permanent teeth due to dental caries (2007-15), were obtained from the Dental Public Health Intelligence Programme using hospital episode statistics (HES). It was decided that de-duplication of episodes within each 12-month period was not required as repeat admission for surgery within 12 months was thought to be uncommon and unlikely to impact on our analysis.

We used established methods to aggregate IMD to higher geographies based on population weighted averages of their constituent LSOA scores(17). Other population level denominator and confounder data were obtained from the ONS (2011 census ethnicity data), and PHE population databases (age- and gender-specific population counts).

### *Data analysis*

Statistical analysis was performed using Stata 14 (Stata Corp, College Station, TX, US). Fuller analysis details are available elsewhere(16) (see also the technical appendix). We calculated summary statistics for each category of fluoride exposure status as follows: for caries outcomes, the proportion of children with  $d_{3\text{mft}} > 0$ , and sample-weighted grand mean of  $d_{3\text{mft}}$ ; for hospital admissions, the crude incidence (density) rate per category of fluoridation exposure, by dividing the episode count by the total persons at risk in 2007-2015.

We used univariate regression and then multivariable models to determine regression coefficients for the association between each category increase of fluoridation exposure and outcomes, the latter adjusted for potential confounders. Proportion data were analysed using a Binomial model with *logit* link, using sample size as the number of 'trials' per LSOA. Inverse inclusion probability weights were not used in regression analyses, as the relationship between the exposure and outcome at the unit of analysis level was of interest, rather than the prevalence of caries itself. Therefore, an unweighted model-based estimate is appropriate and unbiased(18). Robust standard errors were adopted to adjust the standard error for primary sampling unit (PSU) level clustering not accounted for in the primary survey

analysis. The mean dmft data distribution was severely skewed and log transformation was inappropriate due to zero values(19). Therefore, an ordered logistic regression approach was taken using *gologit2* user-written STATA command, which is appropriate for fitting models where the proportional odds assumption is violated(20), as was determined for this analysis using an approximate likelihood ratio test (p value cut off <0.05) (see technical appendix). We analysed hospital admission count data using Negative Binomial models at MSOA-level. For dental extraction admissions a fixed effects model with a cluster option (using the 'parent' LTLA of the MSOA unit of analysis) was adopted to inflate the standard error to account for likely non-independence between the values of the outcome variable for MSOAs within the same LTLA. A backward stepwise procedure was used to fit the most parsimonious model, using the Wald test, taking a significance level of  $p < 0.10$  (see technical appendix). We then modelled the categorical fluoride exposure variable as a linear term, in order to determine whether there was a linear trend in the regression coefficient with each increase in fluoride concentration category.

For all outcomes an *a priori* defined interaction term was fitted between deprivation status and fluoride concentration category and tested for statistically significant evidence of interaction ( $p < 0.10$ ) using a Wald test. When an interaction was confirmed, deprivation quintile stratum-specific regression coefficients, adjusted for covariates, were presented.

All confounders, other than ethnicity, were modelled as categorical variables. Post-estimation, the assumed linear relationship between ethnicity (modelled as the continuous covariate of age-specific population percentage of non-white ethnicity)) and the outcome was checked by confirmation that when ethnicity was successively modelled as a quadratic and cubic function, their coefficients were not significantly different from zero (using a Wald test p value of <0.05). If a non-linear was significantly different from zero, then ethnicity was instead categorised into quintiles and modelled as a categorical variable.

We calculated the preventive fraction (PF) (as a percentage) to indicate the percentage of prevalent cases of caries experience, and extractions due to dental caries, of the study population that could be prevented by exposure to PWS at a concentration of at least 0.7mg/L in a fluoridation scheme, compared to populations exposed to low fluoride concentrations (i.e. of less than 0.2mg/l). 0.7mg/L was selected as this is the concentration at which international evidence suggests we would expect an impact on caries of public health significance(21). Fluoride concentration category was re-coded into a binary variable (<0.2mg/l and  $\geq 0.7$ mg/l) and modelled (adjusted for confounders as above) against proportion of children with caries experience, and extractions due to dental caries, to derive risk ratios. We used Binomial regression with a log link (rather than the logit link used in the non-binary analysis) to determine risk ratios for the caries prevalence outcome. Stratum specific ratios were reported when interaction by deprivation was indicated in the earlier non-binary analyses.

*Post hoc analyses*

In order to investigate the association between fluoride exposure and prevalence of dental caries experience at higher concentrations than 0.7mg/l, we split the highest exposure category into two categories, leaving six in total as follows: <0.1mg/l, 0.1-<0.2mg/l, 0.2-<0.4mg/l, 0.4-<0.7mg/l, 0.7-<0.9mg/l,  $\geq$ 0.9mg/l. This allowed an assessment of the continuation of trend and/or any potential threshold effect.

## Results

Dental survey data were collected for 111,500 five-year-olds (16.5% of the five-year-old 2014 mid-year population), of which 111,455 (99.96%) were allocated a public water supply fluoride exposure status. Distribution of surveyed five-year-olds by fluoride exposure indicator, area level deprivation score and estimated percentage of white ethnicity was broadly similar to the national average for five-year-olds (see supplementary table 1). Prevalence of caries experience (%d<sub>3</sub>mft>0) fell by almost 6% (a relative reduction of 21%) with increasing fluoride concentration (see Table 1), with the highest prevalence (26.3%) seen in areas with a fluoride concentration of <0.1mg/l, and the lowest in areas with the highest fluoride concentrations >0.7mg/l (20.7%). The mean severity was 0.92 d<sub>3</sub>mft (95% CI 0.90, 0.93) in areas with a fluoride concentration of <0.1mg/l and decreased by 36% to 0.59 d<sub>3</sub>mft (95% CI 0.57, 0.60) in areas with the highest fluoride concentrations  $\geq$ 0.7mg/l.

