Effects of a Two-Step Silver Diamine Fluoride Varnish on Shear Bond Strength of Restorations, Dentin and Enamel Hardness, and Biofilm Formation

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ABSTRACT

Introduction:

Dental caries are a limiting factor in maintaining dental and medical readiness in the military. Untreated dental caries can lead to dire health consequences. Consistent and comprehensive access to dental care is often limited due to the intensive operational demands on our nation's warfighters. The standard of care for dental caries is a surgical model where diseased tooth tissue is surgically removed and restored with appropriate restorative materials. While effective, it is not practical in the military operational environment, especially under time constraints. Dental restoratives offer military personnel a simple and preventive treatment of dental caries and are suitable as self-applied first aids. The purpose of this study was to measure the shear bond strengths of two dental restorative materials to human teeth paired with two different fluoride treatments and the hardness and biofilm formation on teeth after applying the fluoride varnishes.

Materials and Methods:

Specimens were made of human molar teeth treated with each of the following four materials: glass ionomer cement GC Fuji II LC Capsules, Filtek Z250, Riva Star steps 1 and 2, or Mark3 NaF varnish. Step 1 of Riva Star consists of silver diamine fluoride and step 2 contains potassium iodide. On human molar slabs, 10 circular specimens of 5 cm in diameter were prepared with restoratives according to manufacturer procedures. Etch-Rite and a proprietary aluminum chloride-based cavity conditioner were used as etchants on tooth surfaces for the Filtek Z250 and glass ionomer cement, respectively. After at least 24 hours underwater, each assembly was removed, and the shear bond strength of the adhesive was measured according to International Organization for Standardization (ISO) 29022.

The hardness was measured according to ISO 14233. Hardness measurements were performed before varnish application, then after storage in an incubator at 37 °C for 4 hours in a demineralization solution (pH = 4.5), and after 1 day in a mineralization solution (pH = 7). A crystal violet staining assay was used to measure biofilm formation of *Streptococcus mutans* bacteria on human molar teeth after the application of fluoride varnish.

Results:

We report a 16% increase in shear bond strength of the Filtek Z250/Riva Star coupled treatment compared to the Filtek Z250/Mark3 NaF coupled treatment. We also demonstrate a significant 84% decrease in bond strength with a GC Fuji II LC/Mark3 NaF treatment compared to control ($P = .0002$), while Riva Star remains statistically unchanged. Enamel and dentinal hardness are significantly improved when Riva Star is applied compared to NaF varnish. A 25%-35% ($P < .0001$) decrease in oral biofilm formation was observed on samples where a Riva Star or NaF varnish was applied.

Conclusions:

Mechanical and antimicrobial testing indicated Riva Star, compared favorably with and in some cases, performed better in the laboratory than a Mark3 NaF varnish. Hardness measurements indicated Riva Star is more effective in dentin tubule occlusion compared to NaF varnish. Our findings help provide practical suggestions to dental treatment, particularly to the unique dental environments seen in the military. Riva Star may be used as an adjunctive treatment prior to placing a final restoration. This study supports the use of Riva Star in conjunction with GC Fuji II LC or Filtek Z250 restorative materials, making it a promising treatment in military dental applications.

INTRODUCTION

Dental caries are the most common dental disease in active duty and reserve military personnel.[1] Consistent and comprehensive access to dental care is limited due to the intense operational demands on servicemen and women. Treatment for infection or tooth loss caused by caries often necessitates root canal therapy or tooth extraction.[2] Such procedures have a high cost and cause a decrease in the quality of life for the patient.[3] The standard of care for caries is a surgical model that involves the removal of the decayed portion of the tooth,

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TABLE I. Chemical List, Composition, Manufacturer, and Lot Numbers of the Fluoride Varnish, Etchant, and Restorative Materials That Were Used in This Study

