

1 **Fluoride exposure in community prevention programmes for oral health using nail clippings**
2 **and spot urine samples: a systematic review and meta-analysis**

3 **Short title:** A Systematic Review with Meta-analysis of Biomarkers of Fluoride Exposure

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13 **Number of Tables:** 4

14 **Number of Figures:** 4

15 **Word count:** 4813

16 **Keywords:** Fluoride, Dental caries, Spot urine, Nail clippings, Biomarker

17

18 **Abstract**

19 Due to practical difficulties in quantifying fluoride exposure in populations, practical and accurate
20 biomarkers can play a major role in the surveillance of fluoride. Among different fluoride biomarkers,
21 spot urine and nail-clippings have gained more attention due to their ease of acquisition. However, there
22 is no robust consensus about the accuracy of these biomarkers for the estimation of fluoride exposure.
23 This systematic review and meta-analysis aimed to synthesize evidence on the association between
24 fluoride exposure and the fluoride concentration of spot urine and nail-clippings. This review was
25 conducted and reported using the PRISMA Statement. Nine databases (Medline, CINAHL, Web of
26 Science, Scopus, ScienceDirect, Sage Journals Online, Campbell Collaboration, Cochrane
27 Collaboration, and Embase); search engines (Google and Google Scholar); and grey literature were
28 searched up to September 2022. All screening, data extraction, and quality assessments were conducted
29 in duplicate. All experimental and observational research studies that reported the correlation between
30 fluoride exposure and fluoride concentrations of spot urine and/or nail clippings were included. The
31 Mixed-Methods Appraisal tool was used to assess the methodological quality of the included studies.
32 A random effect meta-analysis was carried out to determine the relationship between fluoride exposure
33 and fluoride concentration of biomarkers (i.e., spot urine and nail clippings). Forty-four studies met the
34 inclusion criteria. A total of 694,578 participants were included in this review. Twenty-five studies were
35 included in the meta-analysis. The primary meta-analysis showed a moderate correlation of 0.674
36 (95%CI: 0.623-0.725, n=25) between fluoride intake and fluoride concentration of spot urine and a
37 strong correlation of 0.938 (95%CI: 0.520-1.355, n=11) between fluoride intake and the fluoride
38 concentration of nail-clippings in all age groups. The findings of secondary meta-analyses showed a
39 strong positive correlation between fluoride intake and fluoride/creatinine ratio of spot urine in children
40 (0.929; 95%CI: 0.502-0.991; n=2). In conclusion, spot urine and nail-clippings have the potential to be
41 employed as non-invasively obtained biomarkers in populations. However, due to the scarcity of high-
42 quality, relevant studies, more research is needed to establish the validity of these biomarkers.

43

44

45 **Introduction**

46 Fluoride, the ionic form of fluorine, is a natural component of the biosphere and is found in water, soil,
47 and air in varying amounts [Zohoori and Duckworth, 2020]. Despite being present in trace amounts in
48 the body, it has a public health importance due to its role in bone and teeth mineralisation. Fluoride has
49 been well-recognised for the prevention and control of dental caries, which is still the most predominant
50 preventable health condition worldwide [Zohoori and Duckworth, 2020]. Fluoridation schemes such as
51 water-, milk- and salt-fluoridation have been endorsed by many countries to prevent dental caries.
52 However, during critical periods of tooth development (i.e., the first 6 years of life) excessive exposure
53 to systemic fluoride can result in the development of dental fluorosis [O'Mullane et al., 2016]. Hence,
54 there is a clear recommendation for monitoring fluoride exposure, particularly in children, before and
55 after introducing any fluoridation or supplementation programme for the prevention of dental caries
56 [WHO, 2014].

57 The ingested fluoride is mainly absorbed in the stomach and small intestine. The absorbed fluoride is
58 circulated in the body via plasma and incorporated mostly into the calcified tissues, containing 99% of
59 body fluoride. Kidneys are the main route of removal of fluoride from the body, with almost half of the
60 daily absorbed fluoride excreted in the urine [Zohoori and Duckworth, 2020].

61 Since diet (including water) and unintentional ingestion of fluoridated dentifrices are the main sources
62 of fluoride intake in children, it is extremely difficult to quantify fluoride intake from these multiple
63 sources. Therefore, biological markers of fluoride can be of value for identifying and monitoring
64 deficient or excessive fluoride intake.

