The relative toxicity of compounds used as preservatives in vaccines and biologics

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Summary

Background: In vaccines/biologics, preservatives are used to prevent microbial growth.

Material/Methods: The present study examined: (1) the comparative toxicities of commonly used preservatives in US licensed vaccines to human neurons; and (2) the relative toxicity index of these compounds to human neurons in comparison to bacterial cells.

Results: Using human neuroblastoma cells, the relative cytotoxicity of the levels of the compounds commonly used as preservative in US licensed vaccines was found to be phenol < 2-phenoxyethanol < benzethonium chloride < Thimerosal. The observed relative toxicity indices (human neuroblastoma cells/bacterial cells) were 2-phenoxyethanol (4.6-fold) < phenol (12.2-fold) < Thimerosal (>330-fold). In addition, for the compounds tested, except for 2-phenoxyethanol, the concentrations necessary to induce significant killing of bacterial cells were significantly higher than those routinely present in US licensed vaccine/biological preparations.

Conclusions: None of the compounds commonly used as preservatives in US licensed vaccine/biological preparations can be considered an ideal preservative, and their ability to fully comply with the requirements of the US Code of Federal Regulations (CFR) for preservatives is in doubt. Future formulations of US licensed vaccines/biologics should be produced in aseptic manufacturing plants as single dose preparations, eliminating the need for preservatives and an unnecessary risk to patients.

Key words: antimicrobial activity • bacterial contamination • preservatives • toxicity

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Background

For drugs and foods, preservatives are defined as compounds added to kill or to prevent the growth of microorganisms, particularly bacteria and fungi. They are added to vaccine/biologic formulations to prevent microbial growth in the event that the vaccine/biologic is accidentally contaminated, as might occur with repeated puncture of multi-dose vials. In some cases, preservatives are added during manufacture to prevent microbial growth. However, with changes in manufacturing technology in the United States and other developed countries, the need to add preservatives during the manufacturing process has decreased markedly with the introduction of modern, aseptic manufacturing facilities [1].

The US Code of Federal Regulations (CFR) requires, in general, the addition of a preservative to multi-dose vials of vaccines. Indeed, worldwide, preservatives are routinely added to multi-dose vials of vaccine. Historically, tragic consequences, most from the failure to prevent needle contamination between dose withdrawals, have occasionally followed the use of multi-dose vials that did not contain a preservative. These incidents served as the impetus for this requirement [1].

The US requirement for preservatives in multi-dose vaccine was incorporated into the CFR in January 1968 and codified in 1973, although many biological products already contained preservatives prior to 1968. Specifically, the CFR states: “products in multiple-dose containers shall contain a preservative, except that a preservative need not be added to Yellow Fever Vaccine; Poliovirus Vaccine Live Oral; viral vaccines labeled for use with the jet injector; dried vaccines when the accompanying diluent contains a preservative; or to an Allergenic Product in 50 percent or more volume in volume (v/v) glycerin” [21 CFR 610.15(a)] [1]. The CFR also requires “any preservative used shall be sufficiently non-toxic so that the amount present in the recommended dose of the product will not be toxic to the recipient, and in combination used shall not denature the specific substance in the product to result in a decrease below the minimal acceptable potency within the dating period when stored at the recommended temperature” [21 CFR 610.15(a)] [1].

The US Food and Drug Administration (FDA) acknowledges that preservatives do not completely eliminate the risk of contamination of vaccines. The literature contains several reports of bacterial contamination of preserved multi-dose vaccines, emphasizing the need to adhere to aseptic withdrawal techniques in withdrawing vaccine doses from multi-dose vials [2,3]. The US FDA has approved several compounds for use as preservatives in US licensed vaccines, including Thimerosal, phenol, benzethonium chloride, and 2-phenoxyethanol [1]. It is important to note that the US FDA does not license a particular compound for use as a preservative; rather, the product containing that preservative is licensed, with safety and efficacy data generally collected by the manufacturer in the context of a license application for a particular product, in compliance with the applicable drug regulations [1].

