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Computational conformational antimicrobial analysis developing mechanomolecular theory for polymer biomaterials in materials science and engineering

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Abstract

Single-bond rotations or pyramidal inversions tend to either hide or expose relative energies that exist for atoms with nonbonding lone-pair electrons. Availability of lone-pair electrons depends on overall molecular electron distributions and differences in the immediate polarity of the surrounding pico/nanoenvironment. Stereochemistry three-dimensional aspects of molecules provide insight into conformations through single-bond rotations with associated lone-pair electrons on oxygen atoms in addition to pyramidal inversions with nitrogen atoms. When electrons are protected, potential energy is sheltered toward an energy minimum value to compatibilize molecularly with nonpolar environments. When electrons are exposed, maximum energy is available toward polar environment interactions. Computational conformational analysis software calculated energy profiles that exist during specific oxygen ether single-bond rotations with easy-to-visualize three-dimensional models for the trichlorinated bisaromatic ether triclosan antimicrobial polymer additive. As shown, fluctuating alternating bond rotations can produce complex interactions between molecules to provide entanglement strength for polymer toughness or alternatively disrupt weak secondary bonds of attraction to lower resin viscosity for new additive properties with nonpolar triclosan as a hydrophobic toughening/wetting agent. Further, bond rotations involving lone-pair electrons by a molecule at a nonpolar-hydrocarbon-membrane/polar-biologic-fluid interface might become sufficiently unstable to provide free mechanomolecular energies to disrupt weaker microbial membranes, for membrane transport of molecules into cells, provide cell signaling/recognition/defense and also generate enzyme mixing to speed reactions.

Keywords

Conformational analysis; mechanomolecular; lone-pair electrons; bond rotation; inversion; polar; nonpolar

1. Introduction

Conformational stereochemistry molecular bond rotations have the ability with relatively low energies to rapidly expose atoms containing nonbonding lone-pair electron energies or hide such potential energy between atoms [McMurry, 2004; Brown *et al.*, 2009; Zukerman, 2010; Tinoco *et al.*, 2013]. Nonbonding lone-pair electrons are in a stable state within the outer valence shell and do not participate in electron transfer or covalent reactions [McMurry, 2004; Brown *et al.*, 2009]. In order to facilitate analysis for mechanomolecular theory, computational chemistry software is on hand to measure relative energy changes with a molecule during single-bond rotations [Ohlinger, 2013] to provide explanations regarding molecular movement and forces. Subsequent conformational analysis is capable of calculating potential energy profiles during a specific bond rotation along a continuing series of different bond angles [Ohlinger, 2013]. Further, corresponding three-dimensional (3D) molecular models are concurrently available [Ohlinger, 2013] to better understand and demonstrate how nonbonding lone-pair electrons can be exposed or hidden depending on specific bond rotations in relation to potential energy differences. In terms of the electron distributions for the immediate molecular pico/nanoenvironment, as atoms with lone-pair electrons are more exposed, hydrophilic and polar interactions are preferred [McMurry, 2004; Brown *et al.*, 2009]. But, as nonbonding lone-pair electron energies are hidden, hydrophobic and nonpolar interactions are more likely with the surrounding pico/nanoenvironment [McMurry, 2004; Brown *et al.*, 2009].

Conformational stereochemistry routinely uses saturated hydrocarbons as examples without any nonbonding lone-pair electrons for ethane, propane or butane and even the highly nonpolar saturated polyethylene polymer to show carbon-carbon covalent single-bond rotations and also with computer simulations measure changes in relative potential energies that occur [McMurry, 2004; Sperling, 2006; Brown *et al.*, 2009; Zukerman, 2010; Tinoco *et al.*, 2013]. In addition, protein movements characterized as molecular machines described in solid crystals have also been identified due to conformational molecular bond rotation changes from rapid vibratory fluctuating oscillations and described by X-ray crystallography [Williams, 1989; Zukerman, 2010] with nuclear magnetic resonance confirmation in aqueous solutions producing larger motions and fluctuations [Williams, 1989; Zukerman, 2010; Sapienza *et al.*, 2010]. Now, a specific nonpolar molecule, triclosan or 2,4,4'-trichloro-2'-hydroxy-diphenyl-ether, Fig. 1, is considered for conformational 3D analysis through measurements of bond rotations with relative potential energy states. For conformational bond rotation analysis, the oxygen atom ether bonds need recognition for sp^3 bond orbital hybridization similar to carbon bonds and near the tetrahedral 109.5° bond angle but at about 112° [McMurry, 2004; Brown *et al.*, 2009].

Triclosan is a synthetic trichlorinated binuclear aromatic ether with broad-spectrum antimicrobial activity against both gram positive and gram negative organisms at low concentrations [Ciba Specialty Chemicals, 2001; Jones *et al.*, 2000; Russel, 2003; Russel, 2004; European Commission, 2010] with low water solubility of $10\text{ }\mu\text{g/ml}$ at 20°C [Ciba Specialty Chemicals, 2001; Australian Government, 2009]. Triclosan has been in use for over 40 years as the best studied antimicrobial to date with no reliable evidence of risk for resistance to human pathogens following normal exposure to bacterial interaction

[Australian Government, 2009; European Commission, 2010]. Further, triclosan has been identified as an antimicrobial additive for incorporation with polymers, but regrettably mechanical strength and toughness properties for triclosan incorporation are not found [Imazato *et al.*, 1995; Ciba Specialty Chemicals, 2001; Swofford, 2001; Kalyon and Olgun, 2001; Swofford, 2003; Akaki *et al.*, 2005; Malkoc *et al.*, 2005; Slutzky *et al.*, 2006; Australian Government, 2009; IvoclarVivadent, 2012; Kerr Dental, 2012; Dentsply, 2012].

As an alternative source for understanding triclosan properties, rotation of the triclosan oxygen ether bond provides possible mechanical forces through both conjugated planar aromatic rings depending on the surrounding external electron distributions ranging from polar biologic fluids to nonpolar lipid hydrocarbon membranes. Fortunately, triclosan has been investigated at extensive levels as an antimicrobial [European Commission, 2010] to better apply mechanomolecular theory to test results found in literature. Research indicates that triclosan has multiple modes of action as a broad-spectrum antimicrobial and especially affects the cell membrane of bacteria due to hydrophobic or nonpolar interactions that concentrate triclosan inside the cell phospholipid membrane [Villalaín *et al.*, 2001; Guillén *et al.*, 2004; Russel, 2003; Russel, 2004]. Lipids are nonpolar organic molecules with limited water solubility requiring organic solvents for extraction [Sherwood, 2004; McMurry, 2004; Brown *et al.*, 2009]. With biological complexity, phospholipids are components of cell membranes as two fatty-acid inner tail center linkages to outer polar or hydrophilic negatively charged phosphate head groups that can interact with water [Alberts *et al.*, 2002; McMurry, 2004; Sherwood, 2004; Staley *et al.*, 2007; Brown *et al.*, 2009; Michael and Sicar, 2010]. Nonspecific disruptions of the cell membrane have been demonstrated by triclosan without cell leakage [Villalaín *et al.*, 2001; Russel, 2003; Russel, 2004] that include destabilizing movement with interrupted van der Waals interactions between chains, increased lipid chain motion manifested by decreased lipid viscosity, less membrane packing and lower lipid gel to liquid transition temperatures [Villalaín *et al.*, 2001; Guillén *et al.*, 2004]. Further, immiscibility phase separations occur between triclosan and different nonpolar lipid fatty acid chain structures at higher concentrations above 5% [Villalaín *et al.*, 2001; Guillén *et al.*, 2004]. Also, triclosan interacts with mammalian membrane lipids that cause a decrease in melting temperatures [Lygre, 2003]. Triclosan has been shown to accumulate toward the phosphate head groups of the bacterial phospholipid cell membranes probably aligned perpendicular to the lipid chains that appears to be a major disrupting function to compromise membrane reliability [Villalaín *et al.*, 2001; Guillén *et al.*, 2004]. In addition, as triclosan concentrations increase toward higher bactericidal levels, the bacterial cell membrane is disrupted with leakage of intracellular low-molecular-weight constituents interfering with the uptake of essential nutrients and further subsequent overall energy metabolism is interrupted along with hydrolytic enzymes leading to cell death [Regös and Hitz, 1974; Greenman *et al.*, 1997].

Triclosan has shown efficacy as an antimicrobial when incorporated in different polymers with extremely low release into aqueous solutions [Imazato *et al.*, 1995; Swofford, 2001; Kalyon and Olgun, 2001]. In fact, triclosan was reported to provide antibacterial properties even when eluted into water at levels much less than 500 times below the minimum inhibition concentration for the bacterial strain tested [Imazato *et al.*, 1995]. Subsequent

triclosan antibacterial effect at extremely low concentrations was considered a result of inhibiting bacterial growth and reduced adherence to the polymer [Imazato *et al.*, 1995; Sehgal *et al.*, 2007]. As a relevant concern, in order for bacteria to become resistant to a compound, specific bacterial targets are required generally at such lower antibacterial concentrations so that a mutation renders the antibiotic ineffective [Jones *et al.*, 2000; Australian Government, 2009; European Commission, 2010; Goel, 2013]. Consequently, the basis for lack of bacterial resistance to triclosan over extended long periods of use is founded on general disruption of bacterial cell membranes by multiple activities [Jones *et al.*, 2000; Villalain *et al.*, 2001; Guillén *et al.*, 2004; European Commission, 2010].

Although a specific mechanism under laboratory conditions at low triclosan picomolar concentrations has been associated with lipids through inhibiting a protein that is an enzyme as a target [Roujenikova *et al.*, 1999; Stewart *et al.*, 1999; Jones *et al.*, 2000], clinical relevance for bacterial resistance has not been demonstrated in over 40 years of long-term repeated use [Gilbert and McBain, 2002; Russel, 2004; Ciba Specialty Chemicals, 2006; Australian Government, 2009; European Commission, 2010]. Of further interest, the bacterial inhibition association by triclosan with the enzyme is thought to occur through a pi-pi ring stacking interaction between the aromatic triclosan phenol ring and an enzyme cofactor nicotinamide adenine dinucleotide (NADH) ring that also contains two phosphate groups [Roujenikova *et al.*, 1999; Stewart *et al.*, 1999; Dayan *et al.*, 2008]. Electron distributions of nonpolar molecules such as triclosan at close distances can instantaneously be induced to form temporary nonuniform small dipole-dipole attraction forces and pack molecules together [Atkins, 1994; McMurry, 2004; Brown *et al.*, 2009] as a reason for the pi-pi ring stacking. Further, NADH is one of the most important biologic reducing agents as the reduced form of NAD that donates protons and with NAD^+ is the most significant carrier for electron transfer during energy production and fatty-acid catabolism [Walker, 1998; Alberts *et al.*, 2002; Sherwood, 2004; McMurry, 2004; Brown *et al.*, 2009; Murray *et al.*, 2009; Michael and Sircar, 2010]. In fact, all proteins, carbohydrates and fats are broken down to release hydrogen that is carried by NAD as NADH across a membrane [Sherwood, 2004; Michael and Sircar, 2010]. Of importance to possible molecular compatibilization with polymer blending applications [Datta and Lohse, 1996], computational electron distribution mapping for ring stacking shows close similarities between the molecular structures for triclosan and NADH [Roujenikova *et al.*, 1999; Stewart *et al.*, 1999].