65 Considering the body burden of fluoride, the biomarkers of fluoride exposure have then been divided
66 into three categories [Pessan and Buzalaf, 2011, Rugg-Gunn et al., 2011, Lavallo-Carrasco et al., 2021]:
67 contemporary (e.g., blood/plasma, saliva, and urine), recent (e.g., nails and hairs) and historical (e.g.,
68 bone and teeth). Contemporary biomarkers measure present or very recent exposure to fluoride, whereas
69 recent and historical biomarkers measure sub-chronic and chronic exposure to fluoride [Pessan and
70 Buzalaf, 2011, Rugg-Gunn et al., 2011, Lavallo-Carrasco et al., 2021]. Based on pharmacokinetic
71 findings [Villa et al., 2010], 24-hour urinary fluoride excretion is considered a reliable biomarker of
72 fluoride exposure [WHO, 2014]. However, it is extremely difficult to collect 24-hour urine samples
73 from children, especially in younger age groups who are not toilet-trained. As alternatives to 24-hour
74 urine, spot urine and nail clippings are the most studied biomarkers of fluoride exposure due to being
75 non-invasive, ease of acquisition, and their acceptability by study participants [Idowu et al., 2020,
76 Idowu et al., 2021]. In order to establish the reliability of any biomarkers, it is imperative to better
77 understand their associations with fluoride exposure. Our recent scoping review [Kumah et al., 2022]
78 ascertained the nature and extent of the available evidence on how spot urine and nail clippings, as

79 alternatives to 24-hour urine, were used to measure fluoride intake/exposure by mapping the available
80 literature according to their study population, setting, type of study design, methodology, and analytical
81 approach. The review identified 55 articles in which associations between fluoride intake (and/or 24-
82 hour urinary fluoride excretion) and a fluoride biomarker (spot urine and/or nail clippings) were
83 reported, showing that there is enough evidence to explore the association between fluoride intake and
84 fluoride biomarkers to be synthesised in a systematic review. This follow-up systematic review with a
85 meta-analysis aimed to answer the following primary research questions: what is the relationship
86 between fluoride exposure and fluoride concentration of: (i) spot urine; and (ii) fingernail/toenail
87 fluoride?

88 The review also aimed to answer the following secondary research questions: what is the relationship
89 between fluoride exposure and: (i) fluoride/creatinine ratio of spot urine; and (ii) fluoride/specific
90 gravity ratio of spot urine?

91 **Methods**

92 This systematic review and meta-analysis was conducted and reported based on the Preferred Reporting
93 Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [Moher et al., 2009].
94 Objectives, eligibility criteria, and methods of analysis were specified in advance and published in a
95 priori protocol (PROSPERO (CRD42022354454)) [Eskandari et al., 2022].

96 ***Search strategy and selection of studies***

97 The search strategy was developed by two review authors (F.E. and E.A.K.). Search terms included a
98 combination of key concepts in the research question, such as fluoride exposure, fluoride intake,
99 fluoride biomarkers, spot urine, and nail clippings. We searched electronic databases (Medline,
100 CINAHL, Web of Science, Scopus, ScienceDirect, Sage Journals Online, Campbell Collaboration,
101 Cochrane Collaboration, and Embase); search engines (Google and Google Scholar); and Grey
102 literature (OpenGrey, NICE Evidence Search, the Grey Literature Report, Bielefeld Academic Search
103 Engine (BASE), and Australian Bureau of Statistics (ABS)). A detailed search strategy used for
104 searching the databases is presented in Supplementary File 1.

105 The reference lists of eligible articles were also searched for relevant studies. The search for eligible
106 papers was undertaken from 20th May 2021 to 22nd September 2022. The first author (F.E.) performed
107 the searches and imported citations into an Endnote library for reference management. The citations
108 were then exported into Covidence for screening. Duplicates were checked and removed in the Endnote
109 and Covidence software.

110 All titles and abstracts were screened by F.E. and E.A.K. The full texts of seemingly eligible articles
111 were also screened by two independent reviewers (F.E. and E.A.K.). Disagreements between reviewers
112 were resolved through consensus or in consultation with another reviewer (F.V.Z. or L.A.).

