Biological Chemistry | Biomedical Engineering

Triclosan Computational Conformational Chemistry Analysis for Antimicrobial Properties in Polymers

Richard C. Petersen

Departments of Biomedical Engineering, Biomaterials and Restorative Sciences, University of Alabama at Birmingham, Alabama, USA

Triclosan is a diphenyl ether antimicrobial that has been analyzed by computational conformational chemistry for an understanding of Mechanomolecular Theory. Subsequent energy profile analysis combined with easily seen three-dimensional chemistry structure models for the nonpolar molecule Triclosan show how single bond rotations can alternate rapidly at a polar and nonpolar interface. Bond rotations for the center ether oxygen atom of the two aromatic rings then expose or hide nonbonding lone-pair electrons for the oxygen atom depending on the polar nature of the immediate local molecular environment. Rapid bond movements can subsequently produce fluctuations as vibration energy. Consequently, related mechanical molecular movements calculated as energy relationships by forces acting through different bond positions can help improve on current Mechanomolecular Theory. A previous controversy reported as a discrepancy in literature contends for a possible bacterial resistance from Triclosan antimicrobial. However, findings in clinical settings have not reported a single case for Triclosan bacterial resistance in over 40 years that has been documented carefully in government reports. As a result, Triclosan is recommended whenever there is a health benefit consistent with a number of approvals for use of Triclosan in healthcare devices. Since Triclosan is the most researched antimicrobial ever, literature meta analysis with computational chemistry can best describe new molecular conditions that were previously impossible by conventional chemistry methods. Triclosan vibrational energy can now explain the molecular disruption of bacterial membranes. Further, Triclosan mechanomolecular movements help illustrate use in polymer matrix composites as an antimicrobial with two new additive properties as a toughening agent to improve matrix fracture toughness from microcracking and a hydrophobic wetting agent to help incorporate strengthening fibers. Interrelated Mechano-molecular Theory by oxygen atom bond rotations or a nitrogen-type pyramidal inversion can be shown to produce energy at a polar and nonpolar boundary condition to better make clear membrane transport of other molecules, cell recognition/signaling/defense and enzyme molecular "mixing" action. *Journal of Nature and Science, 1(3):e54, 2015.*

Triclosan | antimicrobial | conformational chemistry | bonds | rotation | polar and nonpolar | molecular entanglement

Introduction

Nonbonding lone-pair electrons are stable and do not participate in electron transfer or covalent bond reactions.[1,2] However, nonbonding lone-pair electrons can influence bond positions for exposure in polar/hydrophilic environments while concealing electron polarity in nonpolar/hydrophobic environments.[1-4] The oxygen atom has two sets of nonbonding lone-pair electrons that can interact with the molecular environment.[1,2] The antimicrobial Triclosan is a diphenyl ether with two aromatic rings connected by the ether oxygen atom, Figure 1. Subsequent Triclosan molecular movements are provided by oxygen bond rotations to satisfy energy equilibriums depending on the immediate local polar or nonpolar molecular environments.[5] In fact, protein movements typified as molecular machines described in solid crystals have previously been recognized because of conformation molecular bond rotation alterations from rapid vibratory oscillations seen by X-ray crystallography.[3,6] Further, protein oscillations have been observed with nuclear magnetic resonance corroboration in aqueous media creating even bigger actions and fluctuations.[3,6,7] When molecular environments are at a membrane such as the weak

outer layer of bacteria, polar biologic fluids and nonpolar membrane provide dual uneven conditions for rapid fluctuating Triclosan oxygen bond rotations that can be disruptive to bacteria particularly during cell division.[5] Triclosan has been utilized for more than 40 years as the best examined antimicrobial ever with no dependable proof of danger for resistance to human pathogens after usual bacterial contact.[8,9] Consequently, literature research indicates that Triclosan has several types of properties as a broad-spectrum antimicrobial and particularly influences the cell membrane of bacteria due to hydrophobic or nonpolar interactions that bring Triclosan molecules together inside the cell phospholipid membrane.[10-13]
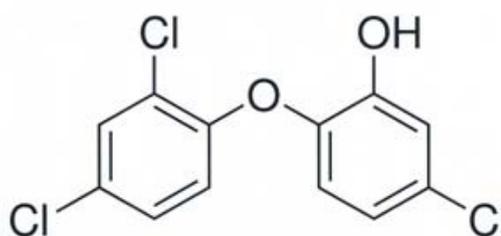

Figure 1. Two dimensional (2D) Triclosan molecular structure. In addition to being a diphenyl ether structure, Triclosan is trichlorinated and has one hydroxyl group in the ortho position.