Practical knowledge for the dominating constituents of a composite structure has clearly demonstrated that structural mechanical strength-type properties are influenced most highly by fibers [Chawla, 1998; Peters, 1998; Petersen and Wenski, 2002; Petersen, 2005; Petersen *et al.*, 2006; Petersen *et al.*, 2007a; Petersen *et al.*, 2007b]. Regarding highly relevant biological interactions, conducting carbon fibers that reinforce developmental biopolymer implants have influenced enhanced cell growth beyond normal limits [Petersen, 2011]. Also, protein fiber form is essential in the metastasis of cancer cells by creating structural extensions that provide leverage through normal tissue [Wienberg, 2007; Lindberg *et al.*, 2008; Petersen, 2013a]. Higher eukaryote cells that include mammalian species have a large evolved cytoskeleton composed of structural protein fibers that resist deformation and transmit mechanical forces whereas prokaryote cells that include bacteria have only

primitive fibers identified within just the last 20 years [Bermudes *et al.*, 1994; Dolan *et al.*, 2002; Alberts *et al.*, 2002; Tuszynski, 2008; Bermudes, 1994; Wickstead and Gull, 2011; Drechsler and McAinsh, 2012; Celler *et al.*, 2013]. As bacteria lack a nucleus, during cell division prokaryote chromosomes are pulled apart rapidly by attachments to the cell membrane [Bermudes, 1994; Alberts *et al.*, 2002; Errington *et al.*, 2003; Wickstead and Gull, 2011; Murray *et al.*, 2009], Fig. 2. Consequently, bacterial cell division potentially can be disrupted by triclosan at different nonspecific cell membrane levels especially without the extensive fibrous cytoskeletal strength that supports mammalian cells. In contrasting evolutionary development, eukaryote chromosomes are pulled apart by a structural fibrous spindle apparatus protected within the cell nucleus further sheltered by extensive cytoskeleton fiber formation that extends to the plasma cell membrane [Bermudes, 1994; Dolan *et al.*, 2002; Alberts *et al.*, 2002; Wickstead and Gull, 2011; Drechsler and McAinsh, 2012]. Because prokaryote microorganisms lack a nucleus and an evolved extensively comprehensive organized fiber cytoskeleton, bacteria duplicate generally by binary fission [Bermudes, 1994; Alberts *et al.*, 2002; Murray *et al.*, 2009; Wickstead and Gull, 2011; Drechsler and McAinsh, 2012; Celler *et al.*, 2013]. During bacterial cell division, two circular chromosomes attached to the cell membrane are separated by an invagination membrane growth that continues to form a septum dividing the cell before cleavage into two separate cells [Alberts *et al.*, 2002; Errington *et al.*, 2003; Murray *et al.*, 2009; Wickstead and Gull, 2011]. In addition, mammalian or eukaryote cells are much larger than bacterial cells [Alberts *et al.*, 2002; Staley *et al.*, 2007; Celler *et al.*, 2013] with relative volume differences greater than 1000 [Alberts *et al.*, 2002] so that relative surface areas for cell membrane targets are many times more exposed with the much smaller bacteria cells [Staley *et al.*, 2007]. Bacterial cells are generally in a range from 1 μm to 10 μm in length whereas mammalian cells broadly exist in a wider larger range from 5 μm to 100 μm [Alberts *et al.*, 2002].

All cell membranes are composed of a bilipid that has two nonpolar fatty acid chains internally attached to phosphate head groups that expose hydrophilic electron distributions to biologic fluids on the outer extracellular side and inner intracellular side [Alberts *et al.*, 2002; Sherwood, 2004; McMurry, 2004; Brown *et al.*, 2009]. Also, lipids are recognized as the universal basis for the membrane lipid bilayer and are synthesized as linear polymers from smaller biological molecular hydrocarbon units for self assembly into larger structures such as membranes [Alberts *et al.*, 2002; Sherwood, 2004; Michael and Sicar, 2010]. Bacterial cell membranes are phospholipids that also contain an outer peptidoglycan coating with protein and disaccharide sugar residues on the outside or extracellular side called the cell wall [Walker, 1998; Staley *et al.*, 2007; Murray *et al.*, 2009]. The disaccharide polymers are further referred to as polysaccharides [McMurry, 2004; Brown *et al.*, 2009]. Because the peptidoglycan coating is a unique bacterial feature, specific antibiotics such as penicillin were developed originally from other microorganisms to target synthesis of the cell wall [Goodman *et al.*, 1985; Baron, 1996; Staley *et al.*, 2007; Greenwood *et al.*, 2007; Goel, 2013]. In fact, enzymes involved in the synthesis of the peptidoglycan layer are targets of several different antibiotics within the bacterial cytoplasm, membrane and outside the membrane [Baron, 1996]. Also, specific antibiotics such as the polymyxins were developed

to particularly target the bacterial membrane with leakage of intracellular components [Baron, 1996; Greenwood *et al.*, 2007; Goel, 2013].

Of interest for bacterial membrane susceptibility to triclosan, during the invagination septal formation, different globular proteins that are enzymes are necessary for peptidoglycan synthesis associated with membrane synthesis required to promote cell division [Murray *et al.*, 2009]. In addition, enzymes are needed afterwards to break the peptidoglycan down that separates the membrane septum [Murray *et al.*, 2009]. In fact, if the peptidoglycan synthesis is inhibited during membrane septal growth, cell division will also be inhibited [Murray *et al.*, 2009]. Extensive previous work on triclosan antimicrobial has shown specific ability to inactivate bacterial membrane stage function at the enzyme-level particularly noted with pi-pi ring stacking of the cofactor ring with NAD/NADH [Roujenikova *et al.*, 1999; Stewart *et al.*, 1999; Dayan *et al.*, 2008]. Other cofactors that contain a ring structure and phosphate groups associated with NADH and enzymes are involved in the catabolism of beta oxidation for unsaturated fatty acids and oxidation metabolism of glucose during energy synthesis [Alberts *et al.*, 2002; Sherwood, 2004; McMurry, 2004; Brown *et al.*, 2009; Michael and Sicar, 2010]. Subsequent pi-pi ring cofactor stacking could possibly form general biologic inactivation through numerous reactions with triclosan as specific antimicrobial interactions. In higher cell forms such as eukaryotes, the proton gradient membrane pump and electron transfer functions for energy synthesis occur in intracellular mitochondria organelles with multiple enzymes required [Alberts *et al.*, 2002; Sherwood, 2004; Michael and Sicar, 2010]. But, for prokaryotes such as bacteria, enzymes necessary for energy synthesis function from the breakdown of molecules at the cell membrane level and some cell operations are generated by related proton hydrogen-pump movements [Walker, 1998; Murray *et al.*, 2009]. As an example for the importance of hydrogen-pump action, during bacterial metabolism protons accumulate outside of the cell and return with movement across the cell membrane with the help of more enzymes while NADH is generated to produce adenosine triphosphate (ATP) [Walker, 1998; Murray *et al.*, 2009] as the energy standard for phosphate bond hydrolysis at just 30.5–31.0 kJ/mol [Alberts *et al.*, 2002; McMurry, 2004; Brown *et al.*, 2009]. Subsequent bacterial metabolism at the cell membrane has potential susceptible inhibition to possible triclosan action that is shown to concentrate at the cell membrane phosphate head groups [Villalaín *et al.*, 2001; Guillén *et al.*, 2004]. Further, at the membrane level of activity, triclosan appears to generally inhibit secreted proteins and polysaccharides required for bacterial growth and substrate binding [Lubarsky *et al.*, 2012]. Also, triclosan has been shown to inhibit biofilm formation on inorganic silica beads by several bacteria species including *Staphylococcus aureus* and that secreted extracellular protein and complex carbohydrate polysaccharides were correlated with the bacterial substrate binding [Lubarsky *et al.*, 2012].

Disruptions of the weak bacterial cytoplasmic membrane identified with triclosan antimicrobial activity should further be considered as a cause for death particularly during the cell division process. The doubling time for bacteria may be very short so that cytokinesis of the septum for two separate cell membranes at the end is extremely fast with complete binary fission development often in about just 20min [Walker, 1998; Staley *et al.*, 2007]. Although triclosan is extremely effective as an antimicrobial at generally relatively

low concentrations, the exact nature for bacterial inhibition has been described by multiple actions creating some extended controversy at times [Stewart *et al.*, 1999; Roujenikova *et al.*, 1999; Australian Government, 2009; European Commission, 2010]. Exaggerated mechanical or structural disruptive rotation forces by triclosan ether bonds on a weak bacterial membrane during a rapid process such as cell division where polar/nonpolar environments between biologic fluids and cell membranes suddenly exchange positions would appear to be a possible explanation for the broad antimicrobial properties demonstrated. In fact, distortion of membrane conformation during ingrowth of the septum is an energy barrier to constriction into two cells [Errington *et al.*, 2003]. Consequently, triclosan localization in the membrane at the phosphate head groups that is thought to probably compromise membrane integrity [Villalán *et al.*, 2001; Guillén *et al.*, 2004] should be a major membrane disruption especially during cell division. Thus, the bacterial phosphate outer area of the cell membrane could be a key area objective for directing triclosan antimicrobial disruptions during continuing bacterial cell division particularly in the septal invagination area where membrane structures with phosphate head groups are being concentrated inward together toward one another. Further, triclosan accumulation near phosphate head groups on the extracellular side or outside of the cell membrane is possible in the septal invagination requiring only minimal transport into the cell membrane. During the stationary phase of bacterial growth with no cell division due to insufficient nutrients with lower metabolism and physical barriers from a build-up of metabolic bacterial wastes [Walker, 1998; Murray *et al.*, 2009], bacteria are more resilient to the inhibitory effects of triclosan [Escalada *et al.*, 2005]. Thus, membranes not undergoing septal binary fission would not be as effectively compromised by accentuated triclosan disruptive localization into the lipid phosphate groups. Consequently, in the bacterial stationary phase triclosan inhibition at the enzyme level and NADH pi-pi ring stacking appears sufficient at such lower concentrations [Escalada *et al.*, 2005].

Because of the possible advantages involved, nonpolar triclosan is considered for an antimicrobial additive with polymer biomaterials by incorporation into nonpolar thermosetting organic resins of related polarity and electron distributions. Relevant resins of interest include vinyl esters and epoxies and also with acrylic liquid/powder chemical cure formulations. Due to similar nonpolar relationships between incorporated triclosan with several of the thermoset and also thermoplastic polymers, the hydrophobic antimicrobial release is extremely very slow into aqueous media [Imazato *et al.*, 1995; Swofford, 2001; Kalyon and Olgun, 2001; Sehgal *et al.*, 2007]. Thus, long-lasting antimicrobial protection for polymers is considered as the ideal solution to prevent bacterial colonization [Imazato *et al.*, 1995; Swofford, 2001; Sehgal *et al.*, 2007]. By comparison, antibiotics have previously been used in bone cement to provide only minor protection of a few days at normal doses and just a few months at high concentrations of antibiotics that in turn reduce mechanical properties [Lawson *et al.*, 2010].

In terms of bacterial colonization, bacterial sizes that occur in a general range of 1 to 10µm appear related to material surface roughness (Ra) properties of similar orders in size that has correlated with adhesive binding of bacterial cells [Nyvad and Fejerskov, 1986; Carlén *et al.*, 2001; Ikeda *et al.*, 2007]. Even small differences for particulate-filled composite

polymers with average Ra values in the 6.4–7.3 μm range were colonized by bacteria at significantly lower levels than similar polymer composites with average Ra values in the 11.7–11.8 μm range [Ikeda *et al.*, 2007]. Because bacterial adhesion has been shown to colonize early in cracks, pits, surface irregularities or gaps between two materials, retention of bacteria in surface depression imperfections of similar dimension or greater than the colonizing bacteria appears to be an important issue [Nyvad and Fejerskov, 1986; Carlén *et al.*, 2001; Ikeda *et al.*, 2007; Kermanshahi *et al.*, 2010]. After a biomaterial is stressed passed the yield point where permanent deformation starts to take place and by long-term repeated loading with fatigue [Beer *et al.*, 2006; Callister, 2007], triclosan antimicrobial protection could be predominantly essential when sufficient surface defects such as microcracking arise over time in a material that could help retain bacteria to culture growth. In fact, the most common cause of failure for polymer matrix composites is by damage accumulation primarily through brittle microcracking of the polymer matrix [Peters, 1998]. As mechanical properties are also of utmost importance in biomaterials for application safety factors, related analyses to prevent bacterial colonization that could develop inside microcracking and larger surface defects could also be evaluated using preliminary flexural strength tests. In addition, apparent observations clearly showed that triclosan antimicrobial addition to resin and particulate-filled composite pastes increased strain-related sample flexural bending strength/toughness and also reduced material resin viscosity particularly observed for much lower paste consistency that could be modeled by mechanomolecular theory. Consequently, triclosan antimicrobial is developed now as a thermoset polymer additive with a new alternate property for use as a toughening agent to prevent polymer microcracking. In addition, hydrophobic wetting agents have been previously proposed to improve fiber and particulate resin impregnation by further reducing percentages of diluent monomer that increase water adsorption [Cope and Linnert, 1980; Cope, 1980] that classifies a new possible property for nonpolar triclosan antimicrobial.