113 ***Eligibility criteria***

114 The inclusion and exclusion criteria were developed based on the population, exposure of interest, and
115 outcomes (PEO) criteria.

116 **Inclusion criteria**

117 ***Participants***

118 We considered studies involving humans as participants for inclusion in this review. Human participants
119 comprised of children and/or adults of any age, gender, or ethnicity.

120 ***Exposure***

121 The review included studies that have examined all forms of exposure to fluoride, such as water, diet,
122 unintentional ingestion of dental products (e.g., toothpaste, mouth-rinses, fluoride-varnish), and air
123 through experimental and environmental means.

124 ***Outcomes***

125 We included studies that assessed the use of spot urine and/or nail clippings to monitor fluoride
126 intake/exposure. Studies also had to report the correlation between fluoride exposure and fluoride
127 concentrations of spot urine and/or nail clippings.

128 ***Study types***

129 All experimental and observational research studies were considered for inclusion in this review. This
130 included but was not limited to, randomised controlled studies, cohort studies with measurements made
131 at a single time point (cross-sectional), pre-post studies, and other longitudinal studies measuring data
132 at multiple time points.

133 ***Data extraction***

134 A standardised data extraction form was developed in the Covidence software to extract relevant
135 information from included studies. Specific information that was extracted included the year of
136 publication, title, aim/objective of the study, study design, country, setting, population demographics,

137 exposure/intake data, methods of data collection, analytical procedures, and outcome(s) of interest to
138 the review questions.

139 The data extraction form was first pilot-tested on 10% of the included articles before commencing data
140 extraction. Data extraction was undertaken by one reviewer (F.E. or S.J.) and verified by another
141 (E.A.K), using the Covidence software.

142 *Assessment of methodological quality*

143 Two reviewers (F.E and E.A.K) independently conducted a quality assessment of each included study
144 using the Mixed-Methods Appraisal (MMA) tool developed by Hong et al. [Hong et al., 2018]. The
145 MMA tool is useful for assessing the quality of studies in reviews with heterogenous study designs and
146 contains specific questions for different study designs [Hong et al., 2018]. Any disagreements between
147 reviewers were resolved by consensus.

148 *Meta-analysis methods*

149 Meta-analyses were conducted to assess the correlations between fluoride intake and: 1) fluoride
150 concentration of spot urine; 2) fluoride concentration of nail clippings (toenail or fingernail); 3)
151 fluoride/creatinine ratio of spot urine; and 4) fluoride/specific gravity ratio of spot urine.

152 Analyses (1) and (2) were considered to be primary analyses, whereas analyses (3) and (4) were
153 secondary analyses. All meta-analyses (primary and secondary) considered studies based on either: (i)
154 studies conducted on adults (≥ 18 years) only; (ii) studies conducted on children (<18 years) only; (iii)
155 studies conducted on mixed adult/children groups. Within each group of studies, some variation in the
156 age range of subjects could exist. For analysis 2, further subgroup analyses were proposed based on the
157 type of nail clippings: toenail and fingernail. Studies that provided aggregate data (e.g., community)
158 level were not included in the meta-analyses.

159 Random effects meta-analyses were conducted using the DerSimonian and Laird estimation method
160 [DerSimonian and Laird, 2015]. These models were chosen to reflect recognised clinical and
161 methodological heterogeneity across included studies, such as the ages of the participants in each
162 constituent study.

163 Unadjusted correlation coefficients were used in the meta-analyses to avoid the introduction of
164 additional heterogeneity caused by variation in included controlling covariates. Where not reported
165 directly, correlation coefficients were calculated from simple regression coefficients and/or t-statistics
166 in conjunction with study sizes. Correlation coefficients were then transformed using the Fisher z-
167 transformation (inverse hyperbolic tangent) for meta-analysis. Back transformations were used to

168 transform the resulting pooled estimate back to the original metric. Correlation coefficients from
169 subgroups reported separately within a single study were combined into a single measure by averaging
170 transformed values and applying a back-transformation to the averaged measure.