A number of previous studies have evaluated compounds used as preservatives in vaccines [4–6]. These studies have raised serious questions as to the safety and potential effectiveness of many compounds commonly used as preservatives in vaccines. As a result, the purpose of the present study was to extend previous research by evaluating: the differences in toxicity of compounds that are commonly used as preservatives in US licensed vaccines to human neurons; and the relative toxicity index of compounds that are commonly used as preservatives in US licensed vaccines to human neurons (human neuroblastoma cells), as these are principle sensitive target cells in the human body, in comparison to bacterial cells (Escherichia coli), as these are a common contaminating bacteria in clinical settings. The importance of these measurements being that ideal compounds for use as preservatives in US licensed vaccines should be relatively non-toxic to human neurons and significantly more toxic to bacterial cells than human cells.

Material and Methods

Human cell cultures

Cultures of SH-SY5Y human neuroblastoma cells from the European Collection of Cell Cultures were purchased from Sigma-Aldrich (St. Louis, MO, USA). The neuroblastoma cells were grown in culture medium that consisted of Dulbecco’s Modified Eagle’s Medium/Ham’s F12, 50/50 1X with L-glutamine (MEM-F12) (Mediatech, Inc., Manassas, VA, USA), 15% fetal bovine serum (FBS) sterile filtered (Equitech-Bio, Inc., Kerrville, TX, USA), and 1% MEM non-essential amino acid (MEM NEAA) solution 100X (Sigma-Aldrich). The cells were grown following a standardized procedure at 37°C, 95% humidity, and 5% CO₂ in 40 mL tissue culture (Nunc™ delta surface) flasks (NUNC™, Rochester, NY, USA). Cells were grown in flasks until nearly confluent and then were trypsinized (Trypsin, INTERGEN® Company, Purchase, NY, USA). The disaggregated cells were seeded evenly into COSTAR® (Corning International, Corning, NY, USA) 96-well [100 mL well⁻¹], cell-culture-cluster, flat-bottom, tissue-culture, treated plates with lids. Prior to treatment with the compounds under study, the cell aliquots seeded in each well were grown following a standardized procedure for at least one day at 37°C, 95% humidity, and 5% CO₂ in the 96-well cell culture plates with appropriate MEM-F12 media and 15% FBS [7].

Bacteria cell cultures

Bacterial cell cultures of E. coli strain N99 were obtained from a stock collection from the National Institute of Health (Bethesda, MD, USA). The bacterial cells were grown to mid-log phase on Nutrient Broth Number 3 (NB3) (Sigma-Aldrich Chemie GmbH, Steinheim, Spain) in sterile test tubes with moderate shaking within an incubator at 37°C. The test tubes with contents were then centrifuged at 5,000 rpm for 10 min. The supernatant was discarded and the pellet was then resuspended in MEM-F12 media, and became the bacterial stock preparation used in the present study. The resultant suspension was tilted by dilution and plating on NB3 agar plates to determine the colony forming units per mL (cfu). It was determined that the suspension contained 1.7×10⁸ cfu/mL.

Compounds

Thimerosal (C₁₂H₁₀HgO₄SNa, CAS No. 54-64-8), phenol (C₆H₅O, CAS No. 108-95-2), 2-phenoxyethanol (C₈H₁₀O₂, CAS No. 122-99-6), and benzethonium chloride
(C_{27}H_{42}ClNO_{2}, CAS No. 121-54-0) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The compounds tested in the present study were highly purified, and were presumed to be >95% pure. Stock solutions were prepared for each compound by dissolving them into or appropriately diluting them with MEM-x culture medium, and the resultant solutions were sterilized by filtration through a pre-sterilized 0.20 mm NALGENE® Filter Unit (Nalge Nunc International, Rochester, NY, USA). The stock solutions prepared and utilized in the present study were freshly prepared for each compound tested.