Computational Chemistry

Triclosan bond rotations of both planar aromatic phenyl groups around the ether oxygen atom are charted from 20° to 90° by Wavefuntion, Inc, Newport Beach, CA according to the relative energies calculated, Figure 2.[5] A three-dimensional (3D) figure of Triclosan is presented to the right at a 50° bond rotation that exposes the oxygen nonbonding lone-pair electrons more than the two-dimensional (2D) structure in Figure 1. A charted energy minimum of about 30° corresponds to the 3D model for Triclosan in Figure 3 that minimally exposes the oxygen lone-pair nonbonding electrons. The yellow arrow shows the dipole directed toward the negative polarity with oxygen atoms for the ether and hydroxyl functional groups. Due to steric hindrance between hydrogen atoms that repulse one another from each phenyl ring below the ether oxygen atom, Triclosan bond rotations were not calculated for less than 20°. Therefore, although the oxygen ether lone pair electrons are more exposed at the 30° oxygen bond rotation rather than at a perfect planar 0.0° rotation, repulsion by two positive hydrogen atoms in mutual proximity undermine the nonpolar Triclosan toward the more stable skewed 30° position.[5] A similar nonplanar skewed Triclosan molecule was predicted earlier from spectrometer measurements with Infrared (IR) and H-nuclear magnetic resonance.[14] Further, the hydroxyl group forms hydrogen bonds between the ether oxygen atom and the chlorine atom in the ortho position.[5,14] One more conformation for Triclosan with the ether oxygen bonds rotated outward is calculated for a higher
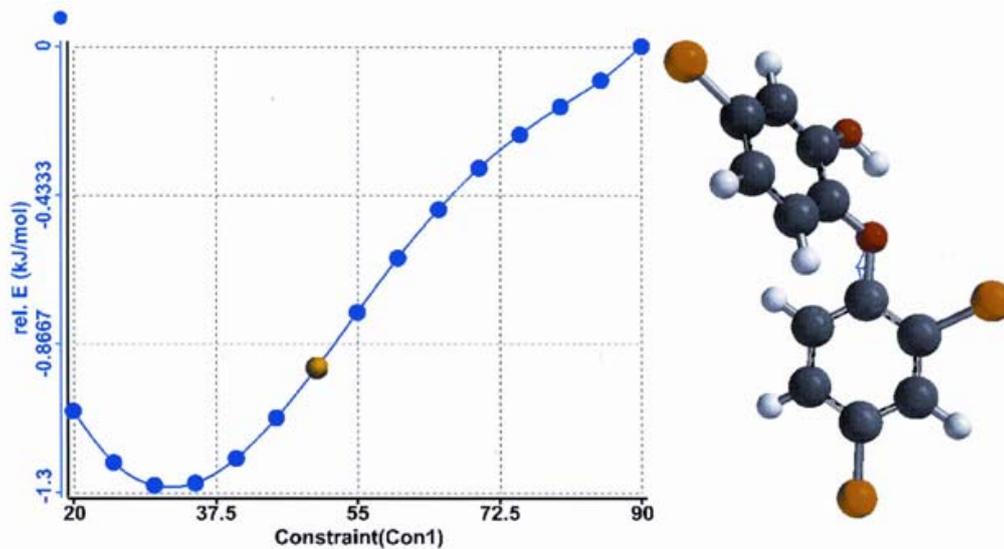

Figure 2. Triclosan energy profile is charted with the oxygen ether bond rotations from 20° to 90° with an energy minimum of about 30°. Triclosan 50° ether oxygen bond rotation to the right.
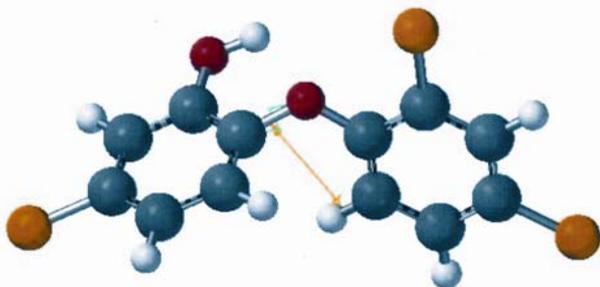

