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ABSTRACT: 2005 IADR/AADR/CADR 83rd General Session & Exhibition

# 2051 *In vitro* QLF observation of remineralizing effect in fluoride applications

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Objectives: The purpose of this *in vitro* study is to evaluate the remineralizing process of incipient enamel lesions in topical fluoride applications with quantitative light-induced fluorescence (QLF). Methods: 160 bovine enamel specimens (5 mm in diameter) were mounted in acrylic rod and polished. Incipient lesions were formed in specimens by 12 to 96 hour immersion in demineralizing solution (Lactic acid: 100 mM, Hydroxiapatite: 3 g/L, Carboxymethyl cellulose sodium salt: 0.2 g, pH: 5.0). These lesions were quantitatively calculated with Δ F (% / ratio of fluorescent loss which describes lesion depth) and divided into 4 groups (Δ F = -8, -16, -24 and -32). These groups were further divided by treatment (control, Fluoride dentifrice, APF and Fluoride dentifrice + APF). Specimens were immersed in artificial saliva for 28 days (KCl: 130 mM, $KH_2PO_4$: 0.9 mM, $CaCl_2$: 1.5 mM, HEPES: 20 mM, pH: 7.0). Surface images of the remineralizing process were recorded on days 3, 6, 9, 12, 15, 21 and 28 with QLF (Inspektor Reserch Systems, The Netherlands). Results: In low demineralized groups (Δ F = -8 and -16), recovery rates reached the plateau within 6 days. In high demineralized groups (Δ F = -24 and -32), recovery rates in the two APF treated groups reached the plateau in 6 days, and in others, the rates kept raising for 2 weeks. In all lesion specimens, rates of the control and Fluoride dentifrice groups were higher than the two APF treated groups. ($p < 0.05$). There was no difference between the two APF treated groups, and neither was there between control group and Fluoride dentifrice group ($p > 0.05$). Conclusion: This *in vitro* study showed that the difference in recovery rates among 4 types of fluoride treatment could be observed using QLF. This suggests the usefulness of QLF in clinical applications.

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# 1069 New Analysis Method of Gingiva with Quantitative Light-Induced Fluorescence

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Objectives:The purpose of this study was to evaluate the condition of gingiva objectively by a new digital image analysis with Quantitative Light-Induced Fluorescence (QLF).

Methods:15 adults (mean age; 22-26 years old) who were informed about the aim of this study and agreed to participate, were selected as the subjects. Photographs of the gingiva were taken with an oral CCD camera (COREFRONT CORPORATION, Tokyo, Japan) and INSPEKTOR PRO(Inspektor Research Systems B.V., Amsterdam, The Netherlands). Subjects were prohibited blushing for 7 days. They were re-examined by same methods after 1, 2, 3, 5 and 7 days. The condition of gingiva was analyzed from the digital images obtained from INSPEKTOR PRO by a computer program (Inspektor-Pro 1.2.0.4, Inspektor Research Systems B.V., Amsterdam, The Netherlands).

Results:On inspection, changes in the gingiva after each day could hardly be observed. However, when analyzing the images of gingiva, it was observed that a change in the color tone of the gingiva was more objectively expressed with red in the gingiva over green in the teeth fluorescence radiance that on the reference point increase, and the change of their gingival inflammation was to quantitatify with this parameter in %.

*Original article*

# Remineralisation study of artificial root caries lesions after fluoride treatment. An *in vitro* study using Electric Caries Monitor and Transversal Micro-Radiography

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**Remineralisation study of artificial root caries lesions after fluoride treatment. An *in vitro* study using Electric Caries Monitor and Transversal Micro-Radiography**

**Aims:** To evaluate and compare remineralisation of root caries lesions after in vitro treatment with various fluoride (F) agents using an Electric Caries Monitor (ECM) and Transversal Micro-Radiography (TMR).

**Materials:** Permanent human teeth were extracted and root surface specimens were sectioned, prepared ($n = 35$), and randomly allocated into seven different experimental groups (groups 1–7).

