Quantification of Alexidine Loss in ReNu Plastic Bottles and the Worldwide Fusarium Keratitis Event of 2004 - 2006

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Abstract

Purpose: The purpose of the present study was to define further the cause of the ReNu with MoistureLoc (RML)-related worldwide Fusarium keratitis event of 2004 - 2006 by:

1) making serial measurements of alexidine concentrations in heated and unheated solutions stored in ReNu bottles; and (2) comparing those results with our prior microbiological inhibitory titers.

Methods: (1) Alexidine solutions, 0.00045% (4.5 parts per million [PPM]) were stored in two identical 60-mL ReNu bottles: one at room temperature (RT, 23°C) and the other at 56°C, for 4 weeks. Serial triplicate measurements of alexidine concentrations were made via liquid chromatography-mass spectroscopy (LC/MS). (2) Previously measured inhibitory titers of alexidine solutions against seven Fusarium organisms were compared with LC/MS measurements of alexidine concentrations.

Results: (1) After 7 days of ReNu bottle storage at 56°C, the alexidine concentration decreased from 4.5 to 0.2 PPM (a 95.6% loss), versus a decrease to 3.2 PPM (a 28.9% loss) when stored at RT (P = 0.000012). The ratio of alexidine solution loss at one week in heated: unheated ReNu bottles was 3.3 and at 4 weeks was 3.2. (2) Inhibitory titers of 1:8 corresponded to an alexidine concentration of 0.4 PPM, which allowed Fusarium growth.

Conclusions: (1) Alexidine loss occurs about three times more in heated than unheated ReNu bottles, with approximately 96% loss by seven days. (2) Since alexidine concentrations ≥ 0.4 - < 0.8 PPM allow Fusarium growth, alexidine loss was likely critical in the disinfection failure of the RML solution and subsequent multi-focal worldwide outbreaks of Fusarium keratitis in 2004 - 2006.

Keywords: Alexidine Loss; ReNu Plastic Bottles; Fusarium Keratitis

Introduction

Between 2004 - 2006, multi-focal worldwide outbreaks of contact lens-related Fusarium keratitis occurred, associated with Bausch and Lomb’s (B and L, Rochester, NY) ReNu with MoistureLoc Multi-Purpose contact lens cleaning solution (RML) containing the bis-biguaniide antimicrobial agent alexidine dihydrochloride (PubChem CID 102678: 1-[N’-[6-[[amino-[[N’-(2-ethylhexyl) carbamimidoyl]amino]methylidene]amino][hexyl] carbamimidoyl]-2-(2-ethylhexyl)guanidine; dihydrochloride [C26H56N10∙2HCl], 0.00045%), an ingredient new to the contact lens solution market at that time [1]. These outbreaks resulted in hundreds of serious cases in Hong Kong [2], Singapore [3], the United States [1,4-6], and the French West Indies [7], with many resulting in permanent blindness. B and L traced these cases to their formulation facility in Greenville, SC [8].
While the Food and Drug Administration (FDA) had recommended “a maximum temperature of 45°C to establish shelf-life,” [9] B and L had stored RML in non-temperature-monitored warehouses [10] (where temperatures may attain 75°C [167°F] [11]) even though the ReNu bottle label stated: “Store at room temperature” [12]. After inspections by the FDA, B and L was cited for improper storage/transport temperatures of RML [10]. They were also cited for numerous other inadequacies including, but not limited to, not having “a test method to evaluate the degradation of alexidine in the RML solution;” failing to perform a “biocidal efficacy study that demonstrates efficacy against clinically significant microorganisms” before bringing RML to the market; failing to notify the FDA about “35 serious (cases) of Fusarium keratitis from Singapore’s Minister of Health;” failing “to report the removal of RML from the market(s) in Singapore and Hong Kong;” and, failing to perform sterility or biocidal testing on product lots or samples of RML implicated in the Hong Kong and Singapore outbreaks [10]. RML was ultimately withdrawn from the world market on May 15, 2006 [13].