Figure 3. Triclosan ether bond angle of approximately 30° corresponds to the energy minimum in Figure 2 and better concealment of lone-pair electrons for the ether oxygen atom.
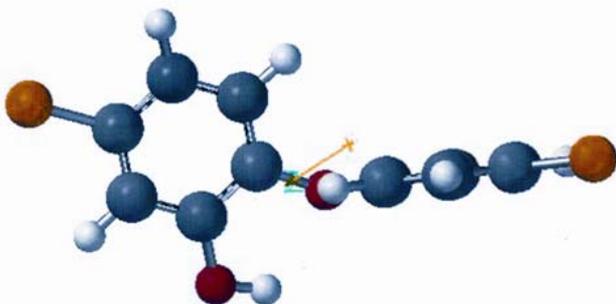

Figure 4. Triclosan ether bond angle of 90° corresponds to the charted energy maximum in Figure 2 to better expose lone-pair electrons for the ether oxygen atom.

energy at 90° in Figure 4 thus maximally exposing the oxygen lone-pair nonbonding electrons. So, the 30° degree bond rotation occurs at a low energy minimum for nonpolar molecular environments and the 90° bond rotation occurs at a high energy maximum for polar molecular environments.[5] Consequently, the polar fluid interface with the nonpolar bacterial membrane is sufficiently unstable for wild fluctuating Triclosan ether oxygen bond rotation movements that can disrupt simple prokaryotic bacteria at several nonspecific cell membrane levels especially during vulnerable cell division.[5]

Triclosan as a Polymer Antimicrobial Additive
Due to potential advantages, nonpolar Triclosan is considered as an antimicrobial additive with polymer Biomaterials by addition into nonpolar thermosetting organic resins of similar polarity corresponding to interactions between the molecular electron distributions. Applicable resins of use include vinyl esters and epoxies and also with acrylic liquid/powder chemical cure formulations. Because of comparable nonpolar relationships between incorporated Triclosan with several of the thermoset and also thermoplastic polymers, antimicrobial release is extremely slow into aqueous solution.[15-18] As a result, long-term antimicrobial defense for polymers is considered a perfect answer to prevent bacterial growth.[15,16,18] In contrast, antibiotics have been used in bone cement to supply just slight protection of a few days at normal doses and only a few months at high concentrations that consequently lower mechanical properties.[19]
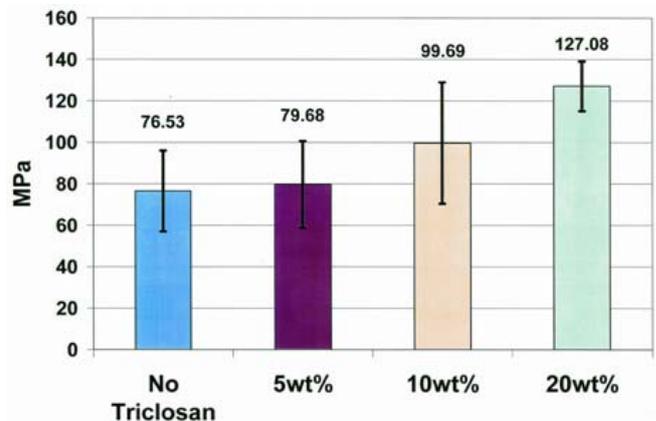

Figure 5. Flexural strengths by adding Triclosan at increasing concentrations to Bis-GMA vinyl ester resin and cured to a polymer.
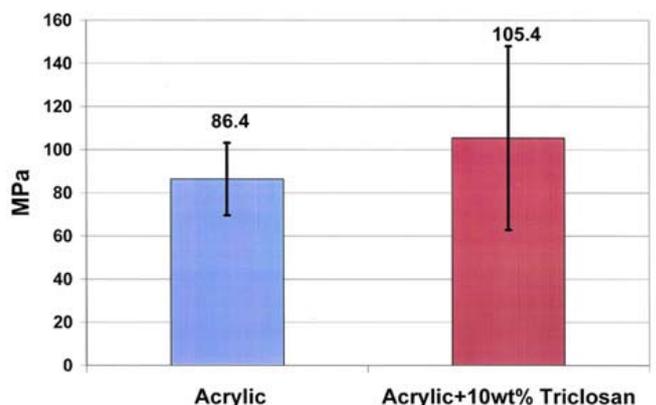