171 For the primary analyses, forest plots were conducted for meta-analyses of the correlation coefficients,
172 reporting the synthesised estimates and associated 95% confidence interval (CI), and a Z-test for the
173 estimated effect (i.e., the correlation between fluoride intake and biomarker). Heterogeneity statistics
174 were also reported, including Cochran's Q test for heterogeneity, the I^2 statistic (proportion of variation
175 across studies ascribed to heterogeneity), and the τ^2 statistic (an estimate of between-study variance).

176 Sensitivity analyses were conducted on the primary meta-analyses to assess the robustness of the
177 derived estimates. Each of the included studies was omitted in turn, and a meta-analysis was conducted
178 based on the remaining studies, with results plotted on an influence plot. Any study which was suspected
179 of excessive influence on the resulting influence plot (considered to be indicated by the point estimate
180 of the "omitted" analysis of a study lying outside the CI of the "combined" analysis) was flagged as an
181 influential study.

182 Heterogeneity was further explored in the primary analyses with Galbraith plots (plots of a standardized
183 effect against the reciprocal of the standard error of the effect) of meta-analyses of primary outcomes.
184 In the absence of substantial heterogeneity, it is to be expected that around 95% of included studies will
185 lie within the shaded area of the plot (95% CI region). Imprecise estimates of effect lie near the origin,
186 and precise estimates are further away.

187 Key findings from subgroup and secondary analyses, were tabulated without graphical representation,
188 including the synthesized effect and 95% CI, heterogeneity as measured by the I^2 statistic and the result
189 of the Z-test for effect. Between-group effects were also calculated where possible for subgroup
190 analyses.

191 **Results**

192 ***Search results***

193 The initial search results yielded 15,177 articles (10,121 after the removal of duplicates). These were
194 then screened by title and abstract independently by two authors (F.E. and E.A.K.) to identify those
195 potentially meeting the inclusion criteria. Following title and abstract screening, 9753 articles were
196 excluded resulting in 368 articles assessed at the full-text screening stage. The full-text screening was
197 conducted by three review authors (F.E., E.A.K., and S.J.), and disagreements between reviewers were
198 resolved through consensus or by another reviewer (F.V.Z.). After the full-text screening, 321 articles

199 were excluded, including 44 articles in the systematic review. The PRISMA flow diagram shows the
200 number of articles at each stage (Fig 1).

201 The detailed study characteristics and outcomes are presented in Supplementary File 2 and
202 Supplementary File 3.

203 ***Study characteristics***

204 Overall, the included studies originated from 21 countries across various continents (Supplementary
205 File 2). Nine studies were conducted in China, seven in India, four in Brazil, three in Canada, two in
206 the UK, two in Mexico, two in Nigeria, two in Ethiopia, one in Japan, one in Germany, one in the USA,
207 one in Serbia, one in Hungary, one in Portugal, one in Poland, one in Slovakia, one in Tanzania, and
208 one in Jamaica. The remaining three studies were jointly conducted in ‘Mexico and Canada’, ‘Belgium
209 and France’, and ‘Brazil and Peru’.

210 A summary of the characteristics of the included studies in the systematic review is presented in Table
211 1. Of the included studies, 70.5% were published after 2014, 77.3% had a cross-sectional design, 59.1%
212 evaluated spot urine as a biomarker for fluoride exposure, and 59.1% were in children.

213 ***Methodological assessment***

214 Based on the MMA tool [Hong et al., 2018], the methodological quality of the included studies was
215 assessed using criteria/items specific to quantitative randomised controlled trials (including cohort
216 studies, cross-sectional studies, before and after studies) and quantitative descriptive studies (including
217 longitudinal studies). As shown in Figure 2, fourteen articles (31.8%) met all quality assessment criteria,
218 and twenty-three studies (52.3%) met six out of the seven assessment criteria. Six studies (13.6%) met
219 five out of the seven criteria, while one study (2.3%) met four of the assessment criteria. Most studies
220 were therefore deemed to be of very good quality, with the risk of selection bias remaining low.
221 Common issues with quantitative non-randomised studies were a lack of information about
222 confounders.