**Methods:** Root surfaces were demineralised in an acidified gel (pH = 5.0) for 3 weeks followed by various F treatments and stored in a standardised remineralising solution at 37°C for 6 weeks. The root surfaces were treated twice daily with different dentifrice slurries for 2 min, either with a neutral placebo dentifrice without F (group 5); or a neutral sodium fluoride (NaF) 1400 p.p.m. F dentifrice (group 1); or a neutral 1250 p.p.m. F dentifrice (group 6); or an acid dentifrice (pH 4.7) with 1400 p.p.m. F containing amine fluoride (AmF) (groups 3 and 4) or a 1250 p.p.m. (pH 4.7) AmF dentifrice (group 6). In groups 1, 2, 5, 6, and 7, the root surfaces were additionally rinsed for 2 min with a neutral non-F placebo solution. In groups 3 and 4, rinsing were performed for 2 min with an acid (pH 4.7) 250 p.p.m. F solution, containing 125 p.p.m. F as AmF and 125 p.p.m. F as potassium fluoride (KF), once or twice per day respectively. ECM was used to measure electrical resistance on root surfaces at baseline and after 3 and 6 weeks respectively. TMR technique was used to measure and compare root surface lesion depths and mineral loss.

**Results:** Six weeks daily treatment with a dentifrice slurry containing AmF followed by rinsing with a combination of equal amounts of AmF and KF solution twice a day showed a statistical significant higher ECM values compared with the other groups. TMR data measuring lesion depths and mineral loss reduction supported the results of the ECM findings.

**Conclusions:** Daily application of a dentifrice slurry containing 1400 p.p.m. F as AmF combined with twice daily rinsing with a 250 p.p.m. F solution containing equal amount of AmF and KF significantly remineralise primary root caries lesions *in vitro*. ECM and TMR are valuable complementary methods in order to analyse the remineralisation processes.

**Key words:** amine fluoride, Electric Caries Monitor, fluoride dentifrice, potassium fluoride, root surface lesions, Transversal Micro-Radiography.

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## Introduction

There is strong evidence that fluoride (F) has a beneficial effect on de-and remineralisation in both enamel and root dentine[1–5]. Root caries seems to be an emerging challenge to the dental profession as studies are showing an increase of root caries, not least in the elderly population with an increased number of teeth with exposed root surfaces being at risk[6–15]. The preventive measures associated with root caries are focused on modifying intra-oral and environmental factors, which are making management complex and multidisciplinary[14,16–23]. In fact, root caries shows similar remineralisation processes

 

after treatment with F as enamel. However root dentine seems to require a significant higher level of fluoride uptake and retention to enhance remineralisation[22,24–26].

As root caries is a significant plaque related disease associated to specific micro-organisms[27–37], combinations of mechanical and/or chemical treatments to control bacterial plaque seem to be of significance for the prevention of root caries[4,12,17,38–41]. The effectiveness of F dentifrice in general shows a dose–response relationship between F concentration and the caries-preventive effect on enamel. However the optimum F concentration for remineralisation of root caries has not yet been definitely defined[38,42–44]. It has been suggested that more F is needed to remineralise root caries lesions and *in vitro* studies have shown that a higher F concentration is needed for caries inhibition in dentine compared with enamel[25,43,45,46]. In fact, to be successful, F concentration as high as 5000 p.p.m. F has been required for the remineralisation of root caries lesions *in vivo*[46–48]. Besides sodium fluoride (NaF) and other F salts such as stannous F and amine fluoride (AmF) have been used for caries prevention of root caries[49–52].

Clinical criteria involving probing to detect the surface texture of root caries lesions and different stages of primary root caries diagnoses have been validated. However, clinical criteria depend on subjective judgements and often require long training procedures for the investigator to acquire the required level of reliability and validity[15,16,53–58]. Therefore, the need for objective methods and tools to measure de-and remineralisation changes in surface lesions are important. Recently new and relevant diagnostic methods have been reviewed for the detection and quantification of caries lesions in enamel[16]. The Electric Caries Monitor (ECM) is an additional diagnostic tool that has been used for the detection of occlusal and fissure caries and has recently been used to measure the electrical resistance of root caries[47,48,59–64].