**Table 1.** Prevalence of caries experience (d<sub>3</sub>mft>0) and mean number of d<sub>3</sub>mft in five-year-olds sampled for the 2014/15 NDEP by mean fluoride concentration (mg/l), England 2014/15

Fluoride conc. (mg/l)	Sample size	Children with d <sub>3</sub> mft>0	d <sub>3</sub> mft>0 prevalence (%) (95% CI)	Mean d <sub>3</sub> mft (95% CI)*
<0.1	33,584	8,837	26.3 (25.8-26.8)	0.92 (0.90-0.93)
0.1-<0.2	42,462	10,819	25.5 (25.1-25.9)	0.89 (0.88-0.90)
0.2-<0.4	16,897	3,675	21.8 (21.2-22.4)	0.71 (0.69-0.72)
0.4-<0.7	5,419	1,316	24.3 (23.2-25.4)	0.81 (0.79-0.84)
$\geq$ 0.7	13,093	2,710	20.7 (20.0-21.4)	0.59 (0.57-0.60)
ALL	111,455	27,357	24.5 (24.3-24.8)	0.83 (0.82-0.84)

\*Weighted by sample size; CI – Confidence interval

The crude odds of caries experience (d<sub>3</sub>mft>0) decreased with increasing fluoride concentration (see supplementary table 2) and following adjustment for potential confounders (table 2). There was an interaction between fluoride concentration and IMD (p<0.001), with increasing fluoride concentration resulting in a larger decrease in odds of d<sub>3</sub>mft in children living in the most deprived areas compared to those from the least deprived areas. Therefore, stratum-specific odds ratios of the fluoride caries association and tests of trend by deprivation are presented. For caries severity, as detailed in the methods section, to allow fitting of a regression model to adjust for the effects of ethnicity and deprivation status, surveyed LSOAs were categorised by the median number of d<sub>3</sub>mft per child and this is summarised in supplementary table 3. The crude odds of being in any higher d<sub>3</sub>mft category compared to 'none' did not show a clear relationship with fluoride concentration (see

supplementary table 4). However, the crude odds of being in 'medium or high' compared to lower categories, or the 'high' caries severity category compared to all lower categories decreased roughly linearly with increasing fluoride concentration above the reference (<0.1mg/l). Crude odds were robust to adjustment and there was an interaction between fluoride concentration and deprivation ( $p < 0.001$ ) as above. Stratum specific odds of being in a 'high' d<sub>3</sub>mft category with each fluoride concentration and tests of trend are shown in table 2. Full results for other d<sub>3</sub>mft categories are in supplementary tables 5 and 6.



**Table 2.** Adjusted odds ratio of caries experience ( $d_{3mft} > 0$ ), and adjusted odds of being in average number of teeth with  $d_{3mft}$  group 'high' versus 'none', 'low' or 'medium' in five-year-olds sampled for the 2014/15 NDEP by mean fluoride concentration (mg/l), stratified by quintile of index of multiple deprivation, England 2014/15

QIMD	Fluoride concentration (mg/l)	Adjusted odds ratio of caries experience (95% CI)*	p for trend	Adjusted odds of 'high' $d_{3mft}$ group (95% CI)*	p for trend
1 (least deprived)	<0.1mg/l	Ref	0.003	0.10 (0.08-0.12)	0.003
	0.1-<0.2mg/l	0.96 (0.87-1.06)		0.10 (0.08-0.11)	
	0.2-<0.4mg/l	0.92 (0.81-1.04)		0.09 (0.07-0.11)	
	0.4-<0.7mg/l	0.99 (0.78-1.26)		0.13 (0.06-0.21)	
	≥0.7mg/l	0.77 (0.61-0.91)		0.08 (0.05-0.11)	
2	<0.1mg/l	Ref	<0.001	0.15 (0.13-0.18)	<0.001
	0.1-<0.2mg/l	1.01 (0.92-1.10)		0.16 (0.14-0.18)	
	0.2-<0.4mg/l	0.92 (0.82-1.03)		0.11 (0.09-0.14)	
	0.4-<0.7mg/l	0.77 (0.64-0.94)		0.08 (0.04-0.13)	
	≥0.7mg/l	0.72 (0.63-0.84)		0.08 (0.05-0.11)	
3	<0.1mg/l	Ref	<0.001	0.21 (0.18-0.24)	<0.001
	0.1-<0.2mg/l	0.99 (0.91-1.08)		0.21 (0.18-0.24)	
	0.2-<0.4mg/l	0.94 (0.84-1.05)		0.21 (0.17-0.25)	
	0.4-<0.7mg/l	0.89 (0.75-1.06)		0.13 (0.07-0.19)	
	≥0.7mg/l	0.73 (0.64-0.83)		0.09 (0.05-0.12)	
4	<0.1mg/l	Ref	<0.001	0.34 (0.30-0.38)	<0.001
	0.1-<0.2mg/l	0.86 (0.79-0.92)		0.28 (0.25-0.31)	
	0.2-<0.4mg/l	0.81 (0.73-0.90)		0.26 (0.21-0.30)	
	0.4-<0.7mg/l	0.93 (0.80-1.09)		0.34 (0.24-0.45)	
	≥0.7mg/l	0.71 (0.63-0.80)		0.17 (0.13-0.22)	
5 (most deprived)	<0.1mg/l	Ref	<0.001	0.71 (0.65-0.77)	<0.001
	0.1-<0.2mg/l	0.62 (0.58-0.67)		0.37 (0.32-0.41)	
	0.2-<0.4mg/l	0.73 (0.66-0.80)		0.46 (0.38-0.54)	
	0.4-<0.7mg/l	0.64 (0.57-0.73)		0.51 (0.38-0.65)	
	≥0.7mg/l	0.48 (0.44-0.53)		0.18 (0.14-0.22)	

\*Adjusted for ethnicity. Robust standard errors.

A *post hoc* analysis for the caries experience outcome of splitting the highest fluoride concentration category into 0.7-<0.9mg/l and  $\geq$ 0.9mg/l revealed strong evidence of an interaction between fluoride concentration and deprivation status ( $p<0.001$ ) as before and that the odds of caries continued to fall at concentrations up to at least 0.9mg/l (see supplementary table 7).