Resulting evidence for triclosan influence on polymer mechanical properties can now help to explain common material test result interactions for polymer toughness increases previously described by molecular entanglement with rotation of bonds for the coiled/kinked polymer chains in the cured and hard solid state [Datta and Lohse, 1996; Ratner *et al.*, 2004; Sperling, 2006; Peacock and Calhoun, 2006; Callister, 2007] and also reduced bond entanglements during the melt stage and rubbery phase [Peacock and Calhoun, 2006] that could also apply to lowering resin viscosity. Subsequent description of mechanomolecular theory involving molecular bond entanglement for strength/toughness then is especially simplified with computational conformational analysis of relative energy levels during bond rotations. The extreme ability of triclosan as a small nonpolar molecule to disrupt much larger membrane structures of bacteria can now be described in proper details with mechanomolecular theory developed through measuring potential relative energies and angles during bond rotations using computational conformational analysis methods. In addition, triclosan computational conformational analysis helps to model biological properties at the cell level by both structural bond entanglements concurrently with rapid molecular fluctuations that disrupt weak bacterial cell membranes. By extension through a more comprehensive mechanomolecular theory, computational conformational analysis assists in developing clear simplified relevant models for molecular transport through the

cell membrane, cell signaling/recognition/defense and enzyme theory by mechanical mixing that speeds up reaction rates with thermodynamic ability to overcome difficult energy barriers.

2. Experimental and Methods

Computational restricted Hartree–Fock self-consistent field (SCF) method calculation was performed using Pulay DIIS extrapolation by Wavefunction Inc., Irvine, CA on Spartan Quantum Mechanics Module software for the triclosan molecule shown in Fig. 1. The bond rotation energy profile calculations were charted along the center oxygen ether bond angle between both aromatic rings from 20° to 90°. Further, more conformational bond rotation analysis was completed with Spartan 3D molecular modeling.

Triclosan was obtained from Ciba Specialty Chemicals Corporation, High Point, NC, as a white crystalline powder and average particle size of 45 µm with 99% minimum purity for incorporation into samples for a photocure vinyl ester resin system, the same photocure resin system but as a particulate-filled composite, the photocure particulate-filled composite with 3.0mm pure quartz fibers and a thermoset chemical-cure powder/liquid acrylic.

The photocure resin was prepared using the resin 2,2-bis[p-(2'-hydroxy-3'-methacryloxy propoxyphenyl)]propane (Bis-GMA) (Esstech, Essington, PA) and diluent viscosity reducer monomer triethylene glycol dimethacrylate (TEGDMA) (Esstech, Essington, PA) with camphorquinone as the initiator and dimethylaminomethacrylate as accelerator (both Sigma Chemical, St. Louis, MO). The BisGMA:TEGDMA ratio was blended at 2:1.

The particulate-filled composite was prepared as a photocure compound using Bis-GMA and TEGDMA with camphorquinone as the initiator and dimethylaminomethacrylate as accelerator. Specifically for the current investigation, particulate-filled photocure paste similar to commercial 3M Corporation dental material Z100® with a 50:50 Bis-GMA:TEGDMA ratio was developed by incorporating 84.5 wt.% or 66 vol.% zirconia silicate (3M Corporation, St. Paul, MN) silanated with 3-methacryloxypropyltrimethoxysilane from the manufacturer. The subsequent particulate-filled composite was also incorporated with 35 wt.% 3.0mm long, 9.0µm diameter pure quartz fibers (JPS Composite Materials Corporation, Slater, SC).

Triclosan was incorporated into BisGMA:TEGDMA photocure resin at increasing concentration levels by adding 0.0wt.%, 5.0wt.%, 10.0wt.% and 20.0wt.% and then to exceedingly high levels from 50wt.% to 90 wt.% to be sure that curing was not significantly inhibited by triclosan. Further, triclosan was incorporated into the BisGMA photocure 84.5wt.% zirconia silicate particulate-filled composite with additions at 0.0 wt.%, 0.2 wt.%, 0.6 wt.%, 1.0 wt.%, 2.0 wt.% and 4.25wt.%. Also, triclosan was added to the fiber-reinforced composite with zirconia silicate particulate filler at 0.0wt.% and 10.0wt.%. Photocure samples were prepared between split molds with two glass plates and release film, clamped and then polymerized on both sides with a 3M ESPE Epilux 2500 halogen curing light (3M Corporation, St. Paul, MN) for a total of 2min. In addition, an Orthodontic

acrylic thermoset powder/liquid mixture (Kerr Corporation, MI) was incorporated with triclosan with addition at levels of 0.0wt.% and 10.0wt.%.

Cured samples with dimensions of approximately 2mm deep $\times$ 2.5mm wide $\times$ 25mm long were measured for width and depth by a micrometer to 0.0254mm accuracy and tested for flexural strength across a 20.0mm span with a load cell using a National Institute of Standards and Technology (NIST) traceable Omega Force Gauge (Omega Engineering, Inc., Stamford, CT) in three-point bending according to the formula.

$$\text{Strength} = (3LF)/(2WD^2), \quad (2.1)$$

where L is the span length, F is the maximum force to break a sample, W is the width of the sample and D is the depth of the sample [Callister, 2007].

Addition of triclosan to the 84.5wt.% particulate-filled composite produced noticeable loss of consistency even at minor concentrations that made sample preparation difficult. Consequently, condensing index values in MPa describing maximum force/unit packing area were tested, but with 84.5wt.% zirconia silicate particulate-filled composite formulated using BisGMA:TEGDMA at a ratio of 2:1 instead of 1:1 to accentuate thickened consistency with less diluent monomer. Condensing index values were measured with the NIST traceable Omega Force Gauge load cell adapted with a round flat condensing instrument for the particulate-filled composite when triclosan was incorporated at 0.0wt.%, 4.25wt.%, 8.41wt.% and 15.31wt.%.

3. Results

3.1. Computational conformational analysis

The results for bond rotation of both planar aromatic groups around the oxygen ether bond angle were charted from 20° to 90° by Wavefunction, Inc. according to the relative energies calculated, Fig. 3. A 3D figure of the triclosan molecule is also presented to the right at a 50° bond rotation and a greater exposure of the ether oxygen from the 2D triclosan model seen in Fig. 1 at 0.0° ether bond rotation and viewed from the opposite side. 3D models as colors for atoms include: Oxygen-Red; Carbon-Grey; Hydrogen-White; Chlorine-Orange and also the possible dipole arrow directing toward the negative polarity-Yellow.

The ether oxygen bond of triclosan is rotated toward an energy minimum at around 30°, Fig. 4, and sequentially outward to 90°, Fig. 5. Between the 20° and 90° angle rotations the highest bond energies are measured at the 90° bond angle rotation. Dipoles directed by negative polarity back towards the ether oxygen atom are skewed toward the aromatic ring with the hydroxyl group.

Figures 6(a)–6(d) are shown at the same 90° ether oxygen bond angle from different stereoscopic 3D views to better appreciate polar access to the exposed lone-pair electrons of the ether oxygen atom.

The energy minimum at 30° instead of 0.0° bond rotation, charted in Fig. 3, was considered with a series of bond rotations in opposite directions from 0.0° rotation through 180°

rotation, Fig. 7(a)–7(f). A common 2D structure for triclosan is presented, Fig. 7(a), that was often positioned with a chlorine atom inside the aromatic rings below the ether oxygen atom [Stewart *et al.*, 1999; Ciba Specialty Chemicals, 2001]. The chlorine atom below the ether oxygen is rotated 180° from Fig. 1 plus viewed with stereoscopic perspective from the opposite side.

The Spartan software was unable to calculate the 3D structure for the 2D triclosan molecule seen in Fig. 7(a). However, as the ether oxygen atom bonds rotate the inner chlorine atom away from Fig. 7(a) through 180° the triclosan molecule is presented for 3D viewing at 45°, Fig. 7(b); then 90°, Fig. 7(c); 120°, Fig. 7(d); 150°, Fig. 7(e); and finally a complete 180 degrees, Fig. 7(f) similar to a conformation in Fig. 1. Further, in all 3D models seen in Figs. 3–5, 6(a)–6(d) and 7(b)–7(f) the hydrogen atom of the hydroxyl group rotates with the oxygen atom bond inward toward the ether oxygen atom lone-pair electrons.

Of importance for understanding triclosan molecular stability for ether oxygen bond rotation, steric hindrance is developed with Fig. 7(f) between two hydrogen atoms in full contact by 3D modeling with computational software calculations that indicate hydrogen atoms and also a chlorine atom in similar positions would interfere for the conformations shown in two dimensions by Fig. 1 and particularly Fig. 7(a), respectively. Thus, the structure shown in Fig. 7(a) is probably rare due to the larger repulsive sizes of a chlorine atom or alternatively hydroxyl group that can be conveniently rotated 180° by ether bonds and drawn in 2D structure. Repulsion of both phenyl ring hydrogen atoms at the 180° rotation angle shown in mild contact by Fig. 7(f) appear to force the stable equilibrium into an energy minimum with slightly more ether oxygen atom exposed back out toward the calculated 30° rotation, charted in Fig. 3, and seen in a similar but nonidentical conformation with Fig. 7(e) from rotation in the opposite direction from Fig. 4. Regardless of the rotation direction of the aromatic groups, the 90° angle bond rotation, Fig. 7(c), nevertheless still appears to expose the ether oxygen atom more than all other conformations which is calculated at the highest relative energy for the chart in Fig. 3 and included as the identical conformation with dipole in Fig. 5 and Figs. 6(a)–6(d).

3.2. Mechanical tests

Incorporation of triclosan to the photocure 2:1 BisGMA:TEGDMA resin increased average flexural strengths by additions of 5wt.%, 10wt.% and 20wt.%, Fig. 8. The weight percent of triclosan in the photocure resin system would correspond to approximately 4.8 wt.%, 9.1 wt.% and 16.7wt.% antimicrobial, respectively. Differences for flexural strength increases between photocure resins without triclosan were not significant for additions of triclosan at 5wt.% and 10wt.% but were statistically significantly different at 20 wt.%, $p < 0.01$. Addition of triclosan to the photocure resin could explain close to 97% of the variability from the average 4 values presented in Fig. 8 for flexural strength, $R^2 = 0.9656$, $p < 0.05$.

Increasing triclosan concentrations substantially noticeably increased the flexural bending observed with all resin samples tested for a lower modulus. Because strengths increased on average during the load-cell force gauge testing with obvious increased bending for all samples, increasing strain energy and toughness could also be inferred without a stress–

strain relationship to integrate deflection with force [Ratner *et al.*, 2004; Beer *et al.*, 2006; Callister, 2007; Petersen *et al.*, 2007b; Petersen, 2013b].

Triclosan incorporated up to a full 90wt.% into the resin would photocure sufficiently without any appreciable free-radical inhibition demonstrated by preparation of solid flexural samples that failed by tensile bending on the lower half with an average of 8.5MPa. However, photocuring was accomplished in a nonpolar resin system and the oxygen ether lone-pair electrons were minimally exposed. Therefore, delocalization of electron radicals through the oxygen ether group atom into the aromatic rings was minimal compared to possible electron access with triclosan in a polarized aqueous fluid.

Incorporation of triclosan to 84.5wt.% zirconia silicate particulate-filled 50:50 BisGMA:TEGDMA photocure composite similar to 3M Corporation Z100® increased flexural strengths at triclosan additions of 0.2 wt.%, 0.6 wt.%, 1.0 wt.%, 2.0wt.% and 4.25 wt.%, Fig. 9. Although a tendency was observed for increased average flexural strength with the addition of triclosan antimicrobial, no significant statistical differences were observed while correlation was low. Compared to triclosan addition to the particulate-filled composite at 0.2wt.%, 0.6wt.%, 1.0wt.%, 2.0wt.% and 4.25wt.%, the amount of triclosan added to the photocure resin was far greater at 1.3 wt.%, 3.9 wt.%, 6.5 wt.%, 12.9wt.% and 27.4wt.%, respectively. Testing was stopped at 4.25wt.% triclosan added as particulate-filled composite compound paste consistency was so entirely reduced toward a more flowable glue-like material that tack becomes completely unmanageable, thus making proper defect-free sample preparations difficult. The subsequent difficult packing during sample preparation producing possible defect stress raisers might explain the low correlation for increasing flexural strength with increasing triclosan.