The Centers for Disease Control and Prevention (CDC) found no evidence of intrinsic product contamination, non-clustering in time of implicated lots, and multilocus genotyping of recovered organisms [1], and suggested “disinfection failure” as the cause of this event [14]. The CDC also postulated “unique properties of the MoistureLoc formula” and “biofilm formation” as possible explanations [1]. If these explanations were indeed complete and correct, and since Fusarium species are distributed worldwide, then cases of Fusarium keratitis would have been epidemiologically traced to all of the B and L RML production sites, including those in: the U.S. (Greenville, SC, supplying Hong Kong, Singapore, the U.S., and the French West Indies); Milan, Italy (supplying Europe); Beijing, China (but not supplying Hong Kong and Singapore); and Bhiwadi, India [12,15,16]. B and L investigators, however (as stated above), determined that all cases appeared to be related to the RML made only in their Greenville, SC facility, which had prepared the RML for distribution to the above cited outbreak regions [8].

Our previous studies indicated that boiling the RML solution for 10 minutes in a glass test tube did not reduce its anti-Fusarium capability [12], but heating the RML solution to 56 - 60°C for 1 - 4 weeks in its high-density polyethylene (HDPE) plastic (but not a glass) bottle resulted in a decreased ability to inhibit seven of the Fusarium organisms recovered by the CDC during the U.S. outbreaks [12,17,18]. However, heating three other commercial contact lens solutions to 60°C for 4 weeks in their plastic bottles showed no anti-Fusarium failure, compared to RML [18].

Because RML had been withdrawn from the market and, thus, would no longer be available for study, we previously performed simultaneous testing of RML (containing alexidine, 0.00045%) and a simulated contact lens solution consisting of a phosphate buffered saline (PBS) solution of alexidine (0.00045%) in glass bottles and in the bottles of another (then available) B and L product, ReNu MultiPlus (RMP), stored at both room temperature (RT, 23°C) and 56°C, for their ability to inhibit Fusarium growth. This study did not demonstrate any statistically significant difference between RML and the PBS-alexidine simulated contact lens solution in any tested condition (P = 0.4801) [19]. We also compared the RML bottle with the RMP bottle and found that these two containers appeared to be identical and were both labeled with the Society of the Plastics Industry, Inc. Resin Identification Code “2” for HDPE plastic [20]. Our independent analysis of the RML bottle by Fourier transform infrared (FTIR) spectroscopy also identified that it was HDPE plastic [17]. These bottles were virtually functionally identical when tested for inhibition of Fusarium growth after both RT (P = 0.6388) and 56°C (P = 1.00) storage of an alexidine solution [19]. Thus, in our later studies we used a PBS solution of 0.00045% alexidine in a RMP container (hereinafter designated as a “ReNu bottle”) as a substitute for RML in its original bottle when examining the effects of time, temperature, and storage containers on the anti-Fusarium activity of alexidine [15,19,21]. We also showed that this statistically significant loss of antimicrobial capability of an alexidine solution (0.00045%) incubated at 56°C in ReNu containers is not specific to Fusarium organisms but occurs with 22 other fungi and bacteria which cause keratitis (12 non-Fusarium fungal isolates [P < 0.0001] and 10 bacterial isolates [P < 0.0001]) [19].

We also later demonstrated that two other contact lens solutions, RMP (containing polyaminopropyl biguanide [PAPB, 0.0001%]) [12,18], and RevitaLens OcuTec (Abbott Medical Optics, Santa Ana, CA, containing both alexidine dihydrochloride [0.00016%] and polyquaternium-1 [0.0003%]) stored in heated ReNu bottles were still able to inhibit Fusarium organisms [15]. Thus, the RevitaLens solution was able to maintain its anti-Fusarium activity under conditions where a higher concentration (0.00045% vs. 0.00016%) of alexidine

alone had been noted to fail, presumably due to the presence of polyquaternium-1, the second antimicrobial agent in the ReVitaLens solution which, apparently, was unaffected by heating in a ReNu plastic bottle. In addition, alexidine solutions heated in seven non-ReNu plastic bottles retained their anti-*Fusarium* capability [15].

Using liquid chromatography with tandem mass spectroscopy (LCTMS), we measured alexidine concentrations in heated (56°C) and unheated (RT) ReNu bottles containing PBS solutions of alexidine (0.00045%). After 4 weeks of storage, the alexidine solution concentration in the bottle stored at RT was 2.8 times as high as that in the heated bottle [21].