The aim of this study was to evaluate the remineralisation effect *in vitro* of various treatments of topical F regimens including toothpaste and rinsing solutions on artificial root caries lesions and to evaluate and compare ECM and Transversal Micro Radiographs (TMR) techniques.

## Materials and methods

### *Demineralisation of root specimens*

Freshly extracted permanent human teeth, premolars and molars, predominantly from adults were collected from the Department of Maxillofacial Surgery, Central Hospital, Halmstad. All teeth were visually examined to ensure that sound root surfaces were free of physical damages from the extraction procedures or other potential defects. The teeth were gently pumiced and washed under running water after the extraction. The entire root surface was painted with an acid protective nail varnish, with the exceptions of small windows. This 'window' had an average surface size of approximately 2 × 3 mm adjacent to the crown of the teeth. Thirty-five teeth were selected and demineralized in a 6% by weight carboxymethylcellulose gel (CMC) (AKZO, Arnheim, the Netherlands) at 37°C for 3 weeks. The gel contained 0.1 M Lactic acid titrated to pH 5 with a 10 M KOH-solution. The gel volume was 20 ml per tooth. After demineralisation, all samples were washed under running water for 30 min. The samples were then allocated randomly in seven experimental groups (groups 1–7).

### *F products, F treatment and remineralisation procedures for different tooth groups*

The following dentifrices and solutions were tested:

Group 1: dentifrice containing 1400 p.p.m. F (NaF; pH 7.0). Groups 2, 3 and 4: 1400 p.p.m. F dentifrice as AmF (pH 4.7) (Elmex sensitive®; Elmex Ltd, Tokyo, Japan). Group 5: placebo dentifrice without F (pH 7.0). Group 6: 1250 p.p.m. F dentifrice (NaF; pH 7.0). Group 7: 1250 p.p.m. F AmF dentifrice (pH 4.7). Additionally teeth in groups 1, 2, 5, 6 and 7 were rinsed with a pH neutral placebo solution once a day and groups 3 and 4 were rinsed with a solution containing 250 p.p.m. F (50% AmF+ 50% KF (Elmex sensitive plus®; Elmex Ltd)] once and twice a day respectively (Table 1).

All dentifrices were separately mixed to form a slurry using 1 ml dentifrice and 2 ml distilled water. The root surfaces were gently brushed using an 'Elmex inter × sensitive' toothbrush for 2 min twice a day, morning and evening simulating natural toothbrushing routines. In between the treatment procedures, the root dentine samples were stored in 10 ml of stirred remineralising solution containing 20 mM N-2-hydoxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 1.5 mM $Ca^{2+}$ as $CaCl_2$ and 0.9 mM phosphate as $KH_2PO_4$ at 37°C for 6 weeks for each tooth sample.

### *ECM, TMR and statistical evaluation*

The ECM (LODE Diagnostics, Groningen, the Netherlands) was used to measure electrical

**Table 1** Application of dentifrice slurries and rinsing solution products in different experimental groups (groups 1–7) with various fluoride (F) agents, fluoride concentrations and pH-levels. Five root samples in each group.

| *Product* | *F concentration (p.p.m. F)* | *Application* | *F-salt* | *pH* | *Branch* | *Group* |
|---|---|---|---|---|---|---|
| Dentifrice + solution | 1400 | Twice per day | NaF | 7.0 | n | 1 |
| | 0 | Once per day | – | 7.0 | ps | |
| | 1400 | Twice per day | AmF | 4.7 | Elmex-sen | 2 |
| | 0 | Once per day | – | 7.0 | ps | |
| | 1400 | Twice per day | AmF | 4.7 | Elmex-sen | 3 |
| | 0 | Once per day | AmF + KF | 4.7 | Elmex-sen+ | |
| | 1400 | Twice per day | AmF | 4.7 | Elmex-sen | 4 |
| | 250 | Twice per day | AmF + KF | 4.7 | Elmex-sen+ | |
| | 0 | Twice per day | – | 7.0 | pd | 5 |
| | 0 | Once per day | – | 7.0 | ps | |
| | 1250 | Twice per day | NaF | 7.0 | | 6 |
| | 0 | Once per day | – | 7.0 | ps | |
| | 1250 | Twice per day | AmF | 4.7 | Elmex-cp | 7 |
| | 0 | Once per day | – | 7.0 | ps | |

n, normal dentifrice; Elmex-sen, Elmex sensitive dentifrice; Elmex-cp, Elmex caries protection; pd; placebo dentifrice; Elmex-sen+, Elmex sensitive plus rinse solution (test); ps, placebo solution; NaF, sodium fluoride; AmF, amine fluoride; KF, potassium fluoride.