Addition of triclosan at 10wt.% to 35wt.% pure quartz fiber-reinforced 84.5wt.% zirconia silicate particulate-filled photocure composite showed a modest increase in flexural strength, Fig. 10, but was not significantly statistically different. In terms of antimicrobial blending, 10wt.% of triclosan incorporated into the fiber-reinforced composite was then an exceedingly greater amount at 64.5wt.% of the photocure resin. Fibers 3.0mm long and 9.0 µm in diameter provide internal compound consistency to hold resin and particulate secure for making samples in a mold. As a result, tack consistency was not found to be a problem for sample preparation with addition of triclosan at 10wt.% into the 35wt.% fiber-reinforced composite.

Incorporation of 10wt.% triclosan to chemical cure orthodontic acrylic produced a modest increase in flexural strength, Fig. 11, but significant statistical difference was not shown. However, increase in flexural strength was offset by a large standard deviation. Further, triclosan incorporation into acrylic made a slightly less viscous paste.

Condensing index, Fig. 12, measuring loss of paste consistency with the addition of triclosan to the 2:1 BisGMA-resin:TEGDMA-diluent, 84.5wt.% particulate-filled zirconia silicate photocure composite was highly significant at 4.25 wt.% triclosan added to the composite or also at 27.4wt.% added to the resin, $p < 0.000001$. Statistical differences were vastly significant at 8.41wt.% triclosan added to the particulate-filled composite but now at 54.2wt.%.

% added to the resin, $p < 0.00000001$. Statistical differences were vastly significant again at 15.31wt.% triclosan added to the particulate-filled composite but at 98.9wt.% added to the resin, $p < 0.00000001$. Correlation for loss of particulate-filled composite consistency by addition of triclosan measured by condensing index appeared exponential rather than linear. Linear correlation was exceeding statistically significant, $p < 10^{-13}$ and $R^2 = 0.862$. Exponential correlation could explain even more of the variability for loss of particulate-filled composite consistency with addition of triclosan, $R^2 = 0.9662$ although a p -value was not available.

4. Discussion

An energy minimum for triclosan molecule that occurs at about 30° oxygen atom ether bond angle rotation of the two aromatic rings seen in Fig. 3 instead of 0.0° rotation can be compared to the common 2D models presented in both Figs. 1 and 7(a). The need to rotate the triclosan ether bond is easily visualized with computational conformational analysis software showing steric interference by hydrogen atom contacts from both opposing aromatic rings, Fig. 7(f).

Although, the oxygen atom with two lone-pair electrons is more exposed at 30° bond rotation instead of the 0.0° rotation viewed with normal 2D conformation drawings, repulsion at close distance by two positive hydrogen atoms or possible other larger groups destabilize the nonpolar Triclosan binuclear aromatic ether molecule towards the more stable 30° minimum energy rotation. Previously, intramolecular OH–O and OH–Cl bonds were examined by spectrometer measurements with Infrared (IR) and H-nuclear magnetic resonance (H-NMR) for chlorinated 2-phenoxyphenols like triclosan [Norden and Edlund, 1987]. The intramolecular hydrogen bond for triclosan by the hydroxyl group is either with the central oxygen ether atom or the ortho position chlorine atom on the opposite aromatic ring, Fig. 13. Further, the size of the H-NMR chemical shift for the hydrogen bond indicates that the molecule is twisted away from the parallel planar aromatic rings conformation [Norden and Edlund, 1987] that was similarly predicted by Spartan software energy relationships in chart Fig. 3 with the approximate 30° bond rotation for the most stable energy minimum state. Thus, the ether oxygen atom rotation skews the triclosan molecule away from the 0.0° parallel aromatic ring conformation [Norden and Edlund, 1987]. Combined with the intramolecular repulsion by the hydrogen atoms between aromatic rings at 0.0° rotation, secondary bonding with the hydroxyl group then prefers stabilization at the conformations where the ether and chlorine groups are shown to interact by intramolecular hydrogen bonding. Also, from conformational 3D analysis, the phenol hydrogen atom of the hydroxyl group rotates inward from the oxygen bond towards the lone-pair electrons of both the ether oxygen atom and opposing phenyl aromatic ortho position chlorine atom.

Intermolecular hydrogen bonding is another consideration for triclosan antimicrobial. Triclosan is sold commercially in 99% pure form as a crystalline powder with an average particle size of approximately 45 μm . The intermolecular hydrogen bonding from the hydroxyl group elevates thermal properties [McMurry, 2004; Brown *et al.*, 2009]. Consequently, the positively charged –OH hydrogen atom from one triclosan molecule is attracted to a lone-pair of electrons on a negatively polarized chlorine atom or perhaps an

oxygen atom of another triclosan molecule that produces a weak attraction force to hold molecules together for increased structural properties [McMurry, 2004; Brown *et al.*, 2009]. Triclosan would normally be expected to exist in a possible liquid state if the hydroxyl group were replaced with a methyl group or a hydrogen atom that cannot form hydrogen bonds. For a simple example, triclosan methyl shown by chemical structure in Fig. 14 replaces the triclosan hydrogen atom of the hydroxyl group with a methyl group to exist in a liquid state [Sigma-Aldrich, 2012].

To better explain mechanomolecular bond rotations for nonpolar triclosan that either conceal or expose the oxygen atom ether lone-pair electrons for nonpolar hydrophobic interactions or polar hydrophilic interactions respectively, the charted energy profile as Fig. 15 is presented. The energy minimum that occurs at approximately 30° ether oxygen bond rotation for the nonpolar and hydrophobic triclosan molecule provides the most stable steric conformation for nonpolar protection of the oxygen lone-pair electrons that would be the conformation in hydrophobic mediums such as hydrocarbons, lipids or cell membranes. Subsequent triclosan ether oxygen bond rotation further to 90° provides the most polar exposure of the oxygen lone-pair electrons that would be the stable conformation in hydrophilic mediums such as water or possible aqueous biologic fluids. Thus, nonpolar and hydrophobic triclosan has mechanomolecular ability to vibrate with free-energy alternating fluctuations when exposed to dual mediums at edges containing both nonpolar and polar materials.

At an interface between a nonpolar cell surface commonly found along the phospholipid membrane with a polar biologic fluid, the oxygen ether lone-pair electron stability for triclosan bond rotation would be difficult to satisfy. Consequently, membrane “perturbations” predicted by triclosan as mechanical energy are thought to destabilize cell membranes by interrupting van der Waals attraction forces that hold lipid chains together with subsequent increasing chain motions [Villalaín *et al.*, 2001; Guillén *et al.*, 2004]. Unstable triclosan fluctuations could entail mechanomolecular energy from rapid alternating movement of either aromatic ring by oxygen ether bond rotations to establish different energy equilibriums during the breakup of lipid chains in an attempt to stabilize lone-pair electron interaction neighbor contacts. Further, lower viscosity triclosan relationships toward lower gel to liquid transition temperatures of a phospholipid chain hydrocarbon that model by interrupting van der Waals forces of attraction [Villalaín *et al.*, 2001; Lygre *et al.*, 2003; Guillén *et al.*, 2004] could occur through similar mechanomolecular alternating directional vibrations making an effort to adjust lone-pair electrons toward more stable polarity compatibility. In fact, from polymer science interruptions of intermolecular forces between polymer chains by weak van der Waals interactions and hydrogen bonding are well-known factors that lower the glass transition temperature related to a decrease in energy requirements for lower gel to liquid transition temperatures with increased chain molecular motion [Sperling, 2006]. Subsequent reduction of bacterial adherence to polymer surfaces incorporated with triclosan [Imazato *et al.*, 1995; Swofford, 2001] might possibly be a result of intermolecular interruptions of van der Waals interactions and hydrogen bonding by triclosan rapid fluctuating bond rotations alternating at an interface between a nonpolar polymer surface and a polar aqueous fluid with nonpolar bacterial membranes. Reduced

particulate-filled composite paste consistency and lower resin viscosity prior to photocure polymerization appear possibly related to triclosan mechanomolecular energy fluctuations that could disrupt weak secondary intermolecular forces of attraction. Before bond rotation molecular entanglement in the more fluid resin state, triclosan mechanomolecular energy bond rotations could upset resin molecular interchain associations by interfering with secondary bonding through van der Waals forces of attraction and hydrogen bonding. Also, improved mixing between resin molecular chains as predicted during polymer blending [Datta and Lohse, 1996] might be heightened from interruptions of secondary bonding between molecular chains by triclosan mechanomolecular rapid fluctuating moments. Before polymerization curing hardens the resin to a solid polymer, compatibilization has been characterized for a dual resin blending model with reduced viscosity, lower interfacial tension between chains, better miscibility for mixing and reduced secondary bonding with less agglomeration due to interrupted van der Waals forces of attraction [Datta and Lohse, 1996]. So, nonpolar triclosan appears to have a new possible polymer additive property as a hydrophobic wetting agent by mechanomolecular energy to reduce resin viscosity toward better fiber and filler polymer coupling and increased loadings while reducing the monomer diluent ratio.

Increased toughness of the cured polymer inferred from mechanical flexural testing that showed increased strength with obvious noted increased exaggerated bending deflections [Ratner *et al.*, 2004; Beer *et al.*, 2006; Callister, 2007; Petersen, 2007b] was particularly noticed with the resin-cured polymer samples. Toughness increases have been observed previously with polymer blends during compatibilization [Datta and Lohse, 1996], when comparing increasing polymer molecular weights and chain lengths [Sperling, 2006] and with elastomers [Callister, 2007]. Polymer toughness has been described due to chain entanglements during compatibilization with polymer blends that can include increased strength [Datta and Lohse, 1996]. Also, increasing polymer chain entanglements that hold the polymer together under stress impart flexibility toughness [Datta and Lohse, 1996; Callister, 2007]. Further, entanglements increase viscosity in the melt state with increasing molecular weights [Sperling, 2006; Peacock and Calhoun, 2006]. In addition, chain bond rotations can produce coiled, kinked and twisted molecular chains for polymer elastomers in the unstressed state that deform extensively where crosslinks prevent excessive chain slippage under stress [Sperling, 2006]. Further, toughness for rubber and other elastomers has been presented with another similar model for polymer chains coiled-up that become taut when stretched [Callister, 2007]. Before yield point during straightening when permanent deformation is reached, polymer chains can spring back into the twisted, coiled state after tension is released [Callister, 2007]. Chain entanglements created by bond rotations that include triclosan molecules would provide similar types of chains tied together loosely with mechanical linkages that also comprise triclosan. When stretched, the bonds would tend to disentangle somewhat so that polymer coiled chains become tightened while extended for increasing flexural toughness with more bending deflections and subsequently return to the coiled positions as bond entanglements go back to normal stable equilibria if tension is removed before yielding. Also, strength improvements are often used as a sign that polymer blend compatibilization is occurring between different domains with better adhesion between chains and increased polymer entanglement [Datta and Lohse, 1996]

noted with all triclosan groups for increasing average flexural strengths. The most common cause of failure for polymer matrix composites is by damage accumulation primarily through microcracking of the polymer matrix due to focused local constraints of the matrix [Peters, 1998]. Consequently matrix toughening has been identified as a key goal toward reducing microcracking in composites [Peters, 1998] so that triclosan incorporation into polymer matrix composites as a toughening agent by bond entanglements appears beneficial.

Triclosan ability to provide antibacterial properties when incorporated into a polymer even when eluted into water at levels many times and orders of magnitude below the minimum inhibition concentrations of the bacteria [Imazato *et al.*, 1995; Swofford, 2001; Kalyon and Olgun, 2001] might be considered a result of simultaneous strong bond entanglements secured with the polymer chain and at the same time also at the surface interface with weak bond entanglements into the bacterial cell membrane. Triclosan low solubility in water at about 10 µg/ml at 20°C [Ciba Specialty Chemicals, 2001] is much higher than measured elution from particle-filled composites almost identical to those tested from the methods section here but at 1.0wt.% triclosan [Imazato *et al.*, 1995]. Triclosan incorporated as 1.0wt.% released from the particle-filled composite in water at just 0.02 µg/ml after 24 h and 0.08µg/ml at 56 h and the minimum inhibitory concentration for the *Streptococcus mutans* strain studied was 14.7µg/ml [Imazato *et al.*, 1995]. Strong polymer entanglements with triclosan would help to explain ultra low antimicrobial leaching from the particle-filled composite into water. Because the nonpolar triclosan works as a hydrophobic toughening agent with minimal release from a polymer, long-term antimicrobial benefits appear particularly useful.