The PF of dental caries if all five-year-olds with drinking water with <0.2mg/l fluoride instead received at least 0.7mg/l from a fluoridation scheme ranged from 17%-28%, lowest in the least deprived quintile (17%, 95% CI 5%-27%), and greatest in the most deprived quintile (28%, 95% CI 24%-32%), see supplementary table 8.

### Hospital admissions

Over 70% of MSOAs lay in WSZs with low (<0.2mg/l) fluoride concentrations, and 10% in WSZs with high (at least 0.7mg/l) fluoride concentrations during the 2007-2015 period of interest (see supplementary table 9). The 0-19 year old population during 2007-2015 (summarised as 'person years') showed a similar distribution.

The crude incidence of cases of children/young people (age 0-19) requiring dental extractions in hospital as a result of caries decreased as fluoride concentration increased from lowest to highest concentration categories (see table 3).

**Table 3.** Crude incidence of cases of hospital admission for dental extractions due to caries in 0-19 year olds in England, by mean fluoride concentration category, England 2007-2015

Fluoride concentration (mg/l)	Cases of extractions due to caries	Person years (millions)	Crude incidence (per 100,000 pyar)	95% CI
<0.1	173,251	40.99	422.7	420.1 – 424.7
0.1-<0.2	123,237	41.13	299.7	298.0 – 301.3
0.2-<0.4	31,215	16.58	188.3	186.2 – 190.3
0.4-<0.7	9,736	4.24	229.6	225.0 – 234.2
$\geq$ 0.7	18,065	11.59	155.9	153.6 – 158.2
Missing	1	0.04	27.81	0.07 – 154.9
Total	355,505	114.53	310.4	309.4 - 311.4

*Pyar* – person years at risk; *CI* – Confidence Interval

The adjusted incidence of admissions for caries-related dental extraction was up to 59% lower (95% CI 33% to 76%) in areas with fluoride of  $\geq$ 0.7mg/l, compared to the reference areas, and there was strong evidence of a linear trend ( $p<0.001$ ) (see table 4). There was no evidence of an interaction between fluoride concentration and deprivation status ( $p=0.40$ ).

**Table 4.** Crude and adjusted incidence rate ratio of cases of hospital dental extractions due to caries in 0-19 year olds, by period mean fluoride concentration (mg/l), England 2007-2015

<b>Fluoride concentration (mg/l)</b>	<b>Crude IRR (95% CI)<sup>†</sup></b>	<b>Adjusted IRR* (95% CI)<sup>†</sup></b>	<b>P for trend</b>
<0.1mg/l	Reference	Reference	<0.001
0.1-<0.2mg/l	0.70 (0.56-0.89)	0.74 (0.62-0.88)	
0.2-<0.4mg/l	0.46 (0.35-0.61)	0.55 (0.44-0.68)	
0.4-<0.7mg/l	0.56 (0.39-0.79)	0.61 (0.46-0.80)	
≥0.7mg/l	0.38 (0.23-0.63)	0.41 (0.24-0.67)	

*IRR – incidence rate ratio*

<sup>†</sup> Cluster robust standard errors derived by clustering on 325 local authority districts

\*Adjusted for age group, gender, ethnicity, index of multiple deprivation

A *post hoc* analysis of splitting the highest fluoride concentration category into 0.7-<0.9mg/l and ≥0.9mg/l revealed that the risk of extractions in hospital due to dental caries did not continue to fall at concentrations up to at least 0.9mg/l.

The adjusted PF of dental caries related admissions for dental extraction if all children and young people with drinking water with <0.2mg/l fluoride instead received at least 0.7mg/l from a fluoridation scheme was 56% (95% CI 25-74%), see supplementary table 10.

As with caries prevalence and intensity, we found a reduction in the mean number of cases of hospital admissions with increasing fluoride concentration across all categories of deprivation (see supplementary figure 1). Unlike our findings for caries prevalence and severity, the relative effect of fluoride did not differ by deprivation status and the benefits of concentrations greater than 0.7mg/l seen for caries prevalence were also not evident for dental extractions.

## Discussion

### Main finding of this study

We found strong evidence for a highly clinically significant reduction in dental caries prevalence/ severity and related hospital admissions for dental extractions, with increasing levels of fluoride in water supplies. We also found a benefit across all categories of deprivation, with a greater effect in areas of greater deprivation. Given that around 70% of the English population have <0.2mg/L in their drinking water and the high levels of dental caries and associated treatment, these findings indicate that many children and young people could benefit from community water fluoridation.

### What is already known on this topic

A recent Cochrane Review (5) found water fluoridation resulted in children having 35% fewer decayed, missing and filled deciduous teeth(5), broadly in keeping with our findings, and there was an absolute reduction in the prevalence of children with

caries experience of 15% (95% CI 11%-19%)(5). This reduction in prevalence is larger than our findings (5-6%) but drew on analysis of studies largely undertaken before 1975. A possible explanation for this difference is that, in contrast to studies from earlier decades, there have been secular changes in diet and an increased use of fluoride containing dental products, particularly toothpaste. This is likely to have contributed to the large reduction in the prevalence of caries over this period, and therefore decreased the absolute benefit of water fluoridation, though the relative effectiveness may still be similar. In support of this, other more contemporary studies(22, 23) have found absolute reductions in caries prevalence of between 3-11% in five-year-olds, closer to the findings of this monitoring report.

The evidence regarding the effects of fluoridation on caries related hospital admissions is more sparse; consequently a greater degree of caution should be used when interpreting our findings. However, the existing evidence appears to be consistent in both direction and strength of association and is strongly supported by the comprehensive evidence base linking reduction in caries and caries severity with water fluoridation. Five studies included in the recent Australian NHMRC review found a reduction in the rate of hospitalisation in areas with fluoridated water supplies(24); in three of these studies effect estimates were reported by the NHMRC of admission rates that were 43-55% lower, consistent with our findings.