Figure 6. Flexural strength after adding Triclosan to chemical-cured acrylic.
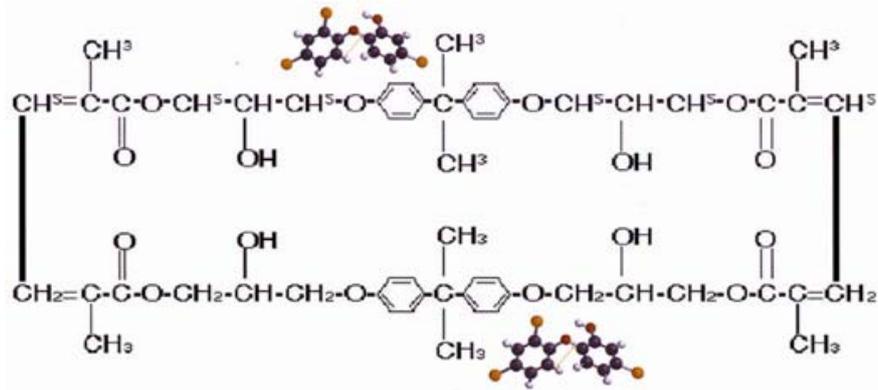
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Figure 7. Representation of a crosslinked Bis-GMA polymer backbone chain with Triclosan for compatibilization bond rotation entanglements. Similar molecular functional groups for both molecules include the two aromatic rings with an ether oxygen atom and also a hydroxyl group located on a ring for Triclosan and nearby for Bis-GMA.

Triclosan as a white crystalline powder has the ability for incorporation into Biomaterial polymers for a vinyl ester Bis-GMA resin system and also a thermoset chemical-cure powder/liquid acrylic.[5] Triclosan was incorporated at increasing concentration levels by adding 0.0 wt%, 5.0 wt%, 10.0 wt% and 20.0 wt% into BisGMA resin with TEGDMA diluent monomer in a 1:1 resin:monomer ratio. Also, an acrylic powder/liquid mixture was incorporated with Triclosan with addition at levels of 0.0 wt% and 10.0 wt%. Cured samples with dimensions of approximately 2 mm deep x 2.5 mm wide x 25 mm long were tested for flexural strength across a 20.0 mm span with a load cell using a National Institute of Standards and Technology (NIST) traceable Omega Force Gauge in three-point bending. Results for the Bis-GMA vinyl ester polymer are presented in Figure 5 and for the acrylic in Figure 6. For both the Bis-GMA vinyl ester and the chemical cure acrylic, Triclosan additions increased the flexural strength. Subsequent increased toughness was inferred from mechanical flexural testing by both cured polymer groups after adding Triclosan that showed increased strength with obvious increased bending.

Toughness increases previously have been noted with polymer blends during compatibilization,[20] when comparing growing polymer molecular weights and chain lengths,[21] and for elastomers.[22] Polymer toughness has been explained due to chain entanglements during compatibilization with polymer blends that may also have higher strength.[20,22] Polymer chain bond rotations can create coiled, kinked and twisted molecular chains for elastomers in the unstressed condition that lengthen out when stressed where crosslinks avert extreme chain slippage.[21] Toughness for rubber and other elastomers has been described with another comparable representation for polymer chains coiled-up that become tight when extended.[22] Prior to yield point during chain straightening when permanent deformation takes place, polymer chains can coil back into the twisted condition after tension is removed.[22] Chain entanglements formed by bond rotations similar to Triclosan molecules would create analogous types of chains joined together loosely with mechanical connections.[5] When stretched, the bonds entangled by Triclosan would tend to unravel to some extent so that polymer coiled chains become taut while lengthened for growing flexural toughness with more bending deflections.[5] Coiled chain arrangements then return as bond entanglements go back to usual steady stability equilibriums when tension is removed prior to yielding.[5] Related strength improvements are often used as an indication that polymers are blending for compatibilization which is occurring between different chain segments with increased adhesion between chains and greater polymer entanglement,[20] noted by all Triclosan groups for increasing flexural strengths. Molecular comparison similarities between Triclosan with addition into the Bis-GMA vinyl ester polymer can be seen in Figure 7 where compatibilization bond rotation entanglements can form during the cure hardened set. The most common reason for failure of polymer matrix composites is by damage accumulation mainly by

