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1 **Matrix and reservoir-type multipurpose vaginal rings for**
2 **controlled release of dapivirine and levonorgestrel**

3
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14
15 **Short title:** Dapivirine and levonorgestrel vaginal rings

16
17 **Keywords:** HIV microbicide; Hormonal contraception; Silicone elastomer vaginal ring;
18 Multipurpose prevention technology; MPT; Formulation development.

19 **Abstract**

20 A matrix-type silicone elastomer vaginal ring providing 28-day continuous release of dapivirine
21 (DPV) – a lead candidate human immunodeficiency virus type 1 (HIV-1) microbicide compound
22 – has recently demonstrated moderate levels of protection in two Phase III clinical studies. Here,
23 next-generation matrix and reservoir-type silicone elastomer vaginal rings are reported for the
24 first time offering simultaneous and continuous *in vitro* release of DPV and the contraceptive
25 progestin levonorgestrel (LNG) over a period of between 60 and 180 days. For matrix-type
26 vaginal rings comprising initial drug loadings of 100, 150 or 200 mg DPV and 0, 16 or 32mg
27 LNG, Day 1 daily DPV release values were between 4132 and 6113 μg while Day 60 values
28 ranged from 284 to 454 μg . Daily LNG release ranged from 129 to 684 μg on Day 1 and 2–91 μg
29 on Day 60. Core-type rings comprising one or two drug-loaded cores provided extended duration
30 of *in vitro* release out to 180 days, and maintained daily drug release rates within much narrower
31 windows (either 75–131 $\mu\text{g}/\text{day}$ or 37–66 $\mu\text{g}/\text{day}$ for DPV, and either 96–150 $\mu\text{g}/\text{day}$ or 37–57
32 $\mu\text{g}/\text{day}$ for LNG, depending on core ring configuration and ignoring initial lag release effect for
33 LNG) compared with matrix-type rings. The data support the continued development of these
34 devices as multi-purpose prevention technologies (MPTs) for HIV prevention and long-acting
35 contraception.

36

37 **Abbreviations**

38 DAC, dual asymmetric centrifuge; DPV, dapivirine; DSC, differential scanning calorimetry;

39 HIV-1, human immunodeficiency virus type 1; HPLC, high performance liquid chromatography;

40 LNG, levonorgestrel; IPM, International Partnership for Microbicides; MPT, multipurpose

41 prevention technology; NNRTI, non-nucleoside reverse transcriptase inhibitor; STI, sexually

42 transmitted infection; SVF, simulated vaginal fluid

43

44 **1. Introduction**

45 Vaginal rings offering sustained or controlled release of antiretroviral drugs have been at the
46 forefront of efforts over recent years to develop microbicide products for prevention of sexual
47 transmission of human immunodeficiency virus type 1 (HIV-1) (Malcolm et al., 2016). A matrix-
48 type silicone elastomer vaginal ring containing dapivirine (DPV; Figure 1A) – an experimental
49 non-nucleoside reverse transcriptase inhibitor (NNRTI) – and intended for 28-day continuous
50 use is being developed by the International Partnership for Microbicides (IPM) (R Karl Malcolm
51 et al., 2012; Nel et al., 2011, 2009). This DPV ring recently completed two Phase III clinical
52 studies (the Aspire Study and The Ring Study) designed to support licensure of the ring for
53 preventing infection with HIV in women (Baeten et al., 2016; Nel et al., 2016b). Results from
54 these studies showed that the ring reduced HIV infection by 27% and 31%, respectively,
55 compared with a placebo ring (Baeten et al., 2016; Nel et al., 2016b). Post hoc sub-group
56 analyses in the Aspire Study revealed a 37% reduced risk after excluding two sites with the
57 lowest rates of retention and adherence, a 56% reduced risk when only women older than 21
58 years were considered, and a 61% reduction in women aged 25 and older (Baeten et al., 2016). In
59 The Ring Study, sub-analysis by age revealed no significant benefit for women younger than 21
60 years, and a 37.5% reduced risk in women aged >25 years (Nel et al., 2016b).

61
62 Despite the fact that a safe and effective vaginal microbicide product to protect against HIV
63 infection has yet to reach market, there is already considerable interest and early-stage
64 development activity around next-generation multipurpose prevention technology (MPT)
65 products that seek to combine HIV prevention with contraception and/or prevention/treatment of
66 other sexually transmitted infections (STIs) (Fernández-Romero et al., 2015; Malcolm and

67 Fetherston, 2013; Malcolm et al., 2016, 2014; Romano et al., 2013; Woodsong et al., 2015).
68 With 86 million unintended pregnancies (Sedgh et al., 2014) and 2.1 million new HIV cases
69 around the world every year (Joint United Nations and HIV/AIDS, 2016), reformulation of the
70 DPV ring to additionally include a continuous-use progestin-only contraceptive is an obvious
71 next step, especially since most existing hormonal birth control methods offer no protection
72 against HIV or other STIs. Furthermore, a vaginal ring with a use indication for both prevention
73 of pregnancy and HIV infection may result in increased user adherence compared with a
74 product preventing only HIV, since women's perceived risk of pregnancy is usually higher than
75 that for HIV infection (Woodsong and Holt, 2015).