In terms of antimicrobial susceptibility to triclosan activity, without a nucleus the bacterial cell membrane is needed for cell division with only a minimal bacterial cytoskeleton compared to evolved fibrous cytoskeletal mammalian cells [Bermudes, 1994; Alberts *et al.*, 2002; Errington, 2003; Murray *et al.*, 2009; Wickstead and Gull, 2011; Drechsler and McAINSH, 2012; Celler *et al.*, 2013]. Then without the intracellular mitochondria organelles for energy synthesis reactions, bacteria require multiple enzyme systems at the cell membrane [Walker, 1998; Alberts *et al.*, 2002; Murray *et al.*, 2009] at risk to triclosan antimicrobial. Further, as the bacterial cell divides, septal invagination of the phospholipid membrane between the chromosomes would tend to concentrate triclosan even more at the phosphate head groups on the extracellular side by squeezing the membranes with triclosan inward and pinching the antimicrobial particles closer together. As triclosan accumulates with the phosphate head groups [Villalán *et al.*, 2001; Guillén *et al.*, 2004], pi-pi aromatic ring stacking appears to occur perpendicular to the membrane phospholipid chains [Roujenikova *et al.*, 1999; Villalán *et al.*, 2001; Dayan *et al.*, 2008] with hydrogen bonding interactions [Norden and Edlund, 1987] in addition to the possibility of ether oxygen bond rotation entanglements into the outside of the bacterial membrane. Consequently, triclosan secured to a bacterial membrane by strong bond entanglements, hydrogen bonding, van der Waals attractions and pi-pi aromatic ring stacking through the surface interface of a tough solid compatible nonpolar thermoset polymer might then force sufficient membrane disruptions during a highly susceptible period in cell division of the bacteria to inhibit microbial growth in a broth solution. Structuring of triclosan into an oil-like bacterial

phospholipid membrane appears to create a possible relatively hard and rigid solid particle-like defect that might interfere with pliability and fluidity that could force a tear when a membrane divides suddenly. With bacterial cytokinesis septal membrane formation and cell division occurring on a scale of possibly just 20min [Walker, 1998; Staley *et al.*, 2007], sudden disruptions near the critical membrane location of the septal invagination next to the implanted chromosomes could be sufficiently disabling toward bacterial inhibition.

In terms of metabolism and membrane synthesis, a cell membrane enzyme has been identified as a specific triclosan target for antimicrobial activity with π - π ring stacking on the cofactor NADH. [Roujenikova *et al.*, 1999; Stewart *et al.*, 1999]. Further, bacterial metabolism occurs with electron transport and proton motive force at the susceptible cell membrane where NADH is the reduced form of the cofactor and helps in catabolism for beta oxidation or breakdown of a carbon-carbon double bond with fatty acids [Walker, 1998; Alberts *et al.*, 2002; Sherwood, 2004; Murray *et al.*, 2009; Michael and Sircar, 2010]. Also proteins and carbohydrate nutrients are broken down by release of hydrogen atoms to NAD as a membrane carrier for a proton [Sherwood, 2004; Michael and Sircar, 2010]. Subsequent bacterial metabolism then becomes particularly vital when nutrients are depleted in the stationary phase [Walker, 1998; Murray *et al.*, 2009] that might selectively occur by entanglements of entrapped bacteria on a polymer surface with triclosan and only minimal leaching of antimicrobial into broth solution. In fact, triclosan has been considered to induce metabolic stress on bacterial cells that indicates related enzyme expression is compromised [Greenman *et al.*, 1997]. In addition, free-radicals should further be considered biologically for lipid crosslinking as a form of polymerization cure shrinkage with covalent bond formation in unsaturated lipid fatty acids with carbon-carbon double bonds [Petersen, 2012] that could secure triclosan closer in to the membrane lipid chains for rotation bond entanglements, hydrogen bonding, van der Waals attractions, π - π aromatic stacking alignments and cell death. Further, from results on high triclosan concentrations in the resin, in a nonpolar lipid membrane environment triclosan ether bond rotation should be minimized to not delocalize electron radicals into a ring and inhibit unsaturated fatty acid crosslinking.

The main polymer chain unit for the BisGMA cured resin crosslinks and polymerizes by free-radical additions at both vinyl groups on each end [Annusavice, 2003]. In order to better explain molecular compatibilization for triclosan, a basic simplified model, Fig. 16, is presented with a relatively larger BisGMA molecule without sp^3 hybridized bond angles. The model loosely shows how initial covalent crosslinks can start at each end of the BisGMA unit and continue free-radical reactive secondary sequence polymerization from the carbon atoms on the other sides of the double bonds into large macromolecules of considerable size [Annusavice, 2003; Sperling, 2006; Petersen, 2012]. More importantly for understanding blending of chemical additive combinations with polymers, analogous molecular similarities between triclosan with two aromatic rings and center oxygen ether bonds match well with the BisGMA molecular polymer unit at midpoint center for compatibilization through similar molecular polarities or electron distributions with two phenyl aromatic rings and associated lateral oxygen ether groups. Subsequently, from basic Organic Chemistry “likes dissolve likes” where electron distributions for corresponding

polarity match best between molecules with similar chemical structures [McMurry, 2004; Brown *et al.*, 2009].

Ether oxygen single-bond rotations by triclosan molecules that stabilize energies related to the dipole and lone-pair electrons might be expected to entangle securely into the BisGMA polymer unit as the resin hardens during cure. Bond entanglements could occur between triclosan and BisGMA by compatibilization with differing ether oxygen single-bond rotations and possible associated carbon single-bond rotations for the resin. As the viscosity of the resin increases during the polymerization reactive secondary sequence cure-shrinkage hardening, triclosan is less able to escape entanglement through mutual single-bond derotations with the BisGMA resin. Once triclosan becomes tightly bound into the solid BisGMA polymer unit by stronger bond-rotation equilibrium entanglements, the bent and twisted polymer chain that develops should have increased strength that was uniformly measured for all triclosan average flexural strength sample groups. At the same time, triclosan incorporated polymer by bond entanglement would be able to stretch out taut for increased toughness and simultaneously increase the bending deflections observed during flexural strength testing. As a result, extremely low triclosan solubility leaching for a particulate-filled composite [Imazato *et al.*, 1995] could then be better explained by mechanical linkages through bond rotation entanglements combined with compatibilization-type blending from similar molecular polarity and electron distributions between the BisGMA polymer and triclosan for strong intermolecular attraction forces.

Electron distributions at close distances for nonpolar molecules like triclosan can be induced to disperse instantaneously for small temporary dipole–dipole attraction forces [Atkins, 1994; McMurry, 2004; Brown *et al.*, 2009]. Consequently, alignment creates pi–pi stacking interactions of aromatic rings [Tinoco *et al.*, 2013] that could entail compatibilization related to triclosan and BisGMA aromatic rings at some level. As explained before, triclosan has been shown to inhibit an enzyme through extensive pi–pi stacking interactions between the phenol ring and the cofactor NADH ring of the enzyme involved [Roujenikova *et al.*, 1999; Stewart *et al.*, 1999; Dayan *et al.*, 2008]. Other general aromatic ring stacking alignment influences crystalline orientation of polymer chains [Sperling, 2006] that might also occur with triclosan. Further, anisotropy in saccharin crystal packing with aromatic ring stacking correlates well with the mechanical property for deformation deflection [Kiran *et al.*, 2010]. Consequently, aromatic pi–pi ring stacking in polymers has demonstrated increased strain toughness deflections with reduced modulus related to compatibilization [Shabbir *et al.*, 2010] that could possibly help to explain increased strain toughness and strength with additions of triclosan to the BisGMA polymer. As a result, the triclosan option for molecular aromatic ring stacking could influence the more complex BisGMA polymer chain to align through the aromatic rings. Further, triclosan incorporation into lipids has been shown to increase crystalline membrane packing not related to entanglements [Villalaín *et al.*, 2001]. Also, at triclosan concentrations greater than 5%, the antimicrobial forms immiscible concentrations towards the phosphate groups in saturated fatty acid esters for phospholipids and aligns perpendicular to the phosphate head groups [Villalaín *et al.*, 2001; Guillén *et al.*, 2004] thus increasing the possibility for pi–pi aromatic ring stacking between triclosan molecules.

Although reduced viscosity was noted for both photocure resin and chemical cure acrylic, the loss of consistency with the particulate-filled zirconia silicate composite similar to the 3M Z100® dental paste was unusually extreme and demonstrated by Condensing Index measurements, Fig. 12. The ability to pack or condense particulate-filled composite is retained by securing intermolecular forces of attraction between the high viscosity BisGMA photocure resin that are assumed to be by hydrogen bonding [Esstech, Inc., 2013a; Esstech, Inc., 2013b] and further by interparticle van der Waals forces of attraction particularly for the smaller nanosize particulate [Milewski, 1973; Atkins, 1994; Petersen, 2006; Petersen *et al.*, 2007a].

The BisGMA resin has an unusually extreme high viscosity measured at about 700,000 cps [Anusavice, 2003; Esstech, Inc., 2013a] or 995Pa [Gajewski *et al.*, 2012] compared to a much lower viscosity of just 775 cps for Ethoxylated Bis Phenol A Dimethacrylate (EBPADMA) [Esstech, Inc., 2013b] or 0.9Pa [Gajewski *et al.*, 2012] for the similar ethoxylated version of the BisGMA molecule. The polymer units for BisGMA and EBPADMA are shown in Fig. 17 as flat linear examples without sp^3 hybridized bond angles.

The chief viscosity difference between BisGMA at 700,000 cps and EBPADMA at 775 cps occurs due to hydrogen bonding between hydroxyl groups that intermesh on the BisGMA chain, Fig. 18, but are not found on EBPADMA. A typical range for hydrogen bonds are between around 20 kJ/mol to 50 kJ/mol [Atkins, 1994; Alberts *et al.*, 2002; Callister, 2007], which are weak compared to covalent bonds, for example carbon-carbon bond dissociation energies are in possible ranges of around 350–375kJ/mol [Alberts *et al.*, 2002; McMurry, 2004; Brown *et al.*, 2009]. Triclosan by mechanomolecular energy then efficiently appears to disrupt weak hydrogen secondary bonds of the BisGMA resin chain by rapid alternating oxygen ether single-bond rotations, Fig. 18. The triclosan large bond rotations of two aromatic rings require at most only –1.3 kJ/mol, Fig. 15, against the continuing instantaneous fluctuating electron lone-pair polarization equilibriums that are difficult to stabilize at a sufficient level without cure-set hardening.

As triclosan entangles in the forming BisGMA polymer chain during polymerization reactive secondary sequence cure shrinkage when covalent bonding occurs, mechanomolecular fluctuating energies are continually reduced where subsequent hydrogen bonding can then possibly even redevelop to an extent during the cure into the hardened solid polymer. Mechanically derived bond entanglements between the polymer chains with triclosan during the cure shrinkage as covalent bonds form then could allow hydrogen bonding to form once again at some level in new equilibrium without the triclosan mechanomolecular disruptions. Subsequent triclosan hydrogen bonds with the BisGMA polymer chain might even work together to facilitate stabilizing bond entanglements and help strengthen/toughen the final polymer. As an alternate biological example for intermolecular bonding interaction, protein folding has been described due to secondary bonding forces and especially hydrogen bonding interactions between chains that restrict protein chain movements [Williams, 1989; McMurry, 2004; Brown *et al.*, 2009]. Hydrogen bonds between triclosan and bacterial membranes are added probable structural restrictive antimicrobial activities to fluid membrane properties that might be particularly effective

during cell division and partially restrict enzymes at much lower antimicrobial concentrations.

Consistency for dental packing of the 3M Corp. Z100® zirconia-silicate particulate-filled photocure composite is developed to a state-of-the-art with multimodal size particle diameters ranging uniformly from 10 nanometers to 3.5 μm [Petersen *et al.*, 2007a]. As a result, high levels of particulate at 84.5wt.% or 66 vol.% are attained where smaller particles fit between larger particles thus producing close particle distances [Milewski, 1973; Petersen *et al.*, 2007a] for well-controlled van der Waals forces of attraction. Van der Waals attraction forces increase exponentially as interparticle distances decrease and are about one-tenth to one-half hydrogen bonds in a typical range from about 2 kJ/mol to 31 kJ/mol [Atkins, 1994; Alberts *et al.*, 2002; Callister, 2007]. Of related interest to the triclosan inverse relationship exponentially with particulate-filled composite viscosity and Fig. 12 flowability Condensing Index, viscosity and temperature relationships for liquids are also inversely related exponentially [Perry *et al.*, 1997]. Again, triclosan mechanomolecular energy with two rapidly alternating aromatic ring ether bond rotations should be expected to interrupt weak van der Waals forces of attraction between particles to produce a much less consistent paste that is more flowable.