microcracking of the polymer matrix from concentrated local limitations of the matrix.[23] As a result, matrix toughening has been recognized as an important objective for reducing microcracking in composites.[23] As a consequence, Triclosan incorporation into polymer matrix composites as a toughening agent with strengthening by bond entanglements is another important goal for an antimicrobial additive.

Triclosan was incorporated into the BisGMA vinyl ester resin and TEGDMA diluent 1:1 system with 84.5 wt% zirconia silicate particulate-filled composite at various low concentrations less than 4.5 wt%.[5] Addition of Triclosan to the 84.5 wt% particulate-filled composite produced noticeable loss of consistency even at minor concentrations that made sample preparation difficult.[5] Consequently, Condensing Index values in MPa describing maximum force/unit packing area were tested, but with 84.5 wt% 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 0.0 wt %, 4.25 wt%, 8.41 wt% and 15.31 wt%, Figure 8. 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, Figure 9. The Triclosan large ether oxygen bond rotations of two aromatic rings require at most only -1.3 kJ/mol, Figure 2, against the continuing instantaneous fluctuating electron lone-pair polarization equilibriums that are difficult to stabilize at a sufficient level without cure-set hardening. So, Triclosan has another polymer additive property as a hydrophobic wetting agent that should help the resin impregnation during the addition of fibers or particulate filler.
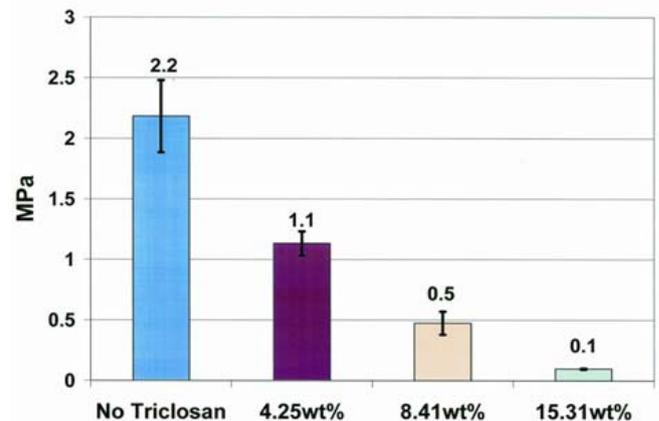

Figure 8. Condensing Index demonstrates increasing loss of paste consistency by adding Triclosan when compressive force is applied.
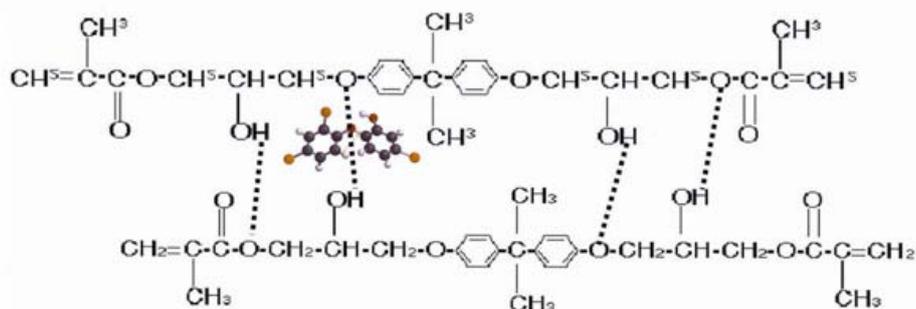
Figure 9. Triclosan ether oxygen atom bond rotations create Mechanomolecular energy to reduce resin viscosity through disruption of hydrogen secondary bonding between the Bis-GMA resin backbone chains that greatly increase the resin viscosity.