#### What this study adds

The nature of water fluoridation is such that the whole population receiving the water supply is able to benefit without the need for individuals to change their behaviour or comply with advice of healthcare professionals, thereby also contributing to the narrowing of dental health inequalities. This is in keeping with our findings of a greater reduction in prevalence and severity of dental caries experience for the most deprived compared to the least deprived five-year-olds. These findings add further weight to the conclusions of recent evidence reviews (24, 25, 26), that there is evidence that fluoridation narrows oral health inequalities in children. The direction and trend in association between fluoride concentration/fluoridation and caries prevalence, severity and dental extractions were similar; this triangulation strengthens confidence in our findings.

The use of hospital admissions as an indicator for assessing the dental health benefits of fluoride in drinking water is relatively novel and potentially more problematic due to data quality considerations. While we observed significant reductions in hospital admissions with increasing fluoride concentration, we did not find a difference in the relative effect across quintiles of deprivation, nor did we observe further reductions at concentrations greater than 0.7mg/L. It is uncertain whether the different patterns of apparent impact between different types of indicator reflect differences in the nature of the outcome (admissions for extraction reflecting the more severe end of the caries spectrum of disease), populations studied or data sources.

## Limitations of this study

While the overall trend was for reduction in caries prevalence/ severity and hospital admissions with increasing fluoride levels in drinking water, there was not a smooth linear relationship, with a flattening of the downward slope at concentrations between 0.2mg/l and 0.7mg/l. This may reflect the true caries-fluoride relationship, instability of associations due to the relatively small population receiving water with these concentrations, limitations in the data (e.g. resulting from differential misclassification of exposure category at these concentrations), or residual/un-measured confounding obscuring the true association. In particular, we were unable to directly adjust for cariogenic dietary factors and oral hygiene behaviours, or area level factors such as differing provision of primary care dentistry or other oral health promotion programmes. Further studies will be needed to confirm the true nature of the relationship. There are known considerations affecting data quality that mean that the dental extraction findings should be treated with caution(27). There is, however, no reason to suppose that services in fluoridated areas are in general likely to record this activity differently to services in non-fluoridated areas(28, 29). The rate of HES extractions performed is only one measure of secondary healthcare need and may under-estimate population caries burden, for example if there are constraints in service delivery such as operating theatre availability limiting the number of extractions undertaken.

## Conclusion

Our findings are consistent with the view that water fluoridation remains a highly effective public health measure to reduce the prevalence and severity of dental caries, and to reduce dental health inequalities in children.

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Technical Appendix

Supplementary tables

Supplementary Figure1



## Supplementary tables and figure 1

**Supplementary Table 1.** Characteristics of National Dental Epidemiology Programme (NDEP) survey of five-year-old children 2014/15 and estimated population of five-year-olds in England using mid-year 2014 population estimates

Characteristic	Fluoride concentration (mg/l)	Surveyed: count (%)	England: count (%)
Fluoride concentration (mg/l)	<0.1	33,584 (30.12)	239,162 (35.49)
	0.1-<0.2	42,462 (38.09)	248,537 (36.88)
	0.2-<0.4	16,897 (15.15)	95,071 (14.11)
	0.4-<0.7	5,419 (4.86)	25,175 (3.74)
	≥0.7	13,093 (11.74)	65,842 (9.77)
	No data <sup>†</sup>	45 (0.04)	169 (0.04)
	TOTAL	111,500 (100.00)	673,956 (100.00)
Deprivation quintile	1 (least deprived)	19,980 (17.92)	120,475 (17.88)
	2	20,487 (18.37)	118,857 (17.64)
	3	21,117 (18.94)	124,584 (18.49)
	4	23,793 (21.34)	141,153 (20.94)
	5 (most deprived)	26,123 (23.43)	168,887 (25.06)
	TOTAL	111,500 100.00)	673,956 (100.00)
Estimated count & percentage ethnicity*	White ethnicity	87,859 (78.80)	530,858 (78.77)

\*Percentage of 0-24 year olds of white ethnicity on census 2011 multiplied by five-year-olds surveyed/five-year-old population; <sup>†</sup>4 LSOAs where 45 children were sampled did not have a fluoride concentration or fluoridation status allocated

**Supplementary Table 2.** Crude odds of caries experience ( $d_{3mft} > 0$ ) in five-year-olds sampled for the national survey, by mean fluoride concentration (mg/l), England 2014/15

<b>Fluoride concentration (mg/l)</b>	<b>Crude odds ratio (95% CI)</b>	<b>p value</b>
<0.1mg/l	Reference	-
0.1-<0.2mg/l	0.96 (0.92-1.00)	0.03
0.2-<0.4mg/l	0.78 (0.74-0.82)	<0.001
0.4-<0.7mg/l	0.90 (0.83-0.97)	0.007
$\geq 0.7$ mg/l	0.73 (0.69-0.77)	<0.001

*Robust standard errors*

**Supplementary Table 3.** Median number of teeth with caries experience, and range, in five-year-olds sampled for the National Dental Epidemiology Programme (NDEP) survey 2014/15, England (n=111,455 five-year-olds in 24,704 LSOAs)

<b>Category of d<sub>3</sub>mft</b>	<b>Median (LQ-UQ) number of teeth with caries experience (d<sub>3</sub>mft)</b>	<b>Range</b>
None	0	0
Low	0.33 (0.25-0.50)	0.05-0.63
Medium	1.00 (0.80-1.17)	0.63-1.5
High	2.40 (2.00-3.50)	1.5-16

*LQ – Lower Quartile; UQ – Upper Quartile*

**Supplementary Table 4.** Crude odds ratios of higher severity d<sub>3</sub>mft category in five-year-olds sampled for the NDEP survey 2014/15, by 2009-2015 period mean fluoride concentration (mg/l), England