With the combined loss of interparticle van der Waals forces of attraction and intermolecular BisGMA resin hydrogen bonding, resultant lower paste consistency viscosity becomes unmanageable and virtually impossible to pack the particulate-filled composite properly into a mold with simple condensing compression placement instruments. Conversely, relatively large amounts of triclosan could be blended with 9 μm 3mm length fiber-reinforced composites where the resin fraction is quite low. For example, 10wt.% triclosan added into a 35wt.% pure quartz fiber-reinforced composite incorporated through a 85.5wt.% zirconia silicate particulate-filled composite required blending during the final mixing stage into the photocure resin at a remarkable 64.5wt.% triclosan that in turn increased flexural strength over the same fiber-reinforced composite without triclosan. Material consistency is not a problem with the resin during sample preparation since molds are poured with low viscosity before the photocure. On the contrary, particulate-filled composites are thickened pastes that require substantial consistency with low tack as the molds are packed or condensed with mild pressure using small instruments that should not stick to the compound before photocure. As a result, loss of proper consistency that starts to develop with minor percents of triclosan at only 0.2 wt.% is a considerable problem during the compressive packing of particulate-filled composites with condensing instruments into a mold. On the other hand, 9 μm diameter 3mm length fibers act as microbarriers to hold photocure resin with particulate at the micrometer level so that consistency is not a concern demonstrated at 10wt.% triclosan. Because fibers appear to work at the micro-level restricting resin flow and containing particulate within the paste compound, application of pressure can even be provided during photocure bonding to an adhesive substrate. In addition, triclosan incorporation should act as a hydrophobic wetting agent for discontinuous fiber-reinforced composite to lower resin viscosity, facilitate incorporating more fibers or particulate, and allow increases in relative percentages of the resin:diluent monomer ratio. As one more point in favor of antimicrobial triclosan addition to fiber-reinforced composite, the diluent

monomer TEGDMA has been implicated in increasing bacterial colonization [Hansel *et al.*, 1998].

In order to better explain bacterial inhibition, oxygen ether single-bond rotations alternating rapidly by a relatively small triclosan molecule at a nonpolar phospholipid membrane/polar biologic fluid interface from one extreme polarization level to the next could be rigorous particularly toward weak bacterial cell membranes. Inhibition of *Strep. mutans* was found following 100 μ l inoculation from suspension into dish-shaped wells on the surface of a composite incorporated with 1wt.% triclosan, incubation at 37°, recovery with agitation for 10 fold dilution, culture onto BHI agar plates with anaerobic incubation for 48 h and measurement of total colony forming units (CFUs) at 6 h, 12 h and 24 h to provide significant statistical differences when compared between controls without triclosan [Imazato *et al.*, 1995]. Also, bacterial adherence to the same polymer composite in broth solution was reduced with triclosan incorporation that was observed by scanning electron microscopy (SEM) [Imazato *et al.*, 1995] that may be partially explained by disruption of secondary bonding adhesion forces of the bacteria to the polymer. Still, nevertheless, as previously described for bond entanglements at the polymer surface, triclosan release from the composite into broth solution after 24 h at 0.02 μ g/ml was extremely well below the minimum inhibitory concentration (MIC) of 11.7 μ g/ml for the *Streptococcus mutans* bacterial strain tested in the bacterial broth [Imazato *et al.*, 1995] suggesting some type of direct interaction possibly at the bacterial cell membrane level with the surface polymer/triclosan interface. Further, secondary bond disruptions by triclosan mechanomolecular energy were tested in the results as a possible reason for reduced resin viscosity and especially much lower paste consistency in particulate-filled composites. Also, triclosan mechanomolecular rapid fluctuating bond rotations described during bacterial membrane disruptions [Villalaín *et al.*, 2001] should be considered as a means to possibly prevent bacterial adherence to a polymer surface observed by SEM [Imazato *et al.*, 1995] by interfering with weak secondary bonding forces of attraction. Preventing bacterial adherence on the polymer composite surface might occur by triclosan free to move with more substantial incorporated particle at the polymer surface by mechanomolecular vibrational interference disrupting secondary bonding required for substrate binding adhesion. Subsequent adherence of bacteria to a substrate is then highly important for biofilm formation [Nyvad and Fejerskov, 1986; Carlén *et al.*, 2001; Ikeda *et al.*, 2007]. In addition, through an alternate study triclosan had shown inhibitory levels 100 times lower than the MICs in a bacterial chemostat broth under constant stirring that reflects actively dividing cells more vulnerable to antimicrobial activity [Greenman *et al.*, 1997]. Overall in the same investigation at low concentrations of triclosan, binding to the bacterial membrane was considered as a possible means by the antimicrobial to enhance membrane fluidity and increase molecular transport through the membrane [Greenman *et al.*, 1997]. Therefore several different opposing membrane changing properties should be considered for triclosan antimicrobial inhibition ranging from increasing overall fluidity by breaking down intermolecular forces for adherence to polymer surfaces and also interrupting secondary bonding between lipid chains to other opposing structural molecular entanglements. In addition, increasing crystalline packing alignment at a cell membrane and to enzyme cofactor NADH from triclosan aromatic pi–pi ring stacking from induced weak electron

distribution dispersion dipole–dipole attraction forces might simultaneously influence antibacterial activity. Thus, triclosan antibacterial properties may include many diverse complex interactions particularly during rapid bacterial cell division.

General trends were consistent for increasing polymer-matrix material flexural strengths with the addition of triclosan antimicrobial. But, significant statistical differences were rarely found in spite of some relatively large average differences. One problem that should be considered could entail the incorporation of air into the resin during the mixing stages to produce large voids or defects acting as stress concentrators. Subsequent air-incorporated porosity with sufficiently large void defects can in turn produce stress concentrators that can interfere with maximum mechanical properties tested [Anusavice, 2003]. Large variability incorporated into samples should then also tend to produce larger standard deviations that reduce probability for statistical significant differences [Daniel, 1999]. Fortunately, porosity defects can be counteracted with vacuum mixing. Mechanical mixing under a vacuum has been shown to remove air bubbles created during mixing [Anusavice, 2003]. Increase in tensile strength of vacuum-mixed material has also been noted [Anusavice, 2003]. Further, one study measured 95% porosity free material with vacuum mixing compared to only 17% porosity free material without vacuum mixing [Anusavice, 2003].

Triclosan commercial powder is supplied at the average particle size of 45 μm described from the supplier. However, the commercial triclosan actually appears from optical microscopy comparisons with seeded triclosan crystals to be shifted and cleaned for the removal of particles generally smaller than at least 40 μm [Zhou and Kovenklioglu, 2004]. So, commercial samples contain a high ratio of larger particulate occurring as needle-like crystals up to 300 μm in length with long aspect ratios [Zhou and Kovenklioglu, 2004]. Such large particulate could possibly produce sufficiently large defects that might reduce material properties during flexural testing. Porosity combined with relatively large triclosan particulate might accentuate problems together as increased stress raiser defects more susceptible to material failure problems. Previous testing has demonstrated that triclosan exists in particle form after incorporation into a thermoset BisGMA:TEGDMA particulate-filled dental composite at an undisclosed size by SEM observations [Akaki *et al.*, 2005]. Also, triclosan breaks down with melting point at 54–57.3°C [Ciba Specialty Chemicals, 2001; Australian Government, 2009]. Thus, when incorporated into a polyethylene thermoplastic polymer in the thermal processing triclosan subsequently recrystallizes and accumulates at the surface after just 60min with continual growth to about one micrometer as isolated particles not coupled with the polymer [Swofford, 2003]. Different particle sizes have indeed been shown to influence composite mechanical properties for strength and modulus with successful commercial particulate-filled composites maintaining particle sizes generally below about 10 μm in addition to particle coupling to the polymer that is important to obtain maximum mechanical composite properties [Anusavice, 2003]. Subsequent future testing with triclosan incorporation into thermoset polymers might include powder regrinding of triclosan down to finer particulate and various mechanical resin mixing processes to break down triclosan during blending integration. Nonetheless, mechanical test development for triclosan additions to a polymer biomaterial is of utmost importance to optimize fracture toughness properties and minimize polymer matrix microcracking that

could in turn promote bacterial colonization. Also, long-term cyclic wear/abrasion resistance testing is a separate concern for antimicrobial protection to minimize possible surface roughness with triclosan incorporation.

From the results, triclosan could generally increase flexural strengths with the photocure BisGMA polymer that appears related to compatible molecular structure properties. Similar electron distribution polarity relationships between molecular structures appear to favor bond rotations for entanglement, eventual secondary hydrogen bonding forces and van der Waals alignment in the cured polymer state with possible pi-pi aromatic stacking. Also, triclosan increased average flexural strengths with zirconia silicate particulate-filled BisGMA photocure composite and zirconia silicate particulate-filled BisGMA photocure composite with 35wt.% chopped 3.0mm pure quartz fibers. But, unsaturated nonpolar chains [Villalaín *et al.*, 2001; Swofford, 2003] and polar systems [Malkoc *et al.*, 2005] appear not to compatibilize with triclosan specifically according to polarities for similar electron distributions as with BisGMA blending. Microban® polyethylene products with triclosan noted continuing phase separation of triclosan particulate over time in the saturated polymer with no aromatic structures and without oxygen available for compatibilization blending between the polymer and triclosan antimicrobial additive [Swofford, 2003]. Triclosan comparisons incorporated with numerous thermoplastic polymers demonstrated separation from more nonpolar polymers than polymers containing more polar related electron distributions particularly for a benzene ring or chlorine atom [Silapasorn *et al.*, 2011]. Further, triclosan was found to be immiscible in a phospholipid saturated fatty acid at concentrations greater than 5% and tends to concentrate near the polar phosphate group [Villalaín *et al.*, 2001]. More recently, two temporary cements [Slutzky *et al.*, 2006; Ivoclar Vivadent, 2012; Kerr Dental, 2012; Garza *et al.*, 2013], and a sealant resin primer [Malkoc *et al.*, 2005; Dentsply, 2012] were made commercially available with triclosan. The sealant resin primer has been disclosed as providing a substantial 2.5–10% triclosan composition [Dentsply, 2012]. However, related to some minor incompatibility, the shear bond strength for the substrate acid-etched/washed/dried resin primer was reduced by addition of triclosan with no statistical significant difference [Malkoc *et al.*, 2005]. Essentially, similar results with the work here caused development of a triclosan adhesive to be discontinued, not related to the polymers for the primer or resin system, but as a result of acid etching techniques that exposed polar domains of the substrate surface that need to be cleaned better in future tests. Although sealant resin improved bonding at a nonsignificant level with addition of triclosan presumably with increasing cohesive strength of the polymerized BisGMA resin, when important polar forms for acid etching of substrates were used to increase mechanically-related bonding strengths for conventional primers, triclosan addition weakened the bond slightly on the adhesive side of the bond. So, compatibility relationships as similar electron distributions with polarity associations of the molecular structures appear to interact toward blending resins and additives together better in a composite. Subsequent compatible blending between molecular structures is necessary to prevent potential phase separation of different domains that could produce defects as stress concentrators with potential reductions in material mechanical strengths and toughness properties or also diminish adhesive bonding to a substrate.

Triclocarban or 3,4,4'-trichlorocarbanilide, Fig. 19, disrupts bacterial cellular membranes and has many similarities with triclosan as trichlorinated binuclear aromatic antimicrobials. Both triclosan and triclocarban have low water solubility [Greenman *et al.*, 1997]. But, nonpolar triclocarban has a lower water solubility of 0.65–1.55mg/L at 25°C compared to more polar triclosan measured at 1.97–4.6mg/L for 25°C [Halden and Paull, 2005] and also by separate analysis at 10mg/L for 20°C [Ciba Specialty Chemicals, 2001; Australian Government, 2009]. Both triclosan and triclocarban are thought to destabilize bacterial membranes and preferentially accumulate at the phosphate head group of the phospholipid fatty acids in the membrane [Villalaín *et al.*, 2001; Orsi *et al.*, 2011]. Instead of the ether linkage between the aromatic rings with triclosan, a symmetric aniline bisamide-type of linkage exists for triclocarban between the asymmetry of trichlorobisphenyl aromatic rings. As a consideration for applying both triclosan and triclocarban, synergy has been reported often with antibiotics producing bacterial inhibition at one fourth the normal doses, but occasionally can produce antagonistic consequences [Baron, 1996].