resistance behaviour during drying with air under conditioned circumstances on root dentin at the start of the trial (baseline) and after 3 and 6 weeks respectively. Each root caries lesion area was analysed 10 times, by randomly positioned measuring points within the exposed root surface area of approximately 2 × 3 mm. Electrical resistance measured was expressed in Ohm units.

Dentine sections of each root surface lesion, 350 μ thick, were cut transversally from the remineralised human root samples after the last measurements with the ECM. TMR of the root samples were made together with an aluminium step wedge on film (Fuji Z&W POS/71337; Fuji Photo Film Co. Ltd, Tokyo, Japan) in the range of 0–300 μm using a monochromatic X-ray generator (Philips PW 1730, Philips Research, Eindhoven, the Netherlands), at 25 kV and 25 mA. After development using D-19 developer (Kodak, Tokyo, Japan) for 10 min, fixing and drying, the film was mounted in a light microscope (Nikon Eclipse, Tokyo, Japan). A charge-coupled device (CCD) camera (Teli CS 8310, Tokyo, Japan) was interfaced to a personal computer equipped with a frame grabber card. Using the CCD camera at standardised settings of magnification ×20 objective magnified macroradiographic images of a blank area and each aluminium step wedge as well as the lesions were digitised into images with 640 × 480 pixels at a resolution of 256 grey levels. The scan area of the dentine was 410 × 340 μ. Further image processing analysis was performed using the National Institute of Health-image program, to calculate lesion depths (microns) and mineral loss (Vol. × μm). Three scans were made of each dentine microradiograph sample.

Differences between the experimental groups were tested for statistical significance by using the non-parametric Wilcoxon Signed Rank test.

## Results

The results are presented in Tables 1–5 including a product specification (Table 1). ECM mean values were similar and not significantly different between experimental groups (groups 1–7) at baseline. After 6 weeks of the varied F treatments and remineralisation, ECM mean values showed a variance between 23.19 MΩ in group 5 (placebo dentifrice plus rinsing with a placebo solution) to a mean of 60.77 MΩ in group 4, treated with a dentifrice containing amine flouride (AMF) (Elmex sensitive®) plus twice daily rinsing procedures with a 250 p.p.m. F solution containing amine fluoride and potassium fluoride (KF) (Elmex sensitive plus®) (Table 2). All F treated root specimens showed statistically significant elevated ECM mean-values compared with the samples in group 5 (Table 3). Lesion depth from the microradiograph was significantly lower in groups 3 and 4 compared with the other groups (Table 4). Statistically significant lower mineral loss (Vol.% × μm) was also found for the specimens in groups 3 and 4 treated with a dentifrice containing AmF (Elmex sensitive®) and rinsed with the Elmex sensitive plus® solution (Table 5).

**Table 2** Electric Caries Monitor (MΩ) mean values (SD) after 10 measurements on each root surface lesion in seven different experimental groups (1–7), see Table 1, at baseline and after 3 and 6 weeks treatment respectively.

| | *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 5* | *Group 6* | *Group 7* |
|---|---|---|---|---|---|---|---|
| Baseline | 6.47 (2.69) | 7.76 (3.63) | 7.32 (3.92) | 7.82 (3.64) | 7.71 (3.11) | 7.77 (3.34) | 9.56 (3.55) |
| 3 weeks | 26.96 (12.47) | 23.95 (11.19) | 30.25 (16.20) | 29.75 (9.44) | 15.76 (6.64) | 17.78 (6.61) | 29.26 (13.19) |
| 6 weeks | 44.77 (19.70) | 54.09 (18.49) | 60.77 (17.50) | 77.16 (17.33) | 23.19 (13.37) | 33.09 (18.84) | 44.17 (14.15) |