<b>D<sub>3</sub>mft severity category</b>	<b>Fluoride concentration (mg/l)</b>	<b>Crude OR (95% CI) of higher d<sub>3</sub>mft category</b>	<b>p value</b>
None vs.	<0.1	Ref	-
Low or	0.1-<0.2	1.07 (1.01-1.13)	0.033
medium or	0.2-<0.4	0.95 (0.88-1.03)	0.186
high	0.4-<0.7	1.25 (1.09-1.42)	0.001
	≥0.7	0.98 (0.89-1.08)	0.699
None or	<0.1	Ref	-
low vs.	0.1-<0.2	1.03 (0.97-1.09)	0.411
medium or	0.2-<0.4	0.82 (0.76-0.89)	0.000
high	0.4-<0.7	0.96 (0.84-1.10)	0.581
	≥0.7	0.66 (0.60-0.73)	0.000
None or	<0.1	Ref	-
low or	0.1-<0.2	0.92 (0.86-0.99)	0.031
medium	0.2-<0.4	0.70 (0.63-0.78)	0.000
vs. high	0.4-<0.7	0.81 (0.68-0.95)	0.012
	≥0.7	0.45 (0.39-0.52)	0.000

*Robust standard errors.*

**Supplementary table 5.** Adjusted odds of being in average number of teeth with d<sub>3</sub>mft group 'low', 'medium' or 'high' vs 'none' in five-year-olds sampled for the NDEP survey 2014/15, by mean fluoride concentration (mg/l), stratified by quintile of index of multiple deprivation, England 2014/15

Deprivation Quintile	Fluoride concentration	Odds*	95% CI	p for trend
1 (least deprived)	<0.1mg/l	0.76	0.67-0.84	0.155
	0.1-<0.2mg/l	0.77	0.70-0.84	
	0.2-<0.4mg/l	0.89	0.77-1.01	
	0.4-<0.7mg/l	1.07	0.70-1.43	
	≥0.7mg/l	0.76	0.61-0.91	
2	<0.1mg/l	1.03	0.93-1.14	0.337
	0.1-<0.2mg/l	1.01	0.91-1.10	
	0.2-<0.4mg/l	1.10	0.95-1.25	
	0.4-<0.7mg/l	1.11	0.80-1.42	
	≥0.7mg/l	0.82	0.66-0.98	
3	<0.1mg/l	1.21	1.09-1.33	0.311
	0.1-<0.2mg/l	1.21	1.10-1.32	
	0.2-<0.4mg/l	1.25	1.07-1.44	
	0.4-<0.7mg/l	1.28	0.93-1.63	
	≥0.7mg/l	1.07	0.87-1.28	
4	<0.1mg/l	1.56	1.40-1.72	0.626
	0.1-<0.2mg/l	1.69	1.53-1.85	
	0.2-<0.4mg/l	1.37	1.17-1.57	
	0.4-<0.7mg/l	1.80	1.31-2.29	
	≥0.7mg/l	1.54	1.25-1.84	
5 (most deprived)	<0.1mg/l	2.51	2.27-2.74	0.340
	0.1-<0.2mg/l	2.28	2.01-2.56	
	0.2-<0.4mg/l	2.25	1.85-2.64	
	0.4-<0.7mg/l	4.01	2.78-5.24	
	≥0.7mg/l	2.51	2.05-2.97	

\*Adjusted for ethnicity

**Supplementary table 6.** Adjusted odds of being in average number of teeth with d<sub>3</sub>mft group 'medium' or 'high' versus 'low' or 'none' in five-year-olds sampled for the NDEP survey 2014/15, by mean fluoride concentration (mg/l), stratified by quintile of index of multiple deprivation, England 2014/15

Deprivation Quintile	Fluoride concentration	Odds*	95% CI	p for trend
1 (least deprived)	<0.1mg/l	0.30	0.26-0.34	0.036
	0.1-<0.2mg/l	0.31	0.27-0.34	
	0.2-<0.4mg/l	0.32	0.27-0.37	
	0.4-<0.7mg/l	0.32	0.19-0.45	
	≥0.7mg/l	0.20	0.15-0.26	
2	<0.1mg/l	0.43	0.38-0.48	<0.001
	0.1-<0.2mg/l	0.44	0.39-0.49	
	0.2-<0.4mg/l	0.42	0.35-0.48	
	0.4-<0.7mg/l	0.34	0.23-0.46	
	≥0.7mg/l	0.28	0.21-0.35	
3	<0.1mg/l	0.60	0.54-0.66	<0.001
	0.1-<0.2mg/l	0.56	0.51-0.61	
	0.2-<0.4mg/l	0.57	0.48-0.65	
	0.4-<0.7mg/l	0.49	0.35-0.64	
	≥0.7mg/l	0.30	0.23-0.38	
4	<0.1mg/l	0.85	0.77-0.94	<0.001
	0.1-<0.2mg/l	0.81	0.74-0.88	
	0.2-<0.4mg/l	0.69	0.59-0.79	
	0.4-<0.7mg/l	0.90	0.66-1.14	
	≥0.7mg/l	0.55	0.44-0.66	
5 (most deprived)	<0.1mg/l	1.64	1.49-1.78	<0.001
	0.1-<0.2mg/l	1.14	1.01-1.27	
	0.2-<0.4mg/l	1.26	1.06-1.47	
	0.4-<0.7mg/l	1.49	1.12-1.86	
	≥0.7mg/l	0.84	0.70-0.98	

\*Adjusted for ethnicity

**Supplementary table 7.** Adjusted odds of caries experience ( $d_{3mft}>0$ ) in five-year-olds sampled for the NDPE survey 2014/15, by mean fluoride concentration (mg/l) extended to 6 categories, stratified by quintile of index of multiple deprivation, England 2014/15