We previously hypothesized that either an alexidine-neutralizing leachate emanates from heated ReNu bottles or alexidine permeates into the walls of heated ReNu bottles [21]. Using Raman spectroscopy (with and without colloidal silver nanoparticles) [21], we were unable to detect any leachates emanating from these heated bottles. In addition, microbiological testing of a PBS solution heated in a ReNu bottle showed no evidence of an alexidine-neutralizing leachate [15].

Using FTIR spectroscopy, we found that a unique alexidine peak occurs between the wavenumbers 150,000 and 165,000 m⁻¹. By comparing the ratios of the areas of the alexidine peaks with a peak corresponding to an ester-based oil additive in the ReNu bottle wall (156,000 m⁻¹ versus 173,900 m⁻¹), we calculated that alexidine permeated into the wall of the methanol-extracted ReNu bottle to a level 3.1 times greater in a heated- versus a room temperature-stored bottle [21]. We also determined that the amount of alexidine was higher in the wall of an original RML bottle (containing the original RML solution [with alexidine, 0.00045%], heated to 60°C for 4 weeks) compared to a bottle wall sample of a RT-stored RMP bottle which had contained the simulated contact lens solution (PBS with alexidine [0.00045%]) (Unpublished data).

Although RML is no longer on the market, its antimicrobial agent, alexidine, is present in the currently available product RevitaLens OcuTec. In addition, the precise chemical composition of the plastic bottles created by the container manufacturer and supplied to B and L for the RML solution bottled in Greenville, SC is unknown. These materials may currently, or could in the future, be used with other thermally labile susceptible pharmaceutical agents. Therefore, it is important to elucidate further the exact mechanism of this product failure. In order to do so, we serially measured alexidine concentrations in heated and unheated solutions stored in ReNu bottles for 28 days to quantify and analyze the rates of alexidine loss from the solutions. We then compared these alexidine concentrations with our prior microbiological inhibitory titers [19] in order to estimate the minimum inhibitory concentration of alexidine required to prevent *Fusarium* growth.

**Methods**

**Measurement of alexidine concentrations in heated and unheated ReNu bottles**

A 10% solution of alexidine in dimethyl sulfoxide (DMSO, Sigma Aldrich, St. Louis, MO) was created using solid alexidine dihydrochloride (Santa Cruz Biotechnology, Santa Cruz, CA), diluted to 0.00045% (4.5 parts per million [PPM]) with PBS, and placed in two empty identical 60-mL ReNu bottles which had been thoroughly rinsed three times with 60 mL of sterile Milli-Q (EMD Millipore, Billerica, MA) water before using. [We had previously determined that DMSO alone did not affect the growth of *Fusarium* [19]]. One bottle was maintained at RT and the other was stored at 56°C. A standard curve with known concentrations of alexidine was used for instrument calibration. Triplicate 5 μL samples were withdrawn from each of these bottles at the following time periods: 0.25 (6 hours), 1, 7 (1 week), 14, 21, and 28 days (4 weeks), and tested on an Agilent 6120B liquid chromatography-mass spectroscopy (LC/MS) system. For each temperature condition, means of the triplicate measurements of the alexidine concentrations were calculated, rounded to the nearest 0.1 PPM, and tabulated in Table 1. Converse plots (Figure) were created by subtracting the mean of the measured solution concentrations from 4.5 PPM (the original concentration), corresponding to alexidine loss from the solutions over time. The red line represents data from the heated (56°C)-stored bottle while the blue line represents the RT-stored bottle.
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Figure: Alexidine loss from solutions stored in heated/unheated high density polyethylene plastic ReNu bottles.

Converse plots were created by subtracting the mean solution concentrations (Table 1) from 4.5 parts per million (PPM) to determine alexidine loss from the heated/unheated solutions (absorption into the ReNu plastic bottles) over time. The red line represents alexidine loss from the heated [56°C]-stored solution and the blue line represents alexidine loss from the room temperature [RT, 23°C]-stored solution. The error bars indicate one sample standard deviation (calculated from the triplicate measurements and rounded to the nearest 0.1 PPM) above and below the mean (depicted by the black dots) for each time-temperature pair.