**Determination of cytotoxicity**

Compound-induced cytotoxicity in human neuroblastoma cells was assessed using the colorimetric 2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) cell assay kit (TOX-2, Sigma-Aldrich). After at least 24 h of growth, the original media was removed from each well in the 96-well cell-culture-plates, and replaced with 100 µL well-1 dilutions of Thimerosal (1 µM–10 µM), phenol (100 µM-10mM), 2-phenoxysyethanol (128 µM–3.2 mM), and benzethonium chloride (11 µM–110 µM) in culture medium that consisted of MEM-F12. For the control wells containing no compound, the same procedure was followed without adding 100 µL of the appropriate cell media was added without adding any compound under study was added. The resultant 96-well cell-culture plates were covered and incubated following a standardized procedure for 24 h at 37 °C, 95% humidity, 5% CO₂, and continuous shaking at 60–80 rpm. The media was then removed from each well, and 50 µL of XTT solution (20% concentration, dissolved in appropriate cell media) were added to each well. The 96-well cell-culture plates were transferred to a VERSAspin tunable microplate reader (Molecular Devices, Sunnyvale, CA, USA) for assaying. The 96-well cell-culture plates were maintained at 37°C and were shaken for 5 s every 15 min. The contents of the study wells in the 96-well culture plates were continuously assayed every 15 min for absorption at 450 nm and 690 nm using Softmax® Pro 5 software until the control wells (without exposure to dilutions) continuously read an absorbance of at least 0.20 (obtained by subtracting the 690 nm absorbance value from the 450 nm absorbance value). The aforementioned procedure was repeated twice for each compound tested. The net values determined for each compound dilution examined (pooled from the two separate 96-well culture plates) were normalized to the average value for the controls, which was set at 100%. The mean results and their uncertainties (SEM) were expressed in terms of percentage control mean:

\[
\frac{([\text{mean}_{\text{test}} \pm \text{SEM}_{\text{test}}])}{[\text{mean}_{\text{control}}]} \times 100\%
\]

**Statistics**

The statistical packages contained in StatsDirect Version 2.7.2 (Cheshire, UK) and SigmaPlot Version 9.0 (San Jose, CA, USA) were used in the present study. Dunnett’s one-way analysis of variance (ANOVA) for multiple comparisons with a control test statistic was used, and a p-value < 0.05 was considered statistically significant. Additionally, where possible, the LC_{50} were determined for cytotoxicity for the different cell types and compounds tested in the present study. The linear regression test statistic from StatsDirect was utilized to examine the linear portion of the curves derived for the assay response curves developed for cytotoxicity for the different cell types and compounds tested to determine the LC_{50}. The LC_{50} derived for the different cell types and compounds tested were used to determine the relative toxicity index of each compound tested for human neuroblastoma cells in comparison to bacterial cells:

**Results**

Figure 1 evaluates cytotoxicity induced by the compounds examined in the present study to human neuroblastoma cells following 24 hr incubation. Overall, the LC_{50} were: phenol (6.38 mM) < 2-phenoxysyethanol (1.47 mM) < benzethonium however, the Thimerosal concentrations studied were not high enough to induce a lethal concentration at which 50% death is produced (LC_{50}) (note: higher concentrations of Thimerosal were not prepared due to safety concerns about the potential toxicity of Thimerosal to lab personnel). Benzethonium chloride was examined (final well concentration up to 110 µM) but no significant reduction in bacterial cell count was observed at the concentrations examined, and higher concentrations could not be evaluated for their relative toxicity to bacterial cells because the viscosity of benzethonium chloride caused effects that interfered with absorbance measurements. The 96-well cell-culture-plates were covered and incubated following a standardized procedure for 20 min at 37°C, 95% humidity, 5% CO₂, and continuous shaking at 60–80 rpm. Subsequently, 20 µL of XTT solution (at 100% concentration) were added to each well (final concentration = -20%). The 96-well cell-culture plates were maintained at 37°C and were shaken for 5 s every 15 min. The contents of the study wells in the 96-well culture plates were continuously assayed every 15 min for absorption at 450 nm and 690 nm using Softmax® Pro 5 software until the control wells (without exposure to dilutions) continuously read an absorbance of at least 0.20 (obtained by subtracting the 690 nm absorbance value from the 450 nm absorbance value). The aforementioned procedure was repeated twice for each compound tested. The net values determined for each compound dilution examined (pooled from the two separate 96-well culture plates) were normalized to the average value for the controls, which was set at 100%. The mean results and their uncertainties (SEM) were expressed in terms of percentage control mean:

\[
\frac{([\text{mean}_{\text{test}} \pm \text{SEM}_{\text{test}}])}{[\text{mean}_{\text{control}}]} \times 100\%
\]
chloride (86 µM) < Thimerosal = (7.41 µM). Figure 2 evaluates the cytotoxicity induced by the compounds examined in the present study to bacterial cells following 20 min incubation. Overall, it was observed that the LC_{50}s were 2-phenoxyethanol = 6.7 mM, Thimerosal > 2.5 mM, and phenol = 78 mM.