Consistency for a 3M Corp. Z100® zirconia-silicate particulate-filled photocure composite is developed to a state-of-the-art with multimodal size particle diameters varying consistently from 10 nanometers to 3.5 micrometers.[24] Consequently, high proportions of particulate at 84.5 wt% or 66 vol% are achieved where smaller particles fit between larger particles to form close particle distances[24,25] for well-controlled van der Waals attraction forces. Such van der Waals forces of attraction amplify exponentially as interparticle distances reduce and are about one-tenth to one-half hydrogen bonds in a characteristic range from approximately 2 kJ/mol to 31 kJ/mol.[22,26] Once more, Triclosan mechanomolecular energy with dual rapid irregular ether bond rotations with force/energy relationships through two aromatic ring moment arms would be predicted to disrupt weak van der Waals forces of attraction between particles to produce a much less viscous paste material that is more flowable. Thus, fluctuating mechanical forces exerted through both planar conjugated aromatic rings not only create intermolecular entanglements during a cure for hardened substances, but also add relatively small energies together during rapid alternating bond rotations to even overcome secondary hydrogen bonding in a resin or weak particulate van der Waals forces in paste.[5]

Triclosan has demonstrated efficacy as an antimicrobial when added into polymers with exceptionally low release into aqueous media.[15-17] Triclosan was described as providing antibacterial properties even when released into water at concentrations far less than 500 times below the minimum inhibition concentration (MIC) for the bacterial strain investigated.[15] Triclosan antibacterial activity at exceedingly low levels was postulated due to inhibition of bacterial growth and less adherence to the polymer surface.[15,17] As a related concern, in order for bacteria to become resistant to a compound, specific bacterial substrates are necessary at typical low antibacterial concentrations whereby a mutation makes the antibiotic ineffective.[8,9,27,28] As a result, the foundation for lack of bacterial resistance to Triclosan over extensive long periods of clinical use is based on general interruption of bacterial cell membranes by numerous molecular actions.[9-11,27] Further, Triclosan has broad-spectrum antimicrobial activity against both gram positive and gram negative organisms at low concentrations.[9,12,13,27,29]

Triclosan Controversy for Bacterial Resistance
Although an explicit method under laboratory circumstances at low Triclosan picomolar concentrations has been linked to lipids by interfering with a protein enzyme as a target,[27,30] clinical significance for bacterial resistance has not been shown in over 40 years of long-standing repetitive use.[8,9,13,29,31] The biochemistry involved shows that the bacterial inhibition by Triclosan with the enzyme is thought to occur by a structuring pi-pi ring-stacking relationship between the aromatic Triclosan phenol ring and an enzyme cofactor nicotinamide adenine dinucleotide (NADH) ring that also has two phosphate groups.[30,32,33] Electron distributions of nonpolar molecules at near molecular distances can instantaneously be induced to form transitory nonuniform small dipole-dipole attraction forces and pack molecules together[1,26] as a reason for the

Triclosan pi-pi ring stacking. Further, NADH is one of the main biological reducing agents as the reduced form of NAD that contributes protons and with NAD+ is the most important carrier for electron transfer during energy production and fatty-acid catabolism.[1,2,34-38] In fact, all fats, carbohydrates and proteins are broken down to liberate hydrogen that is carried by NAD as NADH through a membrane.[36,38] Related to attraction by molecular compatibilization with polymer blending applications,[20] computational electron distribution mapping for ring stacking shows similar comparisons between the molecular structures for Triclosan and NADH.[30,32] Subsequent Triclosan molecules may possibly then form ring stacking with the Bis-GMA resin chain, Figure 7, during cure-set hardening in addition to bond rotation entanglements.