223 ***Meta-analysis 1: Correlation between fluoride intake and fluoride concentration of spot urine***

224 Twenty-five studies that either directly or indirectly reported the correlation between fluoride intake
225 and fluoride concentration of spot urine in children, adults, and mixed groups of children and adults in
226 non-aggregated data were included in this meta-analysis. The meta-analysis revealed a synthesised
227 estimate of the Fisher-transformed correlation coefficient of 0.674 (95% confidence interval 0.623 to
228 0.725). This corresponded to a synthesised estimate of the back-transformed correlation coefficient of
229 0.588 (95% CI 0.553 to 0.620). A Z-test of the standardised mean effect revealed strong evidence (at

230 the 5% significance level) for a non-zero effect ($Z=26.1$; $p<0.001$). Individual estimates for the back-
231 transformed correlation coefficient ranged from 0.310 [Heintze et al., 1998] to 0.995 [Saxena et al.,
232 2012].

233 Cochran's χ^2 test for heterogeneity revealed strong evidence (at the 5% significance level) for statistical
234 heterogeneity ($\chi^2_{(24)}=1979$; $p<0.001$). The I^2 statistic was revealed to be 98.8%, indicating a very high
235 proportion of variation across studies ascribed to heterogeneity. The data is summarised in a forest plot
236 (Fig 3).

237 A sensitivity analysis revealed that the results of Saxena et al. (2012) [Saxena et al., 2012] were exerting
238 excessive influence on the overall effect, with the point estimates of the omitted analysis lying outside
239 the 95% CI associated with the estimate of the combined analysis (Supplementary File 4, Fig 1).

240 A meta-analysis of all included studies except the study of Saxena et al. (2012) [Saxena et al., 2012]
241 revealed that a synthesised estimate of the Fisher-transformed correlation coefficient was 0.569 (95%
242 CI 0.531 to 0.608). This corresponded to a synthesised estimate of the back-transformed correlation
243 coefficient of 0.503 (95% CI 0.486 to 0.543). A Z-test of the standardised mean effect revealed strong
244 evidence (at the 5% significance level) for a non-zero effect ($Z=29.1$; $p<0.001$). Hence the exclusion of
245 the study of Saxena et al. resulted in a reduction of the synthesised estimate of the correlation coefficient
246 of about 14%.

247 The exclusion of the study of Saxena et al. (2012) [Saxena et al., 2012] had no substantive effect on
248 inferences of study heterogeneity as Cochran's χ^2 test for heterogeneity revealed strong evidence (at
249 the 5% significance level) for statistical heterogeneity ($\chi^2_{(23)} = 898$; $p<0.001$). The I^2 statistic was
250 revealed to be 97.4%, indicating a high proportion of variation across studies ascribed to heterogeneity.

251 ***Meta-analysis 2: Correlation between fluoride intake and fingernail/toenail fluoride concentrations***

252 Eleven studies that either directly or indirectly reported this correlation in non-aggregated data were
253 included in the meta-analysis of the correlation between fluoride intake and fluoride concentrations of
254 fingernails and toenails.

255 The synthesised estimate of the Fisher-transformed correlation coefficient was 0.938 (95% CI 0.520 to
256 1.355). This corresponded to a synthesised estimate of the back-transformed correlation coefficient of
257 0.734 (95% confidence interval 0.478 to 0.875). A Z-test of the standardised mean effect revealed strong
258 evidence (at the 5% significance level) for a non-zero effect ($Z=4.40$; $p<0.001$). Individual estimates
259 for the back-transformed correlation coefficient ranged from -0.281 [Sousa et al., 2018] to 0.977
260 [Vidyadharan et al., 2020].

261 Cochran's χ^2 test for heterogeneity revealed strong evidence (at the 5% significance level) for statistical
262 heterogeneity ($\chi^2_{(10)}=480$; $p<0.001$) and I^2 statistic was 97.9%, indicating a high proportion of variation
263 across studies. The data is summarised in a forest plot (Fig 4).

264 A sensitivity analysis revealed that none of the included studies was exerting excessive influence on the
265 analysis, with all point estimates of the omitted analyses lying within the 95% CI associated with the
266 estimate of the combined analysis. Estimates and associated CIs are plotted on an influence plot
267 (Supplementary File 4, Fig 2).

268 ***Subgroup analyses***

269 Tables 2–4 summarise the findings of the primary and secondary subgroup meta-analyses conducted in
270 cases where two or more constituent studies could be identified.