Material	Composition	Manufacturer	Lot
Etch-Rite Etching Gel	38% phosphoric acid	PULPDENT Corporation	200317
Mark3 5% NaF Varnish	Synthetic resin, ethanol, and sodium fluoride	Cargus International, Inc.	D210414V
Riva Star	Step 1: silver diamine fluoride, ammonia, and water Step 2: potassium iodide	SDI Limited	11557551
Filtek Z250 Universal Restorative	Silane-treated ceramic, bisphenol A polyethylene glycol diether dimethacrylate, diurethane dimethacrylate, bisphenol A diglycidyl ether dimethacrylate, triethylene glycol dimethacrylate, and aluminum oxide	3 M ESPE	NA48554
GC Fuji II LC Capsule	2-Hydroxyethyl methacrylate, polybasic carboxylic acid, urethane dimethacrylate, dimethacrylate, and aluminum chloride hexahydrate	GC America Inc.	2102061

followed by filling the area with a restorative material.[4] While effective, it is not practical in deployed operational environments, where the full armamentarium of dental treatments is not always available, and time restrictions prevent treatment of all carious lesions.[5] The work described herein helps close several knowledge gaps in military dentistry, particularly the evaluation of preventive therapies.

A potent fluoride treatment method to prevent caries combines silver diamine fluoride (SDF) and potassium iodide (KI), available from Melbourne-based SDI Limited under the brand name Riva Star (Table I). SDF is a colorless and odorless solution of fluoride, ammonium, and silver, which is antimicrobial in nature.[6] SDF, which is more chemically stable than silver fluoride, interacts with the sulfhydryl groups in bacterial proteins and deoxyribonucleic acid, disrupting hydrogen bonds and slowing respiratory activities, deoxyribonucleic acid replication, and bacterium cell division. These interactions effectively kill bacteria and inhibit biofilm formation.[7] The fluoride in SDF inhibits demineralization caused by cariogenic bacteria, substituting fluorapatite for hydroxyapatite in dentinal tubules.[8,9] During clinical use of Riva Star, SDF is first applied to the tooth during step 1. More recently, KI has been added to the treatment to prevent the dark stains caused by silver in SDF from appearing on the surface of teeth.[10] This is often referred to as "step 2" of Riva Star treatment.[11] Potassium iodide reacts with silver precipitate formed during mineralization to wash away excess stain-causing silver ions. A two-step topical medication ($Ag(NH_3)_2F$ (aq) + $Ca_5(PO_4)_3(OH)$ (s) + KI (aq) → AgI (s) + $2NH_3$ (g) + $Ca_5(PO_4)_3F$ (s) + KOH (aq)), Riva Star can be used to prevent the development of dental caries, promote mineralization of teeth, and treat dentin hypersensitivity.[7]

After its initial use in Japan in the late 1960s, SDF has once again found use in China to treat caries in school children.[12] SDF is generally unpopular because of its dark staining of the teeth but with the addition of KI that can largely be avoided. We expect the application of Riva Star to the warfighter by corpsmen when dentists are unavailable to be beneficial due to SDF's ability to arrest caries progression without the aid of other materials, especially during deployments in austere environments where access to procedural dental care is limited.[5] The purpose of this study was to measure the effects of Riva Star on the bond strength of glass ionomer cements (GICs) and composites to human third molars in good condition and their hardness in an *in vitro* setting. To evaluate antimicrobial efficacy, resistance to biofilm formation was measured on teeth after fluoride application. We hypothesize that the application of both steps of Riva Star will improve mechanical properties of teeth and dental restoratives compared to sodium fluoride (Mark3 NaF) varnish.

MATERIALS AND METHODS

Non-carious extracted human molars were stored in 10% formalin (Azer Scientific Inc., Morgantown, Pennsylvania) before they were set in resin (EpoxiCure, Buehler, Lake Bluff, IL) in sample cups (31.75 mm SamplKups, Buehler) for sectioning. The resin was allowed to cure overnight, and then, coronal sections were made with a linear precision saw (Isomet 5,000, Model 11-2780, Buehler, Lake Bluff, IL) to reveal dentin surfaces. The saw was run at 750 rpm with a feed rate of 2.5 mm/min to cut slabs of tooth-embedded epoxy discs. Each tooth produced two exposed dentin surfaces after polishing at 600 grit, followed by 800 and 1,000 grit.