Table 1 shows an assessment of the relative toxicity of the compounds examined in the present study to human neuroblastoma cells in comparison to bacterial cells. Overall, the relative toxicity index values were: 2-phenoxyethanol (4.6) < phenol (12.2) < Thimerosal (>330).

**DISCUSSION**

The present study was specifically designed to evaluate the relative toxicities of compounds commonly used as preservatives in US licensed vaccines to human neurons and bacterial cells. Among the compounds tested, the relative cytotoxicity of the compounds commonly used as a preservative in US licensed vaccines to human neuroblastoma cells were: phenol < 2-phenoxyethanol < benzethonium chloride < Thimerosal. Where such values could be computed, the overall relative toxicity indices (bacterial cell (E. coli) LC_{50} at 20 minutes/human neuroblastoma cell LC_{50} at 24 hours) of the compounds commonly used as preservatives in US licensed vaccines were: 2-phenoxyethanol < phenol < Thimerosal.

Further, the results of the present study allowed for a determination of the potential relative effectiveness against bacterial contamination of the concentrations of compounds commonly used as preservatives in vaccines/biologics. The results of the present study showed that Thimerosal at a concentration of 1 mg/mL (0.1%), which is ten-fold greater level than that routinely present in vaccines/biologics drug products at a concentration of 100 µg/mL (0.01%), was unable to significantly kill bacterial cells within 20 min. Similarly, the present study showed that phenol at concentration of 7.3 mg/mL (0.73%), which is about 3-fold greater than that routinely present in vaccines/biologics, at a concentration of 2.5 mg/mL (0.25%), significantly killed bacterial cells within 20 min. In contrast, the present study revealed that 2-phenoxyethanol at concentration of 0.93 mg/mL (0.09%), which is about 5-fold lower than the 2-phenoxyethanol concentration of 5 mg/mL (0.5%) routinely used in vaccines/biologics, significantly killed bacterial cells within 20 min.

**Historical survey of preservatives and their published relative toxicities**

The results of the present study regarding the relative toxicity of the compounds tested appear to be similar to previously observed results published as early as the 1930s [8]. For example, investigators compared the resistance of bacteria chlorides to different conditions.
and embryonic tissue to germicidal substances [9]. These investigators observed that Thimerosal was 35.3-times more toxic to embryonic cells than *Staphylococcus aureus* bacterial cells. In addition, these investigators observed that the relative toxicity of Thimerosal to embryonic cells in comparison to bacterial cells was significantly worse than that observed for phenol. Further, these investigators found that Thimerosal was 210-times more toxic to embryonic cells than phenol, a result consistent in magnitude with that observed in the present study, where Thimerosal was found to be 861-times more toxic to human neuroblastoma cells than phenol. Similar results were observed in a series of subsequent studies by investigators from the US FDA [10,11]. It was observed regarding the toxicity of various germicides to guinea pig leukocytes, that Thimerosal was the most toxic among a series of other germicides including: tincture of iodine, hexylresorcinol, potassium mercuric iodide, mercuric chloride, metaphen tincture, phenol, and mercurochrome [10]. Further, another study reported that when comparing the toxicity of various germicides to human leukocytes in comparison to *Staphylococcus*, Thimerosal was the second most toxic germicide tested (out of ten germicides) [11].

Investigators reported on the bacteriostatic and bactericidal actions of some mercurial compounds on hemolytic *Streptococci* [12]. These investigators observed that solutions of mercurochrome, metaphen and Thimerosal failed to kill all the cells in cultures of hemolytic *Streptococci* and described Thimerosal as significantly more toxic to cells it was supposed to protect than to bacterial cells.