271 The findings of subgroup meta-analyses showed a moderately-strong positive significant estimate of
272 the effect of the correlation between fluoride intake and fluoride concentration of spot urine for children
273 only (Table 2). The corresponding estimate of effect was moderate for adults only and mixed adults and
274 children, although the effect was not statistically significant for the mixed group (Table 2).

275 A moderately strong positive effect size was also found for the correlation between fluoride intake
276 and nail clippings fluoride concentrations for all three categories of age groups. However, the
277 estimated effect was not statistically significant for the correlation between fluoride intake and
278 fingernail fluoride concentration for children only and adults only but for the mixed group (Table 3).

279 The findings of secondary meta-analyses showed a strong positive significant effect size for the
280 correlation between fluoride intake and fluoride/creatinine ratio of spot urine for children (Table 4).
281 However, a moderately weak effect size was found for the correlation between fluoride intake and the
282 fluoride/specific gravity ratio of spot urine in adults.

283 **Discussion**

284 This systematic review and meta-analysis explored the relationship between fluoride intake and fluoride
285 concentration of spot urine and nail clippings (i.e., fluoride biomarkers). The included studies were
286 predominantly of high quality (86% of included studies). Results from the meta-analysis indicated a
287 strong correlation between fluoride intake and fluoride concentration of nail clippings in all age groups.
288 The fluoride concentration of spot urine, when normalised to urinary creatinine concentration, was also
289 found to have a strong correlation with fluoride intake in children. However, the findings should be

290 taken with caution as most studies did not estimate total fluoride intake from all sources (e.g. diet,
291 dentifrices), and some used the fluoride concentration of water as a proxy of fluoride intake.

292 Overall, 44 studies originating from 21 countries across various continents met the inclusion criteria
293 and were included in this review. More than 70% of the included studies were conducted after the WHO
294 publication [WHO, 2014] in 2014, and particularly in children (59%). This is mainly because dental
295 fluorosis is an adverse effect of excessive fluoride intake during childhood, hence the need for a simple
296 method for surveillance of fluoride exposure in this age group.

297 The results of our meta-analysis for 25 studies (Fig 3), which explored the overall correlation between
298 fluoride intake and fluoride concentration of spot urine samples (normalised and un-normalised),
299 revealed a positive moderate correlation (0.67) which was statistically significant ($p < 0.001$). The
300 narrow-observed CI (0.62, 0.72) also indicates a good level of precision. When we explored age groups
301 in the subgroup analysis (Table 2), we found that the corresponding correlation was statistically
302 significant in children only and adults only ($p < 0.001$), but not in the mixed adults and children group
303 ($p = 0.143$). The between-group effect analysis also showed a statistically significant ($p < 0.001$)
304 difference between children and adults. This could be explained by the differences in fluoride
305 metabolism between children and adults. Under normal conditions, almost 45% of the fluoride absorbed
306 by healthy children is excreted in the urine, whereas the corresponding value is 60% for adults [Villa et
307 al., 2010]. An analysis of available data for 212 children and 283 adults from different geographical
308 areas showed a strong linear relationship between fluoride intake and 24-hour urinary fluoride excretion
309 for both age groups but with different slopes for young children and adults [Villa et al., 2010]. The
310 finding of our systematic review and the former study suggests that the correlation between fluoride
311 intake and excretion should be investigated separately for different age groups.

312 There was also a very strong and significant positive correlation (0.94, 95% CI 0.52 to 1.36) between
313 fluoride intake and fluoride concentration of nail clippings for the 11 studies which reported this
314 correlation in non-aggregated data (Fig 4). The subgroup analysis based on the type of nail clippings
315 (finger or toe) and age groups also showed a statistically significant strong correlation between fluoride
316 intake and fingernail fluoride when all studies were combined ($n = 6$) but not significant for children
317 ($n = 3$) and adults ($n = 2$) investigated separately (Table 3). However, a moderate correlation was found
318 between fluoride intake and toenail fluoride concentration, which was statistically significant when all
319 studies were combined ($n = 6$) as well as for children only ($n = 4$). Due to the scarcity of relevant studies
320 on these relationships, the findings should be interpreted with caution. A study with 89 children and
321 their parents [Sah et al., 2020], in which total daily fluoride intake and fluoride concentrations of toe-
322 and finger-nails were assessed, found no significant differences in fingernail fluoride in both children
323 and parents but a statistically significant difference in toenail fluoride concentration in parents. This