Two restorative materials and two fluoride varnishes were tested in this study. They were (1) a GIC packaged as an encapsulated liquid and powder, Fuji II LC Capsules (GC America, Alsip, IL); (2) a universal restorative composite, Filtek Z250 (3 M ESPE, St. Paul, MN); (3) a 5% sodium fluoride solution (Cargus International, Congers, NY); and (4) Riva Star consisting of SDF and KI (SDI Limited, Melbourne, Australia). All materials were prepared, applied to molar samples, and handled in accordance with the manufacturer's instructions. Sodium fluoride varnish and Riva Star were applied to tooth surfaces using a 5-cm polypropylene brush. All specimens were stored in 37 °C deionized water for 24 hours before testing. Two mechanical properties (shear bond strength and hardness) were measured on the restorative materials and compared. Oral biofilm formation on molar samples was also measured.

Shear Bond Strength

On molar slabs, 10 specimens in epoxy discs were prepared with restoratives according to manufacturer procedures. Etch-Rite and a proprietary aluminum chloride-based cavity conditioner were used as etchants on tooth surfaces for the restorative materials Filtek Z250 and GIC, respectively. Riva Star or Mark3 NaF varnishes were first applied to tooth surfaces as coatings. TPH Spectra ST A2 (Dentsply, York, PA) was used to form a resin peg by filling half of a 0.13 mL, size #5 gelatin capsule (Torpac, Inc., Fairfield, NJ) with Filtek Z250 or GIC and bonding it to the varnish-coated dentin. Resin pegs were cured with a Dentsply Sirona SmartLite focus for 10 seconds, according to the instructions for use. After the pegs were cured, the epoxy-tooth-peg assembly was submerged in deionized water at $37 \pm 2\,^{\circ}C$. After at least 24 hours underwater, each assembly was removed, and the shear bond strength of the Filtek Z250 or GIC resin pegs was measured according to ISO 29022.[13] The effect of fluoride coatings on the bond strength of resins to molar teeth was determined.

Shear bond strength (SBS) testing was conducted on specimens divided into three groups ($n = 10$) including samples treated with steps 1 and 2 of Riva Star, Mark3 NaF varnish, and untreated samples as a control group. The fluoride varnishes' SBS was determined on the 3 M Filtek Z250 universal composite and GC Fuji II LC glass ionomer capsules. Shear bond strength was measured using the Alliance RT/5 testing instrument (MTS Systems Corporation, Eden Prairie, MN). The load was applied using a 5-kN load cell (model 4,501,029, MTS Systems Corporation) and measured with TestSuite TW Elite software (MTS Systems Corporation). Bond strength was obtained in compressive shear mode and a head speed of 0.5 mm/min.

Hardness

Hardness testing was performed on five specimens from each of the three aforementioned experimental groups, plus one group containing only step 1 (SDF) of Riva Star. Specimens were set in EpoxiCure resin (Buehler, Lake Bluff, IL), and smooth surfaces were prepared by sanding with CarbiMet 400 grit grinding papers (Buehler, Lake Bluff, IL) and then polishing with MicroPolish II alumina particles 1.0, 0.3, and 0.05 μm in diameter (Buehler, Lake Bluff, IL) on an Ecomet 6 grinder with an Automet 3 power head (Buehler, Lake Bluff, IL). Hardness measurements were performed before varnish application, then after storage in an incubator at $37\,^{\circ}C$ for 4 hours in a demineralization solution (pH = 4.5), and after 1 day in a mineralization solution (pH = 7). The demineralization solution consisted of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05 M acetic acid adjusted to pH 4.4 to replicate the pH drop produced by cariogenic bacteria, the main factor in dentin decrystallization.[14] The mineralization solution consisted of 1.5 mM $CaCl_2$, 0.9 mM Na_2HPO_4, and 0.15 mM KCl. Specimens were tested according to ISO 14233 with a pyramidal indenter using the Vickers method at a typical load of 10 mN (Buehler Micromet 5,104, Lake Bluff, IL). Each test consisted of a 20×20 grid of 400 indentations. Hardness values were averaged to 15 data points for each specimen after placement in each storage condition.

Antimicrobial Activity

Human molar slabs were polished and treated with Mark3 NaF varnish or Riva Star ($n = 20$ per group). The ability of Riva Star and sodium fluoride to prevent secondary caries in restored teeth and prevent microbial biofilm formation was tested using *Streptococcus mutans*, the bacteria that primarily causes caries in teeth.[7] In six-well plates, fresh cultured *S. mutans* in tryptic soy broth was added along with the molar slabs. The plates were incubated at $37\,^{\circ}C$ without shaking for 2 weeks. Culture media was replaced daily to allow biofilm growth on enamel. A crystal violet staining assay was used to quantify biofilm formation using a SpectraMax i3x microplate reader (Molecular Devices, LLC, San Jose, CA).