In a subsequent study comparing the toxicity of mercurial antiseptics to human cells, investigators observed that Thimerosal was the most toxic among the compounds tested including: mercurochrome, phenymercuric nitrate, metaphen, bichloride of mercury, mercuriolide, and mercuric cyanide [13]. Further, it was reported that the use of mercurials as preservatives in vaccines and antisera is of considerable interest. It described that these chemicals are added to protect against the introduction of organisms in multi-use containers in particular and, therefore, wondered about their efficacy in actual use. The experimental results showed mercurial preservatives in vaccines and antisera

Table 1. An assessment of the relative toxicities of the compounds study in human neuroblastoma cells in comparison to bacteria.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human cells (LC50)*</th>
<th>Bacterial cells (LC50)*</th>
<th>Relative Toxicity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Phenoxyethanol</td>
<td>1.47 µM</td>
<td>6.7 mM</td>
<td>4.6</td>
</tr>
<tr>
<td>Thimerosal</td>
<td>7.41 µM</td>
<td>&gt;2.5 mM</td>
<td>&gt;330.0</td>
</tr>
<tr>
<td>Phenol</td>
<td>6.38 mM</td>
<td>78 mM</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Relative Toxicity Index = bacterial cell (*E. coli*) LC50 at 20 minutes/human cell (neuroblastoma) LC50 at 24 hours; *Cytotoxicity was measured using the XTT cell assay (following unexposed controls reaching 0.20 nm absorbance).
were negligible after 3 months of storage and an examination of a series of over one thousand bottles of various biologic/vaccine preparations from clinics obtained after use revealed that up to five percent contained viable microorganisms, suggesting that, once these biologics are in the hands of users, the preservative used was not necessarily effective at preventing microorganism contamination [15].

Investigators also described the antimicrobial effectiveness of some preservatives in inactivated human vaccines by application of the "preservative effectiveness" test described in the United States Pharmacopeia (USP) XIX [4]. Five recommended strains as well as three strains isolated from vaccines were used as test strains. Products with hyamine, phenol, Thimerosal, and 2-phenoxyethanol-formaline were investigated. Only phenol met the requirements of the USP XIX test satisfactorily.

The results observed in the present study showing that the compounds tested do not have rapid antimicrobial effects are supported by a number of previous studies. For example, investigators undertook a study to evaluate prooperative sterilization of the perineum by six different antiseptic compounds [14]. These investigators then observed the following reduction in bacterial population following application to their patients: Dettol [alcoholic] (100%), Hibiscrub [concentrated] (98%), Hibiscrub [diluted] (82%), Dettol [aqueous] (77%), Hibitane (68%), Cetavlon (63%), Disadine (60%), Resiguard (54%), Thimerosal [0.1% concentration] (28%), and water, used as a control (24%). In addition, investigators from the US Centers for Disease Control and Prevention (CDC) examined the effectiveness of Thimerosal used as preservative in diphtheria-tetanus-pertussis (DTP) vaccines to kill bacterial contaminants [5]. These investigators reported that preservatives in multi-dose vaccine vials do not prevent short-term bacterial contamination, and that the only feasible and cost-effective preventive measure now available is careful attention to sterile technique when administering vaccine doses from multi-dose vials.

Also, it is important to consider the ability of bacterial species to develop resistance to various antiseptics used as preservatives in vaccine/biological preparations. It is well established that bacterial species tend to easily develop resistance to Thimerosal, and the biological mechanisms for bacterial species resistance to Thimerosal have been elucidated [15,16]. For example, investigators isolated strains of Pseudomonas cepacia from packages of nasal spray preserved with Thimerosal that showed a high degree of resistance to Thimerosal [17]. The isolates of P. cepacia obtained were shown to degrade Thimerosal to metallic mercury, which volatized from the product or assay medium. These investigators then conducted a series of experiments showing the relative ease with which Thimerosal-resistant strains of P. cepacia could be selected for among unadapted cells. In contrast, bacterial species resistance to other antiseptic compounds tested seems to be much more limited, especially, in the case of 2-phenoxyethanol.

Finally, when evaluating the toxicities of the compounds studied following administration of vaccines/biologics to recipients, it is important to evaluate their respective kinetics and toxicity. Thimerosal is known to dissociate into ethylmercury hydroxide or ethylmercury chloride [18], and Thimerosal-preserved drugs are known to contribute to the long-term accumulation of mercury body-burden [19]. Thimerosal can induce potentially toxic levels of mercury in human tissues including the brain [20], and studies have observed persistent mercury residues in the brain for more than 120 days following the last injection of a Thimerosal-preserved vaccine to infant monkeys [21].