Comparison between alexidine solution concentrations and their ability to inhibit Fusarium growth

From a previous study [19], inhibitory titers of alexidine solutions (stored for 28 days [4 weeks] in heated [56°C] and unheated [RT] ReNu bottles) against the seven Fusarium organisms, obtained from the CDC during the Fusarium keratitis event of 2004 - 2006, were compared with the LC/MS concentration measurements of 28 day-stored alexidine solutions at RT and 56°C. (The exact details of the antimicrobial assay are described in our previous study [19]).

Results

Measurement of alexidine concentrations in heated and unheated ReNu bottles

After 7 days (1 week) of heating at 56°C (Table 1) the alexidine concentration decreased from 4.5 to 0.2 PPM. Thus, alexidine loss by one week was 95.6% for the heated bottle; with one week storage at RT, the level decreased to 3.2 PPM, a 28.9% loss (P = 0.000012). Inspection of the Figure shows that the ratio of heated: unheated alexidine loss by 4 weeks was 4.1/1.3 = 3.2.
Quantification of Alexidine Loss in ReNu Plastic Bottles and the Worldwide *Fusarium* Keratitis Event of 2004 - 2006

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Temperature (°C)</th>
<th>Mean Solution Concentration of Alexidine (PPM)</th>
<th>Sample Standard Deviation [44] (PPM)</th>
<th>Temperature (°C)</th>
<th>Mean Solution Concentration of Alexidine (PPM)</th>
<th>Sample Standard Deviation [44] (PPM)</th>
<th>Two-tailed <em>P</em>-value for Student T-test [45]</th>
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<tr>
<td>0</td>
<td>23</td>
<td>4.5</td>
<td>0</td>
<td>56</td>
<td>4.5</td>
<td>0</td>
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<td>0.25 (6 hours)</td>
<td>23</td>
<td>3.1</td>
<td>0.2</td>
<td>56</td>
<td>1.4</td>
<td>0.2</td>
<td>0.000168</td>
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<td>56</td>
<td>0.7</td>
<td>0.2</td>
<td>0.000051</td>
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<tr>
<td>7 (1 week)</td>
<td>23</td>
<td>3.2</td>
<td>0.2</td>
<td>56</td>
<td>0.2</td>
<td>0.1</td>
<td>0.000012</td>
</tr>
<tr>
<td>14</td>
<td>23</td>
<td>3.2</td>
<td>0.1</td>
<td>56</td>
<td>0.2</td>
<td>0.1</td>
<td>&lt; 0.00001</td>
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<tr>
<td>21</td>
<td>23</td>
<td>3.3</td>
<td>0.2</td>
<td>56</td>
<td>0.3</td>
<td>0.1</td>
<td>&lt; 0.00001</td>
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<tr>
<td>28 (4 weeks)</td>
<td>23</td>
<td>3.2</td>
<td>0.1</td>
<td>56</td>
<td>0.4</td>
<td>0.2</td>
<td>&lt; 0.00001</td>
</tr>
</tbody>
</table>

**Table 1:** Alexidine concentrations in solutions stored in heated/unheated high density polyethylene plastic ReNu bottles.

Mean alexidine concentrations were measured (in triplicate, in parts per million [PPM]) by liquid chromatography-mass spectroscopy (LC/MS), in samples stored at both room temperature (RT, 23°C) and 56°C (heated) for from 6 hours (0.25 days) to 4 weeks (28 days). The sample standard deviations are zero at time zero for both the heated and unheated (RT) samples because a solution of exactly 4.5 PPM was formulated as the concentration at the beginning of the experiment. The sample standard deviations were determined [44] and rounded to the nearest 0.1 PPM. The Student T-test [45] was used to determine the P-values comparing the concentrations at the two temperatures (23°C and 56°C) for each time period.