Quintile of Index of Multiple Deprivation	Fluoride concentration (mg/l)	Adjusted odds ratio (95% CI)*	p value	Trend test (p value)
1 (least deprived)	<0.1mg/l	Ref	-	0.001
	0.1-<0.2mg/l	0.96 (0.87-1.06)	0.451	
	0.2-<0.4mg/l	0.92 (0.81-1.04)	0.163	
	0.4-<0.7mg/l	0.99 (0.78-1.26)	0.957	
	0.7-<0.9mg/l	0.85 (0.70-1.02)	0.082	
	≥0.9mg/l	0.63 (0.48-0.84)	0.001	
2	<0.1mg/l	Ref	-	<0.001
	0.1-<0.2mg/l	1.01 (0.92-1.10)	0.865	
	0.2-<0.4mg/l	0.92 (0.82-1.03)	0.132	
	0.4-<0.7mg/l	0.77 (0.64-0.94)	0.009	
	0.7-<0.9mg/l	0.79 (0.66-0.94)	0.009	
	≥0.9mg/l	0.64 (0.51-0.80)	<0.001	
3	<0.1mg/l	Ref	-	<0.001
	0.1-<0.2mg/l	0.99 (0.91-1.08)	0.893	
	0.2-<0.4mg/l	0.94 (0.84-1.05)	0.277	
	0.4-<0.7mg/l	0.89 (0.75-1.06)	0.173	
	0.7-<0.9mg/l	0.71 (0.59-0.85)	<0.001	
	≥0.9mg/l	0.75 (0.63-0.88)	0.001	
4	<0.1mg/l	Ref	-	<0.001
	0.1-<0.2mg/l	0.86 (0.79-0.92)	<0.001	
	0.2-<0.4mg/l	0.81 (0.73-0.90)	<0.001	
	0.4-<0.7mg/l	0.93 (0.80-1.08)	0.362	
	0.7-<0.9mg/l	0.78 (0.66-0.93)	0.004	
	≥0.9mg/l	0.66 (0.57-0.77)	<0.001	
5 (most deprived)	<0.1mg/l	Ref	-	<0.001
	0.1-<0.2mg/l	0.62 (0.58-0.67)	<0.001	
	0.2-<0.4mg/l	0.73 (0.66-0.80)	<0.001	
	0.4-<0.7mg/l	0.64 (0.57-0.73)	<0.001	
	0.7-<0.9mg/l	0.58 (0.49-0.69)	<0.001	
	≥0.9mg/l	0.46 (0.42-0.51)	<0.001	

\*Adjusted for ethnicity. Robust standard errors.

**Supplementary table 8.** Preventive fraction of caries experience ( $d_{3mft} > 0$ ) in five-year-olds in fluoridated areas\*, stratified by index of multiple deprivation, England 2014/15

<b>Quintile of Index of Multiple Deprivation</b>	<b>Preventive fraction %<sup>†</sup></b>	<b>Lower – Upper CI</b>
1 (least deprived)	17%	5%-27%
2	23%	13%-32%
3	22%	14%-30%
4	19%	11%-25%
5 (most deprived)	28%	24%-32%

\*Yes=fluoride concentration  $\geq 0.7$ mg/l AND in water supply zone with fluoridation scheme during 2009-2015,  $n=12,467$  sampled five-year-olds in 2,091 LSOAs. No= fluoride concentration  $< 0.2$ mg/l, fluoride from any source,  $n=76,046$  five-year-olds in 17,709 LSOAs  
<sup>†</sup>adjusted for ethnicity. Robust standard errors.



**Supplementary table 9.** Classification of MSOAs by 2007-2015 period fluoride concentration\* (mg/l), and person years of observation of 0-19 year olds, England

<b>Fluoride concentration (mg/l)</b>	<b>MSOAs</b>	<b>% of total</b>	<b>person years (millions)<sup>†</sup></b>	<b>% of total</b>
<0.1	2,546	37	40.99	36
0.1-<0.2	2,375	35	41.13	36
0.2-<0.4	976	14	16.58	14
0.4-<0.7	277	4	4.24	4
≥0.7	666	10	11.59	10
Missing	1	0.01	0.04	0
<b>Total</b>	<b>6791</b>	<b>100</b>	<b>114.53*</b>	<b>100</b>

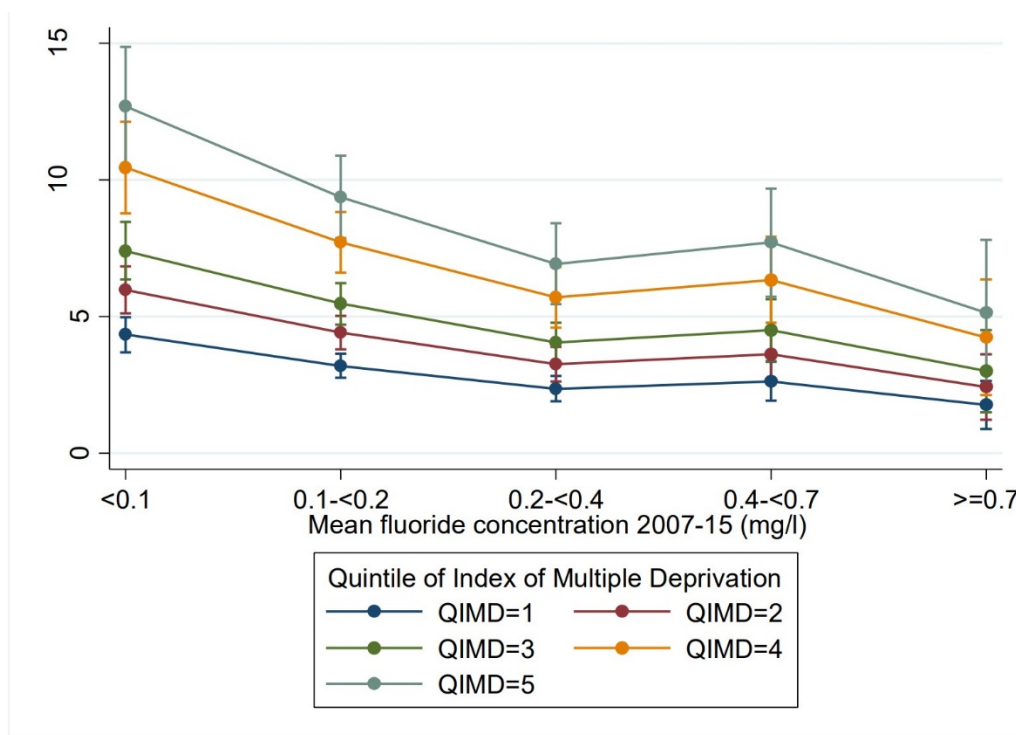
*\*Fluoride concentration derived from the mean fluoride concentration from constituent LSOAs, weighted using 0-19 year old population; <sup>†</sup>May not sum exactly due to rounding.*