**Table 3** Cross tabulation of statistical comparison of Electric Caries Monitor measurements ($n = 10$) on each root surface specimen in different experimental groups (groups 1–7) after 6 weeks of *in vitro* treatment and remineralisation. Non-parametric Wilcoxon Signed Rank test was used. Mean values (MΩ) are within parenthesis.

| | *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 5* | *Group 6* | *Group 7* |
|---|---|---|---|---|---|---|---|
| Group 1 | 44.77 | ns | ns | * | ** | *** | ns |
| Group 2 | ns | 54.09 | ns | * | *** | *** | ns |
| Group 3 | ns | ns | 60.77 | ns | *** | *** | ns |
| Group 4 | * | * | ns | 77.16 | *** | *** | ** |
| Group 5 | ** | *** | *** | *** | 23.19 | ** | *** |
| Group 6 | ns | *** | *** | *** | ** | 33.09 | ** |
| Group 7 | ns | ns | ns | ** | ** | ** | 44.17 |

ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

**Table 4** Micro radiographic parameter, lesion depths (μm): mean (SD) of lesion depth in microns of root surface lesions specimens in different experimental groups (group 1–7) after 6 weeks of treatment and remineralisation *in vitro*. Group 8 is a control group without treatment or remineralisation.

| *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 5* | *Group 6* | *Group 7* | *Group 8* |
|---|---|---|---|---|---|---|---|
| 172.6 (25.0) | 169.9 (24.8) | 157.7* (21.8) | 154.7* (16.1) | 180.8 (14.1) | 166.7 (12.1) | 170.4 (18.3) | 198.3 (23.4) |

*$p < 0.05$.

**Table 5** Micro radiographic parameter, mineral loss value (Vol.% × μm): mean (SD) of root surface lesion specimens in different experimental groups (groups 1–7), after 6 weeks of treatment and remineralisation. Group 8 is a control group without treatment or remineralisation.

| *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 5* | *Group 6* | *Group 7* | *Group 8* |
|---|---|---|---|---|---|---|---|
| 5407 (865) | 5375 (919) | 4661* (714) | 4401* (760) | 6034 (879) | 5723 (766) | 5255 (908) | 6145 (818) |

*$p < 0.05$.

## Discussion

The present short-termed *in vitro* study was designed to simulate clinical situations as much as possible in order to compare the effects of different fluoride containing products to remineralise root caries lesions. Therefore a placebo dentifrice and solution were included in the experiment.

Clinical data have shown that root caries lesions can be remineralised and arrested clinically by reinforcing oral hygiene and other preventive measures[4,18,20,39,41]. It has been further shown that Fs when applied in rinses, gels and dentifrices can control root caries[3,44–46,65]. All F dentifrices tested in this trial had F concentrations of between 1250 and 1400 p.p.m. F but differed with regards to pH and F containing agents (Table 1). Thus, the dentine specimens in groups 2, 3, 4 and 7 were treated with a dentifrice containing AmF at pH 4.7. Lowering of pH has a strong potential effect on F uptake and retention both in enamel and dentine[42,66,67] and thereby promotes remineralisation potential.

This may be one of the explanations for the relatively higher ECM values recorded in groups 3 and 4 (Tables 2 and 3), as the dentifrice used in these groups showed a low pH (4.7), A high ECM value indicates less porosities in the apatite. The additional rinsing with a 250 p.p.m. F solution (pH 4.7) containing AmF and KF is an additional factor which may have favoured the remineralisation processes of root caries lesions as they require a higher F uptake compared with enamel[43,47,68] to promote remineralisation. The optimum F concentration needed for the remineralisation process is still however not defined for root dentine. In enamel, a F concentration higher than 1000 p.p.m. causes less pronounced caries development compared with lower F concentration, although there is evidence that F levels in dentifrices have no effect on progression of radiographically detectable caries lesions[8,43]. High F concentration, up to 5000 p.p.m. F has been suggested for use in gels and dentifrices as a means of preventive management of root caries. Recently, Baysan *et al.*[47] compared the ability of a dentifrice with 5000 and 1100 p.p.m. F to reverse primary root lesions clinically in favour of a 5000 p.p.m. F dentifrice. They were also using ECM to measure electrical resistance behaviour reflecting remineralisation processes in root caries lesions. After 3 months a statistically significant remineralisation of primary root caries lesions exposed to the 5000 p.p.m. dentifrice had occurred.