**Comparison between alexidine solution concentrations and their ability to inhibit *Fusarium* growth**

After RT storage of an alexidine solution in a ReNu bottle for 28 days, the concentration of alexidine decreased from 4.5 to 3.2 PPM (Table 1). A 1:2 dilution of that solution would have thus contained 1.6 PPM which, from our previous study, did not allow growth of any of 21 (7 organisms in triplicate) *Fusarium* cultures [19] (Table 2). A 1:4 dilution would have thus contained 0.8 PPM which, likewise, inhibited *Fusarium* growth [19]. With a 1:8 dilution, or any heated sample, alexidine levels would correspond to ≤ 0.4 PPM, a concentration at which 21 of 21 *Fusarium* organisms grew [19]. Thus, the minimum inhibitory concentration of alexidine required to prevent *Fusarium* growth is > 0.4 - ≤ 0.8 PPM.
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<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dilution of a 4.5 PPM Alexidine Solution (in glass)</strong></td>
<td><strong>Concentration of Alexidine in PPM (from Column A)</strong></td>
<td><strong>Concentration of Alexidine in PPM in a Solution Stored at RT for 28 Days in a HDPE Plastic ReNu Bottle</strong></td>
<td><em><em>Fusarium Growth</em> in a Solution from Column C</em>*</td>
<td><strong>Concentration of Alexidine in PPM in a Solution Stored at 56°C for 28 Days in a HDPE Plastic ReNu Bottle</strong></td>
<td><em><em>Fusarium Growth</em> in a Solution from Column E</em>*</td>
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<td>Full Strength</td>
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<td>3.2*</td>
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<td>1.6</td>
<td>0/21</td>
<td>0.2</td>
<td>21/21</td>
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<tr>
<td>1:4</td>
<td>1.125</td>
<td>0.8</td>
<td>0/21</td>
<td>0.1</td>
<td>21/21</td>
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<td>1:8</td>
<td>0.5625</td>
<td>0.4</td>
<td>21/21</td>
<td>0.05</td>
<td>21/21</td>
</tr>
</tbody>
</table>


**Table 2**: Correlation between mean alexidine solution concentrations and their ability to inhibit Fusarium growth.

After room temperature storage of the alexidine solution in a high density polyethylene (HDPE) plastic ReNu bottle for 28 days, the concentration decreased from 4.5 to 3.2 parts per million (PPM). A 1:2 dilution of that solution thus would have contained 1.6 PPM and allowed no growth in 21 cultures (7 Fusarium organisms in triplicate). Similarly, a 1:4 dilution would have contained 0.8 PPM and allowed no growth. However, when diluted 1:8 (0.4 PPM), the solution allowed Fusarium growth in 21 of 21 cultures, as did all of the heated (56°C) samples. Fusarium growth was determined by visual inspection for fungal growth and graded by a Ph.D. microbiologist (the late B. Laurel Elder, Ph.D., Wright State University, Dayton, OH) as “growth” or “no growth,” based on solution turbidity.

**Discussion**

In a previous paper [21] we reported (using LCTMS) that the alexidine concentration markedly decreases in a solution stored at 56°C for 4 weeks in a ReNu bottle. By FTIR spectroscopic analysis, we showed that alexidine permeates into the heated ReNu bottle wall [21]. In the present paper, we have verified by LC/MS the marked serial decrease of alexidine levels in a solution stored in a heated ReNu bottle. Alexidine loss after 4 weeks (28 days) was 3.2 (4.1/1.3) times more in the heated- than the RT-stored bottle [Figure]. In our previous publication [21], we reported a 4-week loss ratio of 3.1 using FTIR spectroscopic analysis of portions of the heated: unheated plastic bottles themselves. Alexidine loss has, therefore, been shown by two different analytical methods (FTIR and LC/MS) and performed on two different materials (the plastic ReNu bottle walls and the alexidine solutions themselves) to occur about three times greater in heated- than in RT-stored ReNu bottles. Since alexidine levels that are ≥ 0.4 - ˂ 0.8 PPM allow *Fusarium* growth (Table 2), this alexidine absorption by the HDPE plastic ReNu bottles was most likely critical in the disinfection failure [14] of RML and the subsequent multi-focal worldwide outbreaks of *Fusarium* keratitis.

These findings provide further evidence that the disinfection failure [14] of RML was due to solution loss of alexidine by its thermally enhanced absorption into the ReNu plastic bottles used in Greenville, SC. Heating alexidine in seven non-ReNu plastic bottles did not result in antimicrobial failure [15]; neither did heating three other commercial contact lens solutions in their own bottles [18], nor did heating two other contact lens solutions in ReNu bottles result in antimicrobial failure [12,15,18]. This suggests that a unique interaction exists between alexidine and the ReNu HDPE plastic containers bottled in B and L’s Greenville, SC production facility to which B and L had traced the *Fusarium* keratitis cases during the worldwide outbreaks of 2004 - 2006 [8]. Thus, the “unique properties” theory hypothesized by the CDC [1] as an explanation for this event does not appear to be related to the RML formula itself [19], but, rather, appears to be due to the ”unique properties” of the particular ReNu plastic bottles used in Greenville and their interactions with alexidine.