Following incubation, the culture medium was removed from the plate, and slabs were washed gently with deionized water. 0.1% crystal violet staining solution in methanol was added to the plate and incubated for 20 minutes at room temperature. The staining solution was removed, and samples were rinsed three times with deionized water. The slabs were left to dry, and then, 30% acetic acid was added to dissolve the biofilm and incubated for 10 minutes at room temperature. In all, 125 μL aliquots of the solution were transferred to a 96-well plate, and the optical density of the sample solutions was measured at 550 nm to quantify biofilm formation.

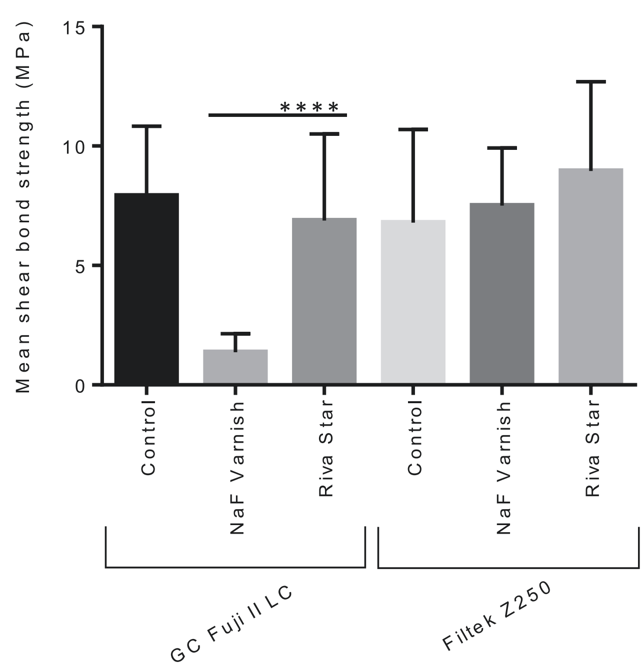

FIGURE 1. Shear bond strength of glass ionomer cement and Z250 composite to human molar teeth ($n = 10$) with and without varnish application. Asterisks indicate the level of significance: $*P < .05$, $**P < .01$, $***P < .001$, $****P < .0001$.

Statistical Analysis

A two-way ANOVA with Tukey's post hoc test was used to compare SBS raw data. Hardness and antimicrobial resistance data were compared using one-way ANOVA with Tukey's post hoc tests. The null hypothesis is that there are no significant differences in the SBS, hardness, and antimicrobial activity between Riva Star and NaF treatments on human molar teeth. Differences with $P \leq .05$ were considered significant.

RESULTS AND DISCUSSION

The SBS of human molar teeth to the Filtek Z250 composite was 24% higher in samples where Riva Star was applied compared to no varnish application (Fig. 1). When compared to a Mark 3 NaF varnish, the SBS to the Z250 composite was 16% higher on teeth where Riva Star was applied. This indicates a possible performance improvement by using the Z250 restorative coupled with Riva Star in place of a traditional sodium fluoride varnish; however, the difference was not determined to be statistically significant.

The SBS of human molar teeth treated with GIC was determined, and no significant difference was observed in the SBS between untreated and Riva Star-treated samples (Fig. 1). However, when Mark3 NaF varnish was applied in conjunction with GIC, a remarkable 83% decrease in the mean SBS was observed compared to samples untreated with Mark3 NaF. Glass ionomer cements set via an acid–base reaction between a basic glass and an acidic ionomer. Moreover, since NaF is a strong base with minimal steric hindrance, it likely has an inhibitory effect on the setting reaction in GIC, significantly lowering the bond strength of the restorative to the tooth. Use of a Mark3 NaF solution smoothens the tooth and GIC surfaces, lowering the overall bond strength.[15] Variable temperature and humidity conditions routinely encountered in the field can also detrimentally affect the setting of GIC.[16] In this study, the application of Mark 3 NaF in combination with GIC resulted in low bond strength of the restorative material to teeth, and it was significantly lower than any other combination.