Alternate mechanomolecular energy can be introduced by triclocarban that might have some similar action with triclosan. Triclocarban provides one set of lone-pair electrons from each nitrogen atom that require stable electron distributions with the immediate pico/nanoenvironment to satisfy polarity compatibilization interactions. Anilines and amides are more stable and less basic than amines due to stabilization through possible delocalization of the lone-pair electrons respectively into the aromatic ring or carbonyl group [McMurry, 2004; Brown *et al.*, 2009]. Amines are sp^3 hybridized and the C–N–C bond angle is 108° [McMurry, 2004; Brown *et al.*, 2009]. However, anilines and amides have restricted rotations with the carbon atom of the aromatic group or carbonyl group respectively to produce a planar oriented equilibrium between a single C–N bond and a double C=N bond for 180° orientation of the N–H bond to the double bonds [McMurry, 2004; Brown *et al.*, 2009]. Although resonance stabilization is considerable with triclocarban, the center carbonyl group is compromised and shared between two nitrogen atoms. Amides also form hydrogen bonds between the hydrogen atom of the electronegative nitrogen atom and the electronegative oxygen atom of the carbonyl group [McMurry, 2004; Brown *et al.*, 2009]. In terms of possible triclocarban biological relevance for intermolecular bonding interactions, hydrogen bonds are specifically required between amide N–H groups and C=O groups that form with long peptide bond sequences in proteins for secondary structural chain folding into the alpha helix, sheets and coils [Alberts *et al.*, 2002; McMurry, 2004; Sherwood, 2004; Brown *et al.*, 2009; Michael and Sicar, 2010; Tinoco *et al.*, 2013]. Such hydrogen bonding interactions between Triclocarban and proteins particularly towards deactivating bacterial enzymes needed for synthesis of membrane lipids and the peptidoglycan cell wall could be important for bactericidal action especially with low triclocarban concentrations.

Mechanomolecular energy similar to oxygen single-bond rotation but by pyramidal inversion is a possibility when amines are in contact with an interface between polar and nonpolar entities such as a cell membrane and biologic fluids, Fig. 20. Perfect alignment by a triclocarban molecule with uneven destabilizing aromatic chlorine-group asymmetry for both nitrogen lone-pair electron stabilities should be considered rare without some intermolecular structuring. Subsequent inversion processes during equilibrium in the single-

bond states for the nitrogen atoms might then continue almost indefinitely until possible pi-pi ring stacking or bond entanglement occurs as the triclocarban molecule is propelled in different directions during the nitrogen inversions near the phosphate head interface of the membrane. Still, a full mechanical test analysis for flexural strength with complete strain energy toughness measurements [Petersen *et al.*, 2007a; Petersen *et al.*, 2007b; Petersen, 2013] need to be determined for the possibility of triclocarban bond inversion entanglements or aromatic pi-pi ring stacking with BisGMA polymer.

Molecules after a carbon-carbon single-bond rotation can generally be resolved but amine pyramidal inversions are so rapid the two separate forms cannot be isolated at room temperature [McMurry, 2004; Brown *et al.*, 2009]. Thermodynamics show that nitrogen pyramidal inversion requires only about 25 kJ/mol [McMurry, 2004; Brown *et al.*, 2009]. By comparison, weak secondary bonds by hydrogen bonding are about 20–51 kJ/mol and van der Waals forces of attraction are about 2–31 kJ/mol [Alberts *et al.*, 2002; Atkins, 1994; Callister, 2007]. Although aromatic aniline resonance stabilization of nitrogen lone-pair electrons occurs along with the central carbonyl group, pyramidal inversion at a relatively higher energy barrier for triclocarban would still appear likely between one of the nitrogen atoms for final compatible polarity electron distributions. Nitrogen inversions by triclocarban would then provide free mechanomolecular energy with the immediate pico/nanoenvironment. To place triclocarban inversions in perspective, rapid protein fluctuations suggests that pyramidal inversions are occurring through the repeating amide peptide bonds measured in time scales for protein fiber contraction during cell division and enzyme action of 10^{-9} s to vibrations of 10^{-11} s seen in protein crystals [Williams, 1989]. Triclocarban possible nitrogen inversions that break secondary bonding should be observed in future testing for increased flowability with condensing index measurements of BisGMA particulate-filled composite pastes.

Although no lone-pair electrons are involved, carbon-carbon single-bond rotations should also be a consideration with entanglement for the long polymer chains overall when interacting with more pronounced rotations developing with lone-pair electrons through oxygen single-bond rotations or nitrogen pyramidal inversions. Butane is one example often used to describe carbon-carbon single-bond conformational changes by bond rotations and relative energies with barriers shown in Fig. 21 applying a Newman projection on the left and relative energies for the different conformations on the right [McMurry, 2004; Brown *et al.*, 2009].

Globular proteins are generally soluble and coiled compared to the insoluble tough more crystalline linear-structured protein fibers of the cytoskeleton and in fact make up the major buffering system for blood as the soluble plasma proteins [McMurry, 2004; Sherwood, 2004; Brown *et al.*, 2009; Michael and Sicar, 2010]. Further, most of the plasma proteins except albumin are glycoproteins that influence properties for solubility to provide transport of other molecules and blood viscosity by virtue of the larger molecular sizes [Michael and Sicar, 2010]. Glycoproteins are proteins with sugar chains attached, Fig. 22, which have mechanomolecular energy potential through numerous oxygen single bonds. In aqueous media, hydrophobic amino acids are hidden deep inside the protein whereas hydrophilic amino acids are exposed on the outside on the protein surface [Tinoco *et al.*, 2013].

Conversely, by extension in nonpolar lipid membranes hydrophobic interactions occur so that hydrophobic amino acids are exposed on the surface whereas hydrophilic amino acids are buried deep inside the protein.

Sugars are generally six carbon atom molecules that are linked together by glycoside oxygen single-bond connections [McMurry, 2004; Brown *et al.*, 2009]. For example, glycogen is a polysaccharide of glucose sugar building blocks up to 100,000 units linked together [McMurry, 2004; Brown *et al.*, 2009]. Bacteria have a complex cell wall peptidoglycan matrix coating outside the cell membrane composed of specific disaccharide sugars polymerized into long polysaccharide chains and crosslinked together by proteins for strength and rigidity [Walker, 1998; Greenwood *et al.*, 2007; Murray *et al.*, 2009]. The crosslinked peptidoglycan cell wall provides structure for the bacteria cell necessary to prevent cell death [Baron, 1996]. Further, glycolipids are long sugar chains connected by glycoside bonds that form off the phospholipid cell membrane [Alberts *et al.*, 2002; Murray *et al.*, 2009]. Mechanomolecular oxygen fluctuation bond rotations from glycoside linkages at the cell membrane/biologic fluid interface consequently have potential energy to help with a variety of important cell functions.

The interface between polar biologic fluids and a nonpolar membrane provides differences in electron distributions that require lone-pair electrons to seek stability as open exposed conformations for hydrophilic aqueous interactions and closed protected conformations for hydrophobic lipid hydrocarbon-type interactions. Subsequent mechanomolecular energy at the cell membrane interface with biologic fluids is required for molecular stabilization of lone-pair electrons that produce uncontrolled fluctuations in the oxygen single-bond rotations and also nitrogen pyramidal inversions for proteins and other nitrogen compounds. Resulting efficient mechanomolecular energy is then available with glycoside oxygen bond linkages and peptide nitrogen amide linkages to provide vibratory movements for transport of other molecules through the membrane and cell signaling/recognition/defense without a great expenditure of energy created by the cell during metabolism. In addition within the globular proteins that are enzymes, mechanomolecular movement can guide molecules with mixing action to speed reactions and overcome difficult thermodynamic energy barriers.

Phospholipid cell membrane differences are shown between gram negative and gram positive bacteria, Fig. 23. Gram negative bacteria have outer and inner cell membranes while gram positive bacteria only have an inner cell membrane. However, the peptidoglycan layer is extensively larger for gram positive bacteria compared to the relatively thin peptidoglycan cell wall layer for gram negative bacteria [Walker, 1998; Staley *et al.*, 2007; Murray *et al.*, 2009]. In terms of acknowledging glycoside and peptide bonds, instead of passive nutrient diffusion through the peptidoglycan cell wall, mechanomolecular energy from polysaccharide oxygen single-bond rotations and protein nitrogen pyramidal inversions should generate efficient vibratory movement to propel nutrients actively toward the cell membrane.

In terms of overall bacterial inhibitory protection from a small molecular weight antimicrobial like triclosan, the apparent distinctive double-cell membrane for the gram negative bacteria might appear to provide enhanced shelter for the inner cell membrane that

is critical to cell division and bacterial metabolism. The complex outer membrane envelope of gram negative bacteria prevents hydrophilic molecules from reaching the inner membrane [Greenwood *et al.*, 2007], but triclosan is nonpolar. Nonetheless, when comparing triclosan minimum inhibitory concentrations (MICs) by an agar incorporation test with 60 gram positive and 53 gram negative bacteria strains [Ciba Specialty Chemicals, 2001], the average MIC for the gram positive group was 5.91 ± 14.78 and for the gram negative group was 52.72 ± 202.69 , although not statistically significant different with $p = 0.10$, Fig. 24. The large standard deviations for the MICs reflect great variability of the effective bacterial inhibition by triclosan against a wide range of both gram positive and gram negative bacteria at low and extremely low concentrations, while a few difficult bacteria may render triclosan antimicrobial actions practically ineffective without much higher doses. Further, when considering the importance of reaching the peptidoglycan coating to inactivate possible enzymes that require cofactor NADH at extremely low triclosan concentrations, the protective properties of an outer cell membrane for gram negative bacteria may possibly be an important barrier obstruction ability when compared to gram positive bacteria. Regardless, the peptidoglycan layer is a common feature of all bacteria and is a specific target for several antibiotics that inhibit enzymes synthesizing peptidoglycans especially gram positive strains of bacteria [Goodman *et al.*, 1985; Baron, 1996; Staley *et al.*, 2007; Greenwood *et al.*, 2007; Murray *et al.*, 2009]. In addition, NADH has been shown to form π - π ring stacking with triclosan [Roujenikova *et al.*, 1999; Stewart *et al.*, 1999; Dayan *et al.*, 2008] and NADH is also involved in electron transfer and proton movement across the inner cell membrane of bacteria during aerobic energy synthesis [Salton and Kim, 1996; Walker, 1998; Murray *et al.*, 2009].

The phospholipid plasma cell membrane for the eukaryote cell is depicted in Fig. 25 showing the inner lipid tails and outer phosphate head groups that form the interface with the outer extracellular and inner intracellular biological fluids. Triclosan and triclocarban are thought to accumulate preferentially in the phospholipid membrane next to the phosphate head groups [Villalain *et al.*, 2001; Orsi *et al.*, 2011]. In addition to linking with proteins as glycoproteins, sugar side chains can link to lipids as glycolipids particularly along the extracellular side of a cell membrane. As a result, numerous mechanomolecular complex movements are possible between the polysaccharide sugar oxygen atoms with single-bond rotations in addition to possible nitrogen inversions with the numerous peptide bond linkages of a protein. One other particular distinguishing characteristic of the mammalian eukaryote cell phospholipid membrane not found in bacterial prokaryote cell membranes are cholesterol molecules [Walker, 1998; Murray *et al.*, 2009] that might confer some protective benefits ranging from maintaining membrane fluidity [Sherwood, 2004] to providing osmotic stability [Walker, 1998]. Consequently, instead of cholesterol induced lubricating membrane fluidity found in mammalian cells, structuring by triclosan with rotation bond entanglements, hydrogen bonding, van der Waals attraction forces and aromatic π - π ring stacking might accentuate into a bacterial membrane to produce a defect that compromises pliability required particularly during the rapid cell division.