Polyethylene-drug interactions have been recognized for over 60 years [22]. Polyethylene has been shown to be very selective in its absorption characteristics, especially with nitrogen compounds, which show relatively high absorption rates [22]. Absorption was noted to be unpredictable, depending on the structure (size/molecular weight) and polarity of the chemical. With complex mixtures involving multiple components, it becomes very difficult to predict absorption a priori. Permeability was also noted to be heat dependent, being an exponential function of temperature [23]. In 1953 Wight, et al. [22] suggested that containers should “suit the contents and not attempt to make the contents suit the container.” In 1963, Autian, concerning pharmaceutical failure related to plastic storage materials, wrote: “It appears that a greater scientific body of knowledge must be accumulated to guide the manufacturer to produce a plastic item which will repeatedly behave in an identical manner under conditions of storage and use and which will insure that no possible harm will directly or indirectly fall upon the patient” [24]. The 1968 British Pharmacopoeia and the second edition of the International Pharmacopoeia required that containers do not react chemically or physically with their contents [25]. In 1970, Polack, et al. wrote: “Although polyethylene is impermeable to water; many other molecules are capable of permeating both from the pure state and from aqueous solutions [26]. In 1971, Friesen and Plein wrote: “...sorption-permeation through polyethylene containers poses a definite problem in the storage of ophthalmic solutions...it is suggested that a policy of storing the solutions under refrigeration...be established. This would reduce the hazard of microbial contamination because of the lack of a preservative level sufficient to inhibit microbial growth [27].” A 1974 report from the World Health Organization warned: “Antimicrobial preservatives (in ophthalmic preparations) must be carefully selected to avoid sorption by the plastic and loss of antimicrobial efficiency [28].” Also in 1974, Norton, et al. wrote: “In view of the present proliferation in numbers and ranges of contact lens solutions it would appear desirable that some form of control of the manufacture and presentation be introduced together with minimum standards of antimicrobial efficiency [29].” Three years later Richardson et al studied the preservative content of commercially available contact lens solutions. They found that 27% (6/27) contained less than 50% of their stated concentration. Of the six solutions containing chlorhexidine (a chemical relative of alexidine), one had about 18% and the other about 38% of preservative present [30]. They concluded: “It is apparent that (several of the preservatives) may be sorbed by... polyethylene...containers which may lead to almost complete loss of preservative on storage [30].” Thus, as long as there are contact lens solutions (or other liquid pharmaceuticals) with varying and novel preservatives and/or other active ingredients stored in highly diverse polyethylene (or other plastic) containers, the potential for such future pharmaceutical failures will exist.

Even though these particular ReNu bottles are no longer used by B and L, it is important for clinicians, drug manufacturers, and governmental regulators to recognize that other potentially thermally incompatible plastic-antimicrobial (or -active ingredient) combinations may occur in the future. Other drugs reported to exhibit significant interactions with plastic materials include, but are not necessarily limited to, insulin, clomethiazole, vitamin A acetate, isosorbide dinitrate, phenothiazines, hydralazine hydrochloride, thiopental sodium [31], nitroglycerine [31,32], warfarin sodium [31,32], diazepam [31-33], midazolam [33], furosemide [34], potassium canrenoate [34], digitoxin [34], digoxin [34], adenosine [34], technetium (Tc99m) succimer [35], fotemustine [36], ceftriaxone [37], propofol [38,39], and miconazole [40].

Since the basic tenets of public health are to prevent the occurrence of disease, to control the spread of disease within the initial population, and to prevent its spread to additional populations [41], it is imperative that pharmaceutical companies perform all of the required packaging testing at realistic storage temperatures [9,42,43] in order to prevent a recurrence of this type of event. Further work is necessary to ascertain the precise properties of the ReNu plastic bottles in order to determine the exact molecular mechanism of this pharmaceutical catastrophe; i.e., chemical attraction of alexidine to some, as yet, unknown component of, versus simple absorption of alexidine into the matrix of, the ReNu HDPE plastic bottle.

**Ethical Consideration**

The authors received no financial support and have no proprietary interests or conflicts of interest related to this submission.

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