324 study also found a statistically significant difference in toenail fluoride concentration in parents but not
325 in children. A review of published studies [Pessan and Buzalaf, 2011] on the relationship between
326 fluoride intake and nail fluoride concentration found higher fluoride concentrations in fingernails than
327 in toenails in three out of the seven included studies. The higher fingernail fluoride concentration could
328 be due to the higher vulnerability of fingernails to external fluoride contaminations (such as soil and
329 nail varnishes), as well as higher blood supply in fingernails and consequently higher uptake of fluoride
330 from plasma. Therefore, more epidemiological studies are needed to assess the sensitivity of nails as a
331 biomarker of fluoride exposure in different age groups and populations with different lifestyles and
332 behaviour. For instance, ingesting soil could be a major route through which young children are exposed
333 to environmental pollutants (such as fluoride) as a result of their hand-to-mouth behaviour.

334 Additionally, the secondary meta-analyses (Table 4) showed a very strong correlation between fluoride
335 intake and fluoride/creatinine ratio of spot urine in children ($n=2$) but a weak correlation between
336 fluoride intake and fluoride/specific gravity ratio of spot urine in adults ($n=2$). The basis for creatinine
337 and/or specific gravity adjustment of concentrations of biomarkers in spot urine samples is to
338 compensate for variation in the urine dilution caused by differences among individuals in their fluid
339 intake, physical activity, temperature, etc. Although both creatinine and specific gravity have been used
340 for clinical diagnosis as well as clinical studies, it has been shown that urinary creatinine fluctuated
341 more than specific gravity by age and gender [Suwazono et al., 2005]. Since a very small number of
342 studies were identified and included in the sub-group analyses and none of these studies included both
343 children and adults, drawing a firm conclusion on the reliability of these biomarkers may not be
344 possible. Additionally, our findings showed overall high heterogeneity levels on the standardized and
345 unstandardized meta-analysis for both spot urine ($I^2=98.8\%$) and nail clippings ($I^2=97.9\%$). Removing
346 one outlier study [Saxena et al., 2012], resulted in a small reduction in the synthesised estimate of the
347 correlation coefficient with no significant effect on inferences of study heterogeneity. The high
348 heterogeneity levels of studies, in this review, could be due to variability across the study designs,
349 fluoride measurement methods, participants' characteristics (age), and more importantly source of
350 fluoride exposure, and methods of exposure assessments (e.g. using water as a proxy of fluoride
351 exposure, duplicate-plate diet collection, food-diaries, etc). Although we carried out several subgroup
352 analyses to help understand the effects of two broad age groups and types of biomarkers, we were
353 unable to explore other key components, such as narrower age groups (e.g., younger- and older-
354 children), type/source of fluoride exposure (e.g. water, diet, toothpaste ingestion) as well as the time of
355 day samples were taken and the number of collected samples, due to a small (or no) relevant studies
356 for such subgroup analyses. In the case of spot urine collection, it is recommended to take them several
357 times within a day to reflect the variation in fluoride intake as well as the period that urine accumulated
358 in the bladder – the shorter the accumulation period, the shorter-lived the peak level of fluoride
359 concentration [WHO, 2014].

360 In general, the fluoride concentration of spot urine and nail clippings can be influenced by several
361 environmental and biological factors. For instance, factors affecting urinary fluoride concentration
362 include environmental temperature, degree of hydration, diet (plant- or meat-based diet), altitude of
363 residence, certain diseases, and acid-base balance [Rugg-Gunn et al., 2011]. The factors influencing
364 nail fluoride concentration include age, gender, geographical area, nail growth rate, length, and site of
365 collection (thumb, toe, finger etc) [Pessan and Buzalaf, 2011].

366 Finally, it should be highlighted that statistical analysis of the relationships between fluoride exposure
367 and fluoride biomarkers (such as urine) has clearly shown that the 95% prediction intervals linked with
368 the regression line do not support biomarkers as an accurate estimator of fluoride exposure on an
369 individual basis but only at population/group levels [Villa et al., 2010, Rugg-Gunn et al., 2011].