Although mammalian eukaryote cells do not have a peptidoglycan coating like bacterial prokaryotes [Walker, 1998; Murray *et al.*, 2009] all eukaryotes have an extensive fiber-

reinforcing cytoskeleton that provides structural support to the cell membrane and also a nucleus with a nuclear membrane and fiber spindles that protect the chromosomes during cell division [Bermudes *et al.*, 1994; Dolan *et al.*, 2002; Alberts *et al.*, 2002; Tuszynski, 2008; Bermudes, 1994; Errington *et al.*, 2003; Wickstead and Gull, 2011; Drechsler and McAinsh, 2012; Celler *et al.*, 2013], Fig. 26. As a result, the cytoskeleton support for the mammalian cell membrane provides a large level of structural safety toward maintaining membrane fluidity with cholesterol. In turn, complex triclosan mechanomolecular ability to improve lipid chain motion [Villalaín *et al.*, 2001; Guillén *et al.*, 2004] might possibly enhance subsequent membrane lubricating properties of cholesterol. Conversely, much weaker bacterial cell membranes without extensive underlying cytoskeleton support or a cell nucleus with nuclear membrane [Bermudes *et al.*, 1994; Dolan *et al.*, 2002; Alberts *et al.*, 2002; Tuszynski, 2008; Bermudes, 1994; Errington *et al.*, 2003; Wickstead and Gull, 2011; Drechsler and McAinsh, 2012; Celler *et al.*, 2013] are generally inhibited by complex triclosan properties at relatively low concentrations [Ciba Specialty Chemicals, 2001]. Further, metabolism occurs with eukaryotes in separate mitochondria organelles surrounded by a second membrane inside the cell whereas bacteria metabolize nutrients for energy at the more vulnerable cell membrane [Walker, 1998; Murray *et al.*, 2009].

Special differences between mammalian eukaryote cells and prokaryote bacteria are the basis for safe treatment so that selective bacterial sites are targeted that do not harm a patient [Goodman *et al.*, 1985; Walker, 1998; Greenwood *et al.*, 2007]. Accordingly, triclosan has shown an exceptional safety profile in microbial testing for human use with excellent toxicology, carcinogenicity, mutagenicity and teratogenicity results and good skin compatibility [Bhargava and Leonard, 1996; Jones *et al.*, 2000; Ciba Specialty Chemicals, 2001; Ciba Specialty Chemicals, 2006; Australian Government, 2009; European Commission, 2010]. Triclosan has further been shown to provide beneficial anti-inflammatory properties [Mustafa *et al.*, 2000]. By comparing common antibiotics ampicillin and tetracycline in an animal model tested with triclosan during acute bacterial infections, triclosan surpassed both antibiotics in reducing bacterial growth rates and bacterial biomass [Sharma *et al.*, 2003]. In addition triclosan increased animal survival time, restored blood parameters and checked liver damage from the infections [Sharma *et al.*, 2003]. By uncovering the potential medically-related health benefits for triclosan with more animal testing results, higher eukaryote cell examinations might even provide additional biological information in regards to unique mechanomolecular interactions.

5. Conclusions

Hartree–Fock computational accuracy provided conformational analysis with oxygen ether bond angles and relative energies for the polymer additive antimicrobial triclosan. Spartan software calculated energy profiles that charted the energy minimum with triclosan oxygen ether bond rotations for both aromatic rings at about 30° out of plane instead of an ideal planar 0.0° rotation. More conformational analysis was completed that showed steric hindrance by hydrogen atoms between the triclosan aromatic rings should create repulsive forces to rotate aromatic rings into a skewed position out of plane further confirmed by earlier literature IR and H-NMR spectroscopy analyses. Lone-pair-electrons concealed at the 30° rotation energy minimum should correspond to ether bond rotations in nonpolar

membranes whereas lone-pair electrons exposed with outward ether oxygen bond rotations should correspond toward the charted energy maximum at a rotation of 90° in polar biologic fluids. From a biologic perspective, unstable equilibriums for triclosan oxygen ether lone-pair electrons at a polar-biologic-fluid/nonpolar-membrane interface provide free vibratory energy for mechanomolecular action. Such rapid alternating fluctuations of the ether oxygen bonds described in biological research as perturbations and disruptions of secondary bonding were considered as a possible property by triclosan to disorganize bacterial membranes. Nonetheless, surface contact with triclosan and minimal leaching were considered to be the main sources of biopolymer bacterial inhibition by molecular structuring properties into a weak bacterial membrane and also reduced microbial adherence by interfering with secondary bonding forces to a surface. Triclosan ability to lower the resin viscosity and reduce particulate-filled composite paste consistency offers important information toward understanding mechanomolecular disruptions of secondary bonding that coincided with basic available information from polymer and composite science on interfering with hydrogen bonding and van der Waals forces of attraction. Further, broad mechanical testing by measures for flexural strength provided information on possible bond rotation entanglement interactions that produced increased strength with toughness properties for several different polymer and polymer matrix composite material combinations. Materials science studied at the polymer surface interface with bacteria nonpolar phospholipid membranes in biologic aqueous fluids combined with unique computational conformational chemistry analysis gives solid support to numerous common theories for polymer bond entanglements that can produce strength and also toughness in addition to resin viscosity reduction by mechanomolecular interruptions of secondary bonding forces of attraction. As a result, triclosan antibacterial properties may include many diverse complex interactions particularly during rapid bacterial cell division. Further, nonpolar triclosan antimicrobial may provide new properties for a future polymer additive as a toughening agent that does not adsorb excess moisture and also a hydrophobic wetting agent toward increasing fiber and filler loadings with reductions in monomer diluents.

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Fig. 1.

Two-dimensional (2D) molecular structure for triclosan showing center oxygen containing two lone-pair electrons and ether bonds between the two conjugated planar aromatic rings. Also, triclosan is trichlorinated and has one hydroxyl group on an aromatic ring in the ortho position.

Fig. 2.

Bacteria with no nucleus attach chromosomes to the cell membrane that subsequently invaginates inward between the two circular-like chromosomes with a septum during the rapid binary fission process.

Fig. 3.

The energy profile for triclosan is charted with bond rotation about the oxygen ether atom comparing the relative bond energies for bond angles between 20° to 90° rotation that gave an energy minimum at about 30° bond angle rotation.

Fig. 4. Bond angle of approximately 30° rotation maintains hidden oxygen ether atom and lone-pair electrons with the dipole moment also concealed between aromatic rings corresponding to energy minimum observed in the charted results for Fig. 3.

Fig. 5.
90° bond rotation of both phenyl rings through oxygen ether bonds demonstrating how oxygen lone-pair electrons are completely exposed with dipole moment corresponding to the high bond energy value observed in the charted results for Fig. 3.

Fig. 6.

(a)–(d) Four identical conformations of triclosan at 90° oxygen ether bond rotation viewed from different angles for a stereoscopic perspective on polar access to both lone-pair electrons of the center ether oxygen atom with exposed dipole moment viewed from the same side as Fig. 1.

Fig. 7.

Triclosan oxygen ether bond rotations from 2D 0.0° rotation through to 180° in 3D: (a) Common 2D structure 0.0° ether bond rotation. (b) 3D structure 45.0° ether bond rotation. (c) 3D structure 90.0° ether bond rotation. (d) 3D structure 120.0° ether bond rotation. (e) 3D structure 150.0° ether bond rotation. (f) 3D structure 180.0° ether bond rotation with steric interaction between hydrogen atoms.

Fig. 8.

Increasing average flexural strength values are measured as triclosan is incorporated into the BisGMA/TEGDMA photocure resin up to 20wt.%.

Fig. 9.

Increases in average flexural strength is noted when adding triclosan into the photocure 84.5wt.% zirconia silicate particulate-filled composite up to 4.25wt.%.

Fig. 10.

A slight increase for average flexural strength is measured when adding 10 wt.% triclosan into the 35wt.% quartz fiber-reinforced particulate-filled photocure composite.

Fig. 11.

Increase in average flexural strength with 10 wt.% triclosan to chemical-cure acrylic formulation.

Fig. 12.

Condensing index shows increasing loss of paste consistency during compressive insertion of force gauge measurement tool that appears significantly exponential for the regression from 0.0wt.% to 15.31wt.% triclosan additions into the 84.5wt.% zirconia silicate particulate-filled composite.

Fig. 13.

Triclosan oxygen ether bond rotation of 30° is further influenced by intramolecular secondary hydrogen bonding from the hydroxyl group with the ether oxygen atom that connects both aromatic rings or the chlorine atom on the opposite phenyl ring to increase stability at the energy minimum position from any further rotation away from the initial 2D 0.0° rotation planar state shown by Fig. 1.

Fig. 14.

Chemical structure for triclosan methyl is a liquid at room temperature showing a methyl group replacing the hydrogen atom on the hydroxyl phenol group of triclosan that exists as a crystalline powder.

Fig. 15.

Low-energy minimum at 30° bond rotation is nonpolar and hydrophobic while the high-energy at 90° bond rotation is polar and hydrophilic. Changes in bond rotation between maximum and minimum relative energies are approximately 60° at -1.3 kJ/mol.

Fig. 16.

Triclosan molecule oxygen ether bond rotations compatibilize well with the BisGMA polymer unit for aromatic rings and similar ether functions for single bonds that rotate to influence nonbonding mechanically-related entanglement to normally increase the strength of polymers and possibly toughness.

Fig. 17.

(a) Top structure BisGMA resin chain polymer unit has a viscosity of 700,000 cps related to hydroxyl groups that form hydrogen bonds. (b) Lower structure EBPADMA resin chain polymer unit without hydroxyl groups has a viscosity of just 775 cps that is almost three orders of magnitude lower than BisGMA.

Fig. 18.

Triclosan mechanomolecular energy disrupts hydrogen bonding between BisGMA resin chains thought responsible for the extremely high viscosity of 700,000 cps.

Fig. 19.

Molecular structure of triclocarban similar to bisaromatic triclosan but with diamide-style linkage instead of an ether linkage and no hydroxyl group.

Fig. 20.

Pyramidal inversion of nitrogen atom to expose lone-pair electrons for polar or hydrophilic media and alternatively protect the lone-pair electrons from nonpolar or hydrophobic media.

Fig. 21.

(a) Newman projection shows bond rotations for butane. (b) Relative energies during butane bond rotation. Bond rotations favor a minimum with methyl groups in butane as far away from one another as possible. The highest least stable conformation occurs when the methyl groups in butane are eclipsed directly in front of one another. Relative energies for a methyl group eclipsed with hydrogen require 16 kJ/mol and both methyl groups eclipsed 19 kJ/mol.

Fig. 22.
Basic globular protein or glycoprotein represented with sugar polysaccharide molecules attached (with Permission National Institutes of Health/Department of Health and Human Services).

Fig. 23.

Comparison of the similarities for cell membrane envelopes between gram negative and gram positive bacteria. Triclosan molecules outside of the inner membrane side are shown for relative contrast of sizes to better appreciate the possible forces involved through the broad aromatic ring moment arms during oxygen ether bond rotation. Triclosan forces appear to accumulate in perpendicular alignment by aromatic pi-pi ring stacking and hydrogen bonding into the membrane near the phosphate head groups. Note the extra outer membrane for the gram negative cell (with permission from Salton and Kim [1996]).

Agar Incorporation Test for Triclosan

Fig. 24.

Comparison between gram positive and gram negative bacterial strains for triclosan minimum inhibitory concentrations by agar incorporation testing [data from Ciba Specialty Chemicals, 2001].

Fig. 25.

Plasma cell membrane showing relationships for phospholipids, sugars, proteins and cholesterol. The eukaryote plasma cell membrane has some basic difference from the bacterial prokaryote cell membrane with cholesterol that prevents crystallization of lipid chains in addition to a comprehensive intracellular linkage with an extensive cytoskeleton not shown. For a comparative example relationship, triclosan molecules shown on the extracellular side of the lipid membrane tend to accumulate in membranes near the phosphate headgroups.

Fig. 26.

Eukaryote cells that include mammals have a nucleus and extensive cytoskeleton protein fiber network that supports the cell membrane. (a) Drawing model emphasizes the nucleus that protects chromosomes and cytoskeleton network that supports the cell membrane. (b) Microscope image includes nucleus with microtubules of the cytoskeleton accentuated by special dyes (with permission from National Institutes of Health/Department of Health and Human Services).