**Supplementary table 10.** Preventive fraction of cases of caries related dental extractions in 0-19 year olds in fluoridated areas England 2007-2015

Fluoridation status*	Preventive fraction % <sup>†</sup>	Lower – Upper CI
Yes	56%	26%-74%

\*Yes=fluoride concentration  $\geq 0.7$ mg/l AND in water supply zone with fluoridation scheme during 2007-2015, in 628 MSOAs with 10.96 million person years of observation. No= fluoride concentration  $< 0.2$ mg/l, fluoride from any source, in 4,893 MSOAs with 82.12 million person years of observation; <sup>†</sup>adjusted for age group, gender and ethnicity. Cluster robust standard errors derived using clustering term on 289 local authority districts

**Supplementary figure 1.** Deprivation quintile stratum-specific predicted mean count of cases of 0 to 19-year-olds requiring caries-related dental extraction per MSOA, by period mean fluoride concentration (mg/l), England 2007 to 2015.



Adjusted for age group, gender, ethnicity, and size of 0-19 year old population; Cluster robust standard errors derived using clustering term on 325 Local Authority Districts; QIMD – Quintile of Index of Multiple Deprivation (1 least deprived, 5 most deprived)

## Technical appendix

### Exposure assessment

#### Fluoride exposure indices based on concentration and fluoridation scheme flagging data

The information most relevant to the exposure of interest was the fluoride concentration of water from public water supplies for residents of England. Exposure indicators were estimated by combining fluoride concentration obtained from routine fluoride monitoring data from 1995-2015, provided by the Drinking Water Inspectorate (DWI), and population data obtained from the Census and related mid-year estimates computed by the Office for National Statistics (ONS).

Public water supplies are delivered through a system of defined zones known as water supply zones (WSZs). Since 2006, the DWI has retained annual records that identify, via a flag, those WSZs that have fluoridation schemes. As no new fluoridation schemes have been initiated since 1995, flagged WSZs were considered to have been fluoridated from at least 1995 continuously to 2015, unless there was known to be significant operational disruption in those zones.

Water companies in England have a duty to monitor the fluoride concentration of public water supplies in the WSZs they supply; WSZs are sampled from randomly chosen sampling points (typically consumers' taps), that must be representative of the WSZ as a whole. Concentration testing must meet minimum standards for accuracy and precision.

#### Water Supply Zone boundary data used to define exposure geography

The DWI supplied copies of water company WSZ boundary files in digital format from 2004-2015, of which we were able to prepare 2005-2015 for analyses.

#### Allocating fluoride exposure to statistical and administrative areas in England

It is important to perform analyses using the smallest sized geography available as the unit of analysis, in order to maximise statistical power and to allocate the data that best describes the attributes and exposure of the population of interest, but at a large enough geography such that the statistical models used are capable of achieving a reasonable fit to the data. The smallest geographical unit of analysis was the Lower layer Super Output Area (LSOA), and analyses at larger geographical areas were performed by using LSOA level fluoride, health and population data as 'building blocks', aggregated to form their larger 'parent' Middle layer Super Output Area

(MSOA) and Lower Tier Local Authority (LTLA) areas (with which their borders match). We used Geographic Information Systems (GIS) point-in-polygon (PIP) methods to assign fluoride concentration data to statistical areas using the population weighted centroid of each LSOA. The population weighted centroid of each LSOA ('point'), which assigns a single geographic point to each LSOA based on the largest aggregation of its population, was overlaid onto WSZs ('polygons'), thereby allocating a fluoride concentration from a WSZ to an LSOA (and their populations).

The geographic footprints of WSZs are not fixed over time. WSZs may be aggregated or dis-aggregated to ensure continuity of supplies, hence the number and geographic boundaries of zones may change, making tracking of fluoride concentration data from individual WSZs over time challenging. Each water company gives each WSZ a site reference code but these codes may not be unique across all water companies in England. Similarly water companies have merged or ceased to operate at various points in time so WSZs are not perpetual. To overcome the issue of WSZs changing shape and size over time, point-in-polygon analysis was repeated for each year of available (mapped) WSZ data (2005-2015). The linked LSOA-WSZ pairs were then merged with the DWI fluoride concentration and fluoridation scheme flagging dataset, using concatenated site reference and water company coding (i.e. creating a unique identifier by conjoining the site reference and water company acronym) by year to identify common WSZ years. Arithmetic mean period fluoride concentrations for the exposure period of interest were then aggregated from LSOA to higher geographic levels, weighted by the exposed population.

We further sought to match exposure data to the dental outcomes; for caries in 5-year-old children 2014/15 mean fluoride concentration were calculated for calendar years 2009-2015; for hospital admission (extraction) fluoride concentrations were calculated for 2007-15. Fluoride concentration in water supply, regardless of source, was grouped into the following *a priori* defined categories: 0.0-<0.1mg/l, 0.1-<0.2mg/l, 0.2-<0.4mg/l, 0.4-<0.7mg/l, ≥0.7mg/l.

## Analysis of the association between fluoride concentration and health outcomes

Statistical analysis was performed using Stata 14 (Stata Corp, College Station, TX, US)

## Descriptive epidemiology

### Dental caries prevalence and severity

We calculated summary statistics (proportion of children with  $d_{3mft} > 0$ , and sample-weighted grand mean of  $d_{3mft}$ ) for each category of fluoride exposure status. Summary crude statistics were not weighted by inverse inclusion probability weights (the inverse of the probability of subject selection), to take account of varying selection probabilities between survey strata) because of unknown selection probabilities of the surveyed five-year-old children. As such, the prevalence estimates are valid only for the children surveyed, and can be extrapolated only after determining how representative the surveyed children are of the wider population. We therefore calculated the proportion of surveyed five-year-olds within each fluoride concentration category, deprivation quintile, and of white ethnicity, and repeated this for all five-year-olds in England (using mid-year population estimates for 2014), to compare the characteristics of the two groups. For proportion of white ethnicity surveyed and in the general five-year-old population, this was estimated by multiplying the proportion of 0 to 25-year-olds of white ethnicity in each LSOA on census 2011, by the count of five-year-olds surveyed, and the mid-year estimate of five-year-olds, respectively. The 0-25 year age band was the closest available age grouping by ethnicity at LSOA level.