Using the ECM, the electrical resistance measurements have been performed on fissure caries in enamel[59,61–64]. In general, electrical resistance increases considerably when the pore volume decreases in enamel or dentine, similar to the remineralisation process of caries lesions. The relationship between clinical criteria used to detect primary root caries with electrical measurements has been investigated by Baysan *et al.*[47] and a significant correlation for ECM readings and early caries lesions in root dentine were found.

However, as electrical resistance in biological apatites is determined by the fluid saturation and the conductivity of the fluid, measurement variations of the ECM can therefore be expected[68]. Variation of electrical resistance values in teeth also depends on the presence and variation of pore structure and pore volume. This seems to differ locally in both enamel and root dentine surfaces within relatively small distances (mm). Variations of ECM output measurements also reflect the grade of standardisation and measurement precision. Each experimental surface was measured 10 times with the ECM unit and the precision of the method was calculated as ±7–15% for root dentine. Therefore the ECM can be used for diagnostic purposes to detect early caries lesions and consequently follow and determine caries lesion progression in both enamel and dentine.

Lesion depths (μm) and mineral loss values (Vol.% × μm) were measured from microradiographic analyses (Tables 4 and 5). Only the specimens receiving a combination of AmF dentifrice and rinsing solution in groups 4 and 5 showed a statistically significant lower lesion depth. This effect was also significant for the mineral loss parameter, indicating a remineralising effect of the AmF compound. The mineral loss values observed in the different experimental groups tested were also valid for the variation found in the lesion depths.

AmF has been shown to have a caries inhibiting effect by both promoting the formation of calcium fluoride ($CaF_2$) and by the intrinsic antiglycolytic activity on bacteria[49,51,52]. Whereas most of the studies performed with this substance investigated the effect of individual products on dental enamel, the present study investigated the effect of a treatment regime (Elmex® sensitive) consisting of a dentifrice and a dental rinse solution (Elmex sensitive plus®) on remineralisation of root surface lesions.

The favourable effect in this study on root lesion remineralisation after daily application of a dentifrice containing AmF (Elmex sensitive®) and rinsing with a solution containing AmF and KF (Elmex sensitive plus®) is clear. *In vivo* AmF by itself also shows similar anti-cariogenic properties by having an antiplaque effect[31]. As previously studied, the lowered pH (4.7) is also a potential factor to promote remineralisation by forming $CaF_2$[42,45,66,67,69].

Although, *in vitro* studies do not reflect the physiological situation in the human oral environment, they are often necessary to facilitate an understanding of chemical principles and reactions and are therefore practised to screen and compare the effect of various pharmaceutical agents with different properties[43].

The short treatment and follow up time (6 weeks) is normally too short to record remineralization processes using clinical criteria. However, the use of the ECM makes it possible to monitor small changes of electrical resistance within small areas of root surfaces even after only a short experimental time. Indeed, Baysan *et al.*[47] in a clinical study recorded statistically significant changes of primary root caries lesions by measuring electrical resistance after 3 months. Although the lesion depths between the different groups did not

differ significantly for the group receiving both AmF dentifrice and rinsing solution, the average values are comparable with lesion depths presented by Nyvad *et al.*[20] for arresting root surface caries *in situ*.

## Conclusions

All F-containing toothpaste products tested led to a relatively higher degree of remineralisation of root surface lesions compared with placebo products as measured using the ECM method. Additional rinsing twice a day with an AmF-KF solution (250 p.p.m. F) was superior when compared with an AmF-dentifrice alone.

The ECM measuring electrical resistance behaviour in the tooth is a useful diagnostic tool to study variations of de-and remineralisation in biological apatites.

The TMR data support the remineralisation efficacy for the combination of a dentifrice containing AmF and rinsing twice daily with a solution containing AmF and KF with a total concentration level of 250 p.p.m. F.

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