370 ***Strength and limitations***

371 To our knowledge, this is the first systematic review and meta-analysis to quantitatively examine the
372 association between fluoride intake and fluoride concentrations in spot urine and nail clippings as
373 biomarkers of fluoride exposure.

374 This review employed a rigorous methodology, which included a comprehensive database search,
375 yielding 15,117 studies with no restriction on year of publication. It included a large overall sample size
376 (n=694,578 individuals), with no age restriction applied. All screening, data extraction, and quality
377 assessments were conducted in duplicate using standardised protocols. Additionally, included studies
378 represented a range from low- to high-income countries across various continents, and most studies
379 (86%) were considered as having high quality.

380 However, it also contains some limitations, as only studies published in the English language were
381 included and therefore relevant and important data from studies in other languages may have been
382 missed. Likewise, the cross-sectional nature of most of the included studies might prevent drawing
383 conclusions about the causality and direction of associations. However, the significant associations
384 found, in this systematic review, between fluoride intake and fluoride concentration of spot urine and
385 nail clippings are promising and could form a useful starting point for future research into the causal
386 pathways between fluoride exposure and spot urine/nail clippings as fluoride biomarkers. A greater
387 focus on longitudinal studies would be therefore highly encouraged.

388 **Conclusions**

389 Finding viable and accurate biomarkers for fluoride exposure has gained much attention over the past
390 several decades due to the expansion in knowledge of fluoride metabolism, technical advances in
391 fluoride measurements and the importance of fluoride surveillance in populations.

392 Spot urine and nail clippings have the potential to be employed as non-invasively obtained biomarkers
393 in populations. This systematic review found fluoride concentrations in spot urine (when normalised to
394 urinary creatinine excretion) and nail clippings were strongly correlated with fluoride intake in a group
395 of children and adults. However, due to the shortage of related studies and the high heterogeneity of
396 the included studies, more research is needed to establish the validity of these biomarkers. Future
397 research should explore the cost-effectiveness and generalizability of these biomarkers for different
398 fluoridation schemes. In particular, high-quality studies are needed to explore the different methods of
399 fluoride delivery (e.g., dentifrice, fluoridated-salt or -water), different settings /geographical areas, and
400 targeted populations.

401

402 **Conflict of Interest:** No conflicts of interest.

403 **Funding:** This research was funded by The Borrow Foundation [grant number NFB/KH]

404 **Statement of Ethics:** An ethics statement is not applicable because this study is based exclusively on
405 published literature.

406

407

408 **Author Contributions:** F.V.Z. and L.A. conceptualised the study and formulated the research
409 questions and contributed to developing the PROSPERO protocol. F.E. performed the searches. F.E.
410 and E.A.K. conducted the title, abstract and full-text screens independently. F.E., E.A.K., and S.J.
411 independently extracted data. F.E., and E.A.K conducted quality assessment. J.S. conducted meta-
412 analysis. F.V.Z. and F.E. led the writing of the manuscript and all authors have commented on drafts of
413 the manuscript. All authors read and approved the final manuscripts.

414

415 **Data Availability:** All data generated or analyzed during this study are included in this article and its
416 supplementary material files. Further enquiries can be directed to the corresponding author.

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594 **Tables:**

595

596 **Table 1.** Summary of characteristics of included studies

597 **Table 2.** Subgroup analysis* of effect estimates of correlation between fluoride intake and fluoride
598 concentration of spot urine

599 **Table 3.** Subgroup analysis of effect estimates of correlation between fluoride intake and fluoride
600 concentration of nail clippings

601 **Table 4.** Secondary outcomes of effect estimates of correlation between fluoride intake and
602 fluoride/creatinine ratio of spot urine, as well as fluoride/specific gravity ratio of spot urine,
603 respectively

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605

606 **Figure captions**

607

608 **Figure 1.** Flow diagram outlining the study selection process (adapted from Moher et al [11]).
609 (n=number of studies)

610 **Figure 2.** Quality assessment scores for included articles using the Mixed Methods Assessment Tool

611 **Figure 3.** Forest plot for meta-analysis of correlation between fluoride intake and fluoride
612 concentration of spot urine

613 **Figure 4.** Forest plot for meta-analysis of correlation between fluoride intake and fingernail/toenail
614 fluoride concentrations

615