Hardness was measured on the enamel of teeth treated with SDF (step 1 of Riva Star), Mark3 NaF varnish, or steps 1 and 2 of Riva Star (Fig. 2A). All three fluoride treatments exhibited a statistically significant increase in the hardness over a 24-hour period after acid wash exposure ($P = .0002$-$.0011$) compared to control at 24 hours ($P < .0001$), whereas a 29% decrease in the hardness was observed without any fluoride treatment (control group, $P < .0001$). No significant difference in the enamel hardness was observed between samples treated with Riva Star or Mark3 NaF varnish at the same time point. With respect to dentinal hardness, application of Riva Star (one step or two step) showed a statistically significant increase over 24 hours ($P = .0003$ and $.0167$); the mineralization solution worked to reinforce the mechanical bonds created between the restorative material and dentinal microtubules in all specimens treated with a fluoride varnish (Fig. 2B). The control showed a statistically significant decrease in hardness after placement in demineralization solution for 4 hours but recovered sufficiently by 24 hours, still significantly less than either Riva Star treatment (Fig. 2B).

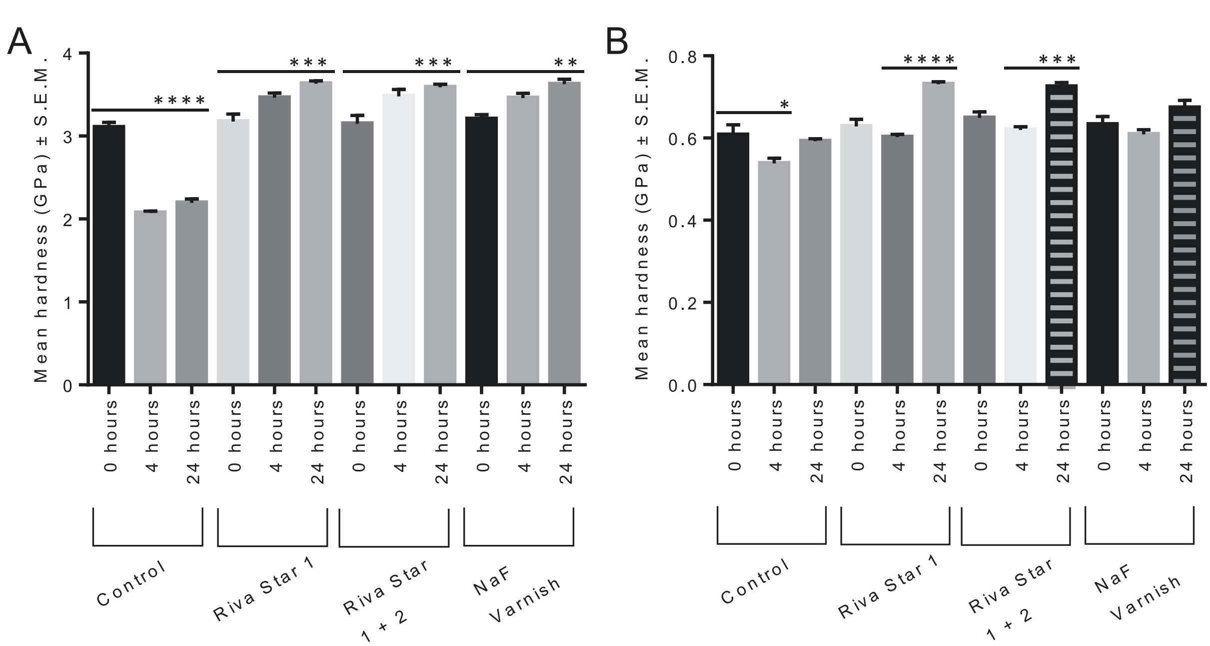

FIGURE 2. Hardness of molar teeth measured before and after demineralization (4 hours) and remineralization (24 hours) ($n = 5$). (A) Enamel hardness of untreated teeth or teeth treated with Riva Star or sodium fluoride. (B) Dentin hardness values of untreated and fluoride-treated teeth. Black bars indicate a significant difference between Riva Star and NaF varnish determined by a one-way ANOVA test ($P < .05$). Asterisks indicate the level of significance: *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$.