Bacterial Inhibition by Triclosan
To better elucidate on the property for bacterial inhibition, oxygen ether single-bond rotations fluctuating rapidly by a comparatively small Triclosan molecule at a nonpolar phospholipid membrane and polar biologic fluid interface from one excessive polarization level to the next should be thoroughly detrimental especially on frail bacterial cell membranes.[5] Although Triclosan incorporated into a polymer composite did not generate inhibitory zones on agar with the disk diffusion method, greater bacterial inhibition was observed under the Triclosan incorporated polymer disc after removal for a few hours.[15] Also, bacterial growth was reduced in broth solution by immersion of a polymer composite with only 1 wt% Triclosan.[15] Further, bacterial adherence to the identical polymer composite in broth solution was lower with Triclosan incorporation and observed by scanning electron microscopy (SEM)[15] that may be due to disruption of secondary bonding adhesion forces of the bacteria with the polymer. Nonetheless, with bond entanglements to the polymer surface, Triclosan release into broth solution after 24 hours at 0.02 ug/ml was well under the minimum inhibitory concentration (MIC) of 11.7 ug/ml for the *Streptococcus mutans* bacterial strain in the bacterial broth.[15] Subsequent bacterial inhibition without release from the polymer indicated a type of interaction at the bacterial cell membrane level with the surface interface of the polymer incorporated with Triclosan. Secondary bond interruptions by Triclosan mechanomolecular energy is suggested as a possible reason for reduced resin viscosity and especially much lower paste consistency in particulate-filled composites.[5] Corresponding Triclosan mechanomolecular rapid alternating bond rotations related to bacterial membrane disruptions[10] can be considered in the same way as a means to possibly interfere with bacterial adherence to a polymer surface observed with SEM[15] by interfering with weak secondary bonding forces of attraction. Inhibiting bacterial adherence on the polymer composite surface might occur by Triclosan free to move with mechanomolecular vibrational interference disrupting secondary bonding required for substrate binding adhesion. Subsequent adherence of bacteria to a surface is then critical for biofilm structure.[39-41] Through another investigation Triclosan demonstrated inhibitory levels 100 times lower than the MICs in a chemostat bacterial broth with steady
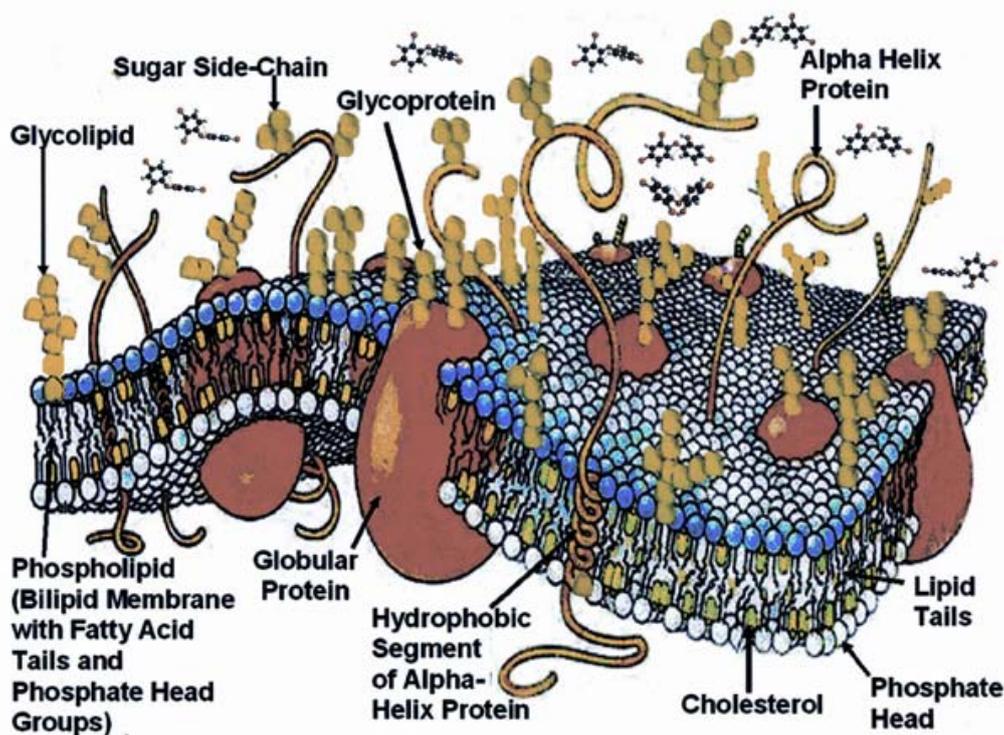
Figure 10. Representation of a mammalian or eukaryotic cell membrane.