### Hospital episodes for dental extractions for caries reasons

Case counts were aggregated by fluoridation status to calculate a crude incidence (density) rate per category of fluoridation exposure, by dividing the episode count by the total persons at risk in 2007-2015.

## Analytic epidemiology

### General approach

We used univariate regression to determine crude regression coefficients of the outcome for each category increase of fluoridation exposure.

Multivariable models were then constructed to determine regression coefficients and their 95% confidence intervals, adjusted for all *a priori* selected potential confounders. A p value was calculated using a z test to indicate the strength of the evidence against the null hypothesis that the regression coefficient did not vary by exposure to fluoride/fluoridation. The regression technique chosen depended on the underlying distribution of the dependent variable data. Regression coefficients were converted to odds ratios (following logit-Binomial regression) or risk ratios (following Negative Binomial regression) using STATA's '*eform*' option.

A backward stepwise procedure was used to fit the most parsimonious model, using the Wald test to determine whether the coefficient(s) of the

independent variable was significantly different from zero (and therefore were assumed likely to improve the model fit to the data), taking a significance level of  $p < 0.10$ .

For all the dental extractions outcome, a fixed effects model with a cluster option (using the 'parent' LTLA of the MSOA unit of analysis) was adopted to inflate the standard error to account for likely non-independence between the values of the outcome variable for MSOAs within the same LTLA.

We then modelled the categorical fluoride exposure variable as a linear term, in order to determine whether there was a linear trend in the regression coefficient with each increase in fluoride concentration category.

An interaction term was fitted between fluoride concentration category and a *priori* specified potential effect modifiers, and the resulting joint term tested for statistically significant evidence of interaction ( $p < 0.10$ ) using a Wald test. If a statistical interaction was present, the stratum specific estimates of the effect modifier were presented.

All confounders, other than ethnicity, were modelled as categorical variables. Post-estimation, the assumed linear relationship between ethnicity (modelled as a continuous covariate) and the outcome was checked by confirmation that when ethnicity was successively modelled as a quadratic and cubic function, their coefficients were not significantly different from zero (using a Wald test  $p$  value of  $< 0.05$ ). If a non-linear term resulted in superior model fit, then ethnicity was instead categorised into quintiles and modelled as a categorical variable.

#### Prevalence of caries experience, and mean caries severity

Proportion data were analysed using a Binomial model with logit link, using sample size as the number of 'trials' per LSOA. We calculated unadjusted odds ratios (for prevalence of caries experience), for each category increase of fluoridation exposure, at LSOA level.

The mean  $d_3mft$  data distribution was severely skewed. Log transformation was inappropriate due to zero values(1). Therefore an ordered logistic regression approach was taken, by splitting the outcome into ordered categories of '0' for zero values, and then 3 further categories ('1', '2' and '3'), formed from equal tertiles of the remaining outcome data. The proportional odds assumption was tested across response categories using an approximate likelihood ratio test, a  $p$  value of  $< 0.05$  being the cut-off used to reject proportionality of odds. This being the case, data were modelled using

*gologit2* user-written STATA command, which is appropriate for fitting models where the proportional odds assumption is violated(2).

For all dental outcomes, an *a priori* interaction between deprivation status (measured by quintile of index of multiple deprivation) and fluoridation status was tested using the methods outlined above. When an interaction was confirmed, deprivation quintile stratum-specific regression coefficients, adjusted for covariates, are presented.

Robust standard errors were adopted to adjust the standard error for PSU level clustering not accounted for in the primary survey analysis. Note, inverse inclusion probability weights were not used in regression analyses, as the relationship between the exposure and outcome at the unit of analysis level was of interest, rather than the prevalence of caries itself. Therefore, an unweighted model-based estimate is appropriate and unbiased(3).

#### Public health impact measures

The preventive fraction (as a percentage) in children exposed to fluoride was calculated to indicate the percentage of prevalent cases of caries experience, and extractions due to dental caries, in children in each year group (five-year-olds or children and young people aged 0-19 years for extractions) of the study population that could be prevented by exposure to drinking water fluoridated at a concentration of at least 0.7mg/L compared to populations exposed to low fluoride concentrations (i.e. of less than 0.2mg/l). 0.7mg/L is a level at which international evidence suggests we would expect an impact on caries of public health significance(4). Fluoride concentration category was re-coded into a binary <0.2mg/l and ≥0.7mg/l and modelled against proportion of children with caries experience, and extractions due to dental caries, to derive risk ratios respectively. For the prevalence outcome, risk ratios were determined using a Binomial regression with a log link instead of the logit link used in the main analysis, with the former an acceptable applied method to derive risk ratios while the latter is preferred for modelling proportion data. Stratum specific ratios were reported when interaction by deprivation was present in the primary analyses described above.

The following formula was used to calculate the preventive fraction:  $1 - RR$  (lower CI =  $1 - RR$  upper CI; upper CI =  $1 - RR$  lower CI). Where RR is the risk ratio of the outcome for the exposed (fluoride ≥0.7mg/l) compared to the unexposed group (fluoride of <0.2mg/l), and CI is the confidence interval around this risk ratio.

### *Post hoc* analyses

Prevalence of dental caries experience and incidence of dental extraction admissions related to dental caries

In order to investigate the association between fluoride exposure and prevalence of dental caries experience at higher concentrations than 0.7mg/l, we split the highest exposure category into two categories, leaving six in total as follows: <0.1mg/l, 0.1-<0.2mg/l, 0.2-<0.4mg/l, 0.4-<0.7mg/l, 0.7-<0.9mg/l,  $\geq$ 0.9mg/l. This allowed an assessment of the continuation of trend and/or any potential threshold effect.

### References

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