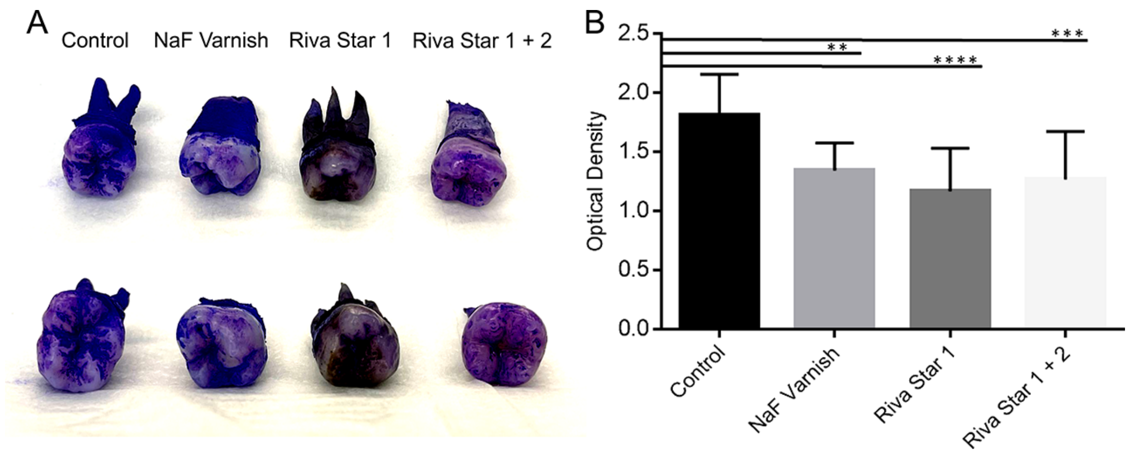

FIGURE 3. Human molar biofilm activity of *Streptococcus mutans* bacteria strain with and without application of a fluoride varnish ($n = 20$ per group). (A) Crystal violet staining on the surface of human molars as a visual indicator of oral bacteria concentration. (B) Optical density measurements of crystal violet staining taken using a plate reader. Asterisks indicate the level of significance: $*P < .05$, $**P < .01$, $***P < .001$, $****P < .0001$.

Crystal violet staining of the most common oral bacteria strain, *S. mutans*,[7] provided a visual indication of the reduction of bacteria after fluoride was applied (Fig. 3A). Teeth treated with step 1 of Riva Star only showed silver staining, but KI was effective in eliminating gray staining in step 2. A 25%-35% decrease in oral biofilm formation was observed on samples, where SDF or Mark3 NaF varnish was applied ($P < .00001$, Fig. 3B). The antimicrobial effects of fluoride on teeth are well known.[17–19] No significant difference was observed between Riva Star and Mark3 varnishes.

CONCLUSIONS

When coupled with Filtek Z250 restorative and a phosphoric acid etchant, Riva Star increased SBS to human molars. SBS testing revealed the ability of Riva Star to increase the bond strength of GIC to teeth, while our results suggest Mark3 NaF inhibits the setting reaction of GIC. Hardness measurements indicated Riva Star is more effective in dentin tubule occlusion compared to Mark3 NaF varnish. Our findings help provide practical suggestions to dental treatment, particularly to the unique dental environments seen in the military. *In vivo* testing is needed to confirm that the laboratory results obtained in this experiment apply to clinical applications and provide guidance for dental practitioners. We therefore hypothesize that roughening the tooth surface with a burr after Mark3 NaF application is necessary to expose the dentinal microtubules and allow the restorative material to mechanically interlock inside porous dentin. Riva Star may be used as an adjunctive treatment prior to placing a final restoration. Herein, the results indicated that it provides antimicrobial action and mineralization characteristics that may prevent recurrent caries or help arrest carious lesions that are questionable to treat at the time of the examination. This can reduce the number of patient encounters, particularly in operational environments where there are no dental practitioners.[1] Additionally, it can help troops to be operationally ready for longer periods of time between patient encounters. Lastly, the need for less treatment can potentially save dental treatment facilities time and decrease the cost of care. Although further research is needed to evaluate clinical performance using Riva Star and various restorative materials, it is promising as an adjunctive therapy.

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CONFLICT OF INTEREST STATEMENT

There are none to report.

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