stirring that is a sign of actively dividing cells more susceptible to antimicrobial action.[42] In general for the same investigation at low concentrations of Triclosan, binding to the bacterial membrane was described as a method for the antimicrobial to increase membrane fluidity and enhance molecular transport through the membrane.[42] As a result, many different contrasting membrane altering properties can be considered for Triclosan antimicrobial inhibition due to enhancing general fluidity that includes interrupting intermolecular forces for adherence to polymer surfaces and also through interfering with secondary bonding between lipid chains and other structural molecular entanglements.[5] Further, increased 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 increase antibacterial activity as hard defects that interfere with vital molecular movements. As a result, Triclosan antibacterial properties may include many diverse complex interactions particularly important during rapid bacterial cell division.[5]

Mechanomolecular Energy at a Membrane and for Enzymes
The boundary conditions for polar biologic fluids and a nonpolar membrane provide differences in electron distributions that necessitate nonbonding lone-pair electrons seek stability as open conformations for hydrophilic aqueous relationships and closed conformations for hydrophobic lipid hydrocarbon-type interactions. As a result, Mechanomolecular energy at the cell membrane border with biologic fluids is needed for molecular stabilization of lone-pair electrons that create unrestrained alternating vibratory rotations in oxygen single-bonds and also more pronounced nitrogen pyramidal inversions for proteins and other nitrogen-containing amine molecules. Amines are organic molecules that are sp^3 hybridized with a nitrogen atom containing tetrahedral bond angles of 108° and one set of lone pair electrons.[1,2] Consequently, the nonbonding lone pair electrons need stability through pyramidal bond inversions to comply with polarity compatibilization relationships for the immediate near molecular environment.[5] Nitrogen pyramidal inversions are so rapid that the two bond positions cannot be resolved at room temperature.[1,2] Subsequent free mechanomolecular energy is then offered at the membrane interface with glycoside oxygen bond linkages and

peptide nitrogen amide linkages in resonance stabilization as an amine to supply vibratory movements for membrane transport of other molecules.[5] In addition mechanomolecular energy can provide the needed alternating vibration interactions for cell signaling/recognition/defense without a large outflow of energy produced by the cell during metabolism.[5] Further, within the globular enzyme proteins, mechanomolecular energy can direct molecules with mixing action to lower reactions times and overcome complicated thermodynamic energy barriers.[5]

The eukaryote phospholipid plasma cell membrane is represented in Figure 10 illustrating the inner lipid tails with the outer phosphate head groups that form the interface with the inner intracellular and outer extracellular biological fluids. Triclosan and another trichlorinated antimicrobial, Triclocarban, are thought to concentrate in the phospholipid membrane close to the phosphate head groups.[10,43] Oxygen atoms connect proteins with sugar side chains as glycoproteins and can bond to lipids as glycolipids especially on the extracellular side of a cell membrane.[36,38]. Consequently, many mechanomolecular intricate movements are possible involving the polysaccharide sugar oxygen atoms with single-bond rotations along with nitrogen inversions in resonance with the peptide bond linkages of a protein.[5] Another unique trait of the mammalian eukaryote cell phospholipid membrane not evident in bacterial prokaryote cell membranes are cholesterol molecules[34,37] that provide protective benefits including maintaining membrane fluidity[36] and supplying osmotic stability.[34] As a result, structuring by Triclosan with oxygen rotation bond entanglements, hydrogen bonding and aromatic pi-pi ring stacking might accentuate into a bacterial membrane to produce a critical crystalline-type flaw that interferes with flexibility especially needed for the rapid cell division by bacteria.[5] Therefore, in addition to Triclosan molecular perturbations with fluctuating bond rotation mechanomolecular movements at a weaker bacterial membrane, alternative Triclosan structuring would appear to be another mode of action that could produce a decisive hard defect in susceptible bacterial membranes and also enzymes with similarities for inactivation of the cofactor NADH.

Conclusions
Triclosan mechanomolecular energy through the oxygen ether atom bond rotation is sufficiently active as a broad-spectrum

antimicrobial by vibratory fluctuating movements to disrupt normal bacterial membranes. Further, Triclosan can pack into crystalline form by ring stacking to interfere with enzymes and the NADH cofactor at extremely low concentrations. Because Triclosan has numerous antibacterial actions bacterial resistance by mutation is not a possibility under normal conditions. Mechanomolecular Theory can further be considered for free energy to provide membrane transport of molecules and cell signaling/recognition/defense. Further, mechanomolecular energy can be used to describe mixing action by enzymes to bring reactants together and speed reaction rates.

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