In contrast, the other compounds tested in the present study such as phenol, 2-phenoxyethanol, and benzethonium chloride are fairly rapidly broken down and excreted from the human body. For example, investigators evaluated the distribution of 2% 2-phenoxyethanol antiseptics applied to newborn infants [22]. These investigators observed that the urinary concentration of 2-phenoxyethanol was <2 ppm in all samples, while urinary 2-phenoxyacetic acid concentrations reached 5-95 ppm. These investigators concluded that 2-phenoxyethanol undergoes extensive oxidative metabolism to 2-phenoxyacetic acid in the human body. As another example, investigators evaluated the excretion of phenol metabolites following administration of phenol [23]. These investigators observed following administration of phenol that there was a fairly rapid significant increase in the urinary excretion of phenol sulfate, phenol glucuronide, and hydroquinone glucuronide.

Furthermore, in comparative studies of the toxicities of the compounds examined in the present study to in human clinical trials, investigators reported on the rate of local and systemic reactions in a randomized double-blind trial where Thimerosal-preserved saline (0.01% Thimerosal) was compared to phenol-preserved saline (0.4% phenol) [24]. Overall, 331 volunteers received Thimerosal-preserved saline during the study and 41 (12.4%) had reactions; in contrast, 326 volunteers received phenol-preserved saline during the study and only 4 (1.2%) had reactions. These investigators concluded that considering the high frequency of hypersensitivity reactions to Thimerosal (i.e. at a rate more than 10-fold higher than those receiving phenol-preserved saline) in their study population, Thimerosal should be replaced as a preservative.

**Strengths/limitations**

In considering the procedure developed to test the relative toxicities of compounds commonly used as a preservative in US licensed vaccines in comparison to previous studies, conditions were developed to be as realistic as possible to an actual in vivo setting. Namely, human neuroblastoma cells were incubated for 24 hrs with exposure to the compound and bacterial cells were incubated for 20 min with exposure to the compound. These time periods were chosen, so that for each cell type, about one cell replication cycle was examined for cytotoxicity. Further, the 20 min incubation for bacterial cells for the compounds tested was also chosen because this is a realistic time period for a preservative to show its antimicrobial effectiveness in an in vivo setting in a healthcare provider’s facility. Namely, if significant bacterial contamination was to occur in a multi-dose vial of a vaccine/biologic, the contaminated product would likely be given to the next recipient within minutes to hours after its contamination. Thus, any true antimicrobial used as a preservative in a vaccine/biologic should have kill a significant number of bacterial cells prior to the next administration.
Further, other variables such as media or serum concentrations were minimized, since both cell types were exposed to compounds in the same type of media without serum. Finally, the assay used to measure cytotoxicity was held constant between the two cell types examined.

The present study also had the limitation that only one type of human and bacterial cells were used. As a result, it is possible that other types of cells may yield different results than those obtained in the present study. The consistency of the results obtained in the present study with previous observations argues that the present results are genuine and not the result of particularly unusual cell types.

**CONCLUSIONS**

The present study was specifically designed to evaluate the relative toxicities of compounds commonly used as preservatives in US licensed vaccines, to human neurons and bacterial cells. Overall, none of the compounds commonly used as preservatives can be considered ideal preservatives. They were all found to be significantly toxic to human neurons, and worse they were all found to be significantly more toxic to human neurons than bacterial cells. In addition, for all compounds used as preservatives in vaccines/biologics, except 2-phenoxyethanol, the concentrations necessary to induce significant killing of bacterial cells were significantly higher than those routinely present in vaccine/biological preparations. It is possible that other results may be observed with different human and bacterial cell types. Despite this possibility, it is doubtful that any of the compounds commonly used as preservatives in US licensed vaccines/biologics would comply with the CFR requirements for preservatives. The results of the present study indicate that future formulations of vaccines/biologics should be produced in aseptic manufacturing plants as single dose preparations, eliminating the need for preservatives and minimizing the risk to patients.

**Acknowledgement**

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**Conflict of interest**

Potential Conflict of Interest: None of the authors owns a financial interest in any antimicrobial products. David Geier and Dr. Mark Geier have been involved in vaccine/biological litigation.

**REFERENCES:**

13. Engley FB: Mercurials as disinfectants: Evaluation of mercurial antimicrobial action and comparative toxicity for skin tissue cells. Chicago: 42nd Mid Year Meeting of the Chemical Specialties Manufacturer’s Association, 1956