76
77 Many of the MPT products currently undergoing development, including a number of vaginal
78 ring devices, have prioritised use of levonorgestrel (LNG; Figure 1B) as the contraceptive
79 hormone component based on its historical record of safety and effectiveness and its suitability
80 for continuous use without need for a monthly withdrawal period (Mansour, 2012; Romano et
81 al., 2013; Ugaonkar et al., 2015; Woodsong et al., 2015). In addition to its current use as a long-
82 acting contraceptive in intrauterine devices and subdermal implants (Eisenberg et al., 2015;
83 Gonzalo et al., 2002; S Koetsawang et al., 1990; Rose et al., 2009), LNG has also previously
84 been investigated extensively for delivery from silicone elastomer vaginal rings (Bounds et al.,
85 1993; S Koetsawang et al., 1990; S. Koetsawang et al., 1990a, 1990b; Mishell et al., 1975;
86 Murphy et al., 2016b). Recently, as part of continued efforts to develop a MPT vaginal ring
87 offering simultaneous release of DPV and LNG, we reported on various formulation strategies to
88 reduce the extent of LNG binding to addition cure silicone elastomer materials (Murphy et al.,
89 2016b). Here, we report for the first time assessment of the preclinical feasibility of matrix-type

90 and reservoir-type silicone elastomer vaginal rings offering continuous release of both DPV and
91 LNG for at least 60 days and preferably at least 90 days in quantities anticipated to offer clinical
92 effectiveness.

93

94 **2. Materials and methods**

95 **2.1. Materials**

96 Micronised DPV was supplied by S.A. Ajinomoto OmniChem N.V. (Wetteren, Belgium). Non-
97 micronised LNG (Batch No: 120101) was supplied by Haorui Pharma-Chem Inc. (Irvine, CA,
98 US). MED-4870 and DDU-4320 silicone elastomer kits were purchased from NuSil Technology
99 LLC (Carpinteria, CA, US). HPLC-grade acetonitrile, HPLC-grade isopropanol and potassium
100 dihydrogen orthophosphate (AnalaR analytical reagent) were purchased from VWR International
101 Ltd. (Dublin, Ireland). Phosphoric acid (85% w/w in water) was purchased from Sigma-Aldrich
102 (Gillingham, UK). A Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK) was used
103 to obtain HPLC-grade water.

104

105 **2.2 Ring release rate targets**

106 The aim of this study was to develop a MPT vaginal ring offering at least 60-day *in vitro* release,
107 and preferably 90-day release, of DPV and LNG at levels likely to be effective for HIV
108 prevention and contraception. In comparison, the existing Dapivirine Vaginal Ring-004 contains
109 only 25 mg DPV and is intended for 28 days of use (Nel et al., 2009). For the DPV component of
110 the MPT ring, the *in vitro* release rate on Day 60 or Day 90 was targeted to be equal to or greater
111 than the Day 28 *in vitro* release value from the Dapivirine Vaginal Ring-004 (i.e. 200 µg). This
112 value was determined from historical data across multiple batches of Ring-004 and measured

113 experimentally under the same *in vitro* release conditions as those used to test the MPT rings
114 described in this study. Two target (lowest acceptable) *in vitro* release rates – 35 µg/day and 70
115 µg/day – were defined for LNG based on our analysis of previously reported data in the
116 scientific literature (Clark et al., 2014; Eisenberg et al., 2015; Jackanicz, 1981; S Koetsawang et
117 al., 1990; S. Koetsawang et al., 1990a; Landgren et al., 1994a, 1994b; Xiao et al., 1985). Vaginal
118 rings with *in vitro* LNG release rates ranging from 20–30 µg/day have been investigated
119 previously (Clark et al., 2014; Jackanicz, 1981; S. Koetsawang et al., 1990a; Landgren et al.,
120 1994a, 1994b; Xiao et al., 1985). Systemic LNG levels peaked at between 300 to 800 pmol/L
121 shortly after ring insertion and remained relatively stable with an average decline of 23–26%
122 during the 3 months of use (S Koetsawang et al., 1990; Landgren et al., 1994b; Xiao et al.,
123 1985). However, new ring designs targeting higher LNG *in vitro* release rates (e.g. 35 µg/day)
124 have been advocated due to concern with the higher pregnancy rates observed among heavier
125 women in clinical trials (Brache et al., 2000).

126

127 **2.3. Differential scanning calorimetry**

128 Samples of micronised DPV, non-micronised LNG and physical mixtures of the two drugs at
129 10% w/w intervals were prepared for DSC analysis. Each mixture was mixed thoroughly, first by
130 hand using a spatula and then in a Speedmixer™ at 3000 rpm. Samples were analyzed by DSC
131 (TA Instruments 2920 modulated DSC) in standard heating ramp mode. Approximately 5–10 mg
132 of each sample was accurately weighed into an aluminum pan and heated from 20 to 250°C at a
133 rate of 10°C per min alongside an empty reference pan. For each sample, the following
134 parameters were noted for any melting transitions that were observed: onset temperature (°C),
135 peak temperature (°C) and enthalpy (ΔH , J/g). A minimum of four replicates was used to

136 calculate mean values for each sample mixture. DSC analysis was similarly performed on
137 silicone elastomer samples loaded with various concentrations and ratios of DPV only, LNG
138 only and DPV+LNG in order to characterize the nature of the drugs in the rings.

139

140 **2.4. Matrix-type vaginal ring manufacture**

141 The DPV-only matrix-type vaginal ring (Ring-004) that recently completed being tested in two
142 Phase III clinical trials in Africa contains 25 mg DPV and is intended for 28-day use (Baeten et
143 al., 2016). In order to extend DPV release from a matrix-type device out to at least 60 days, it
144 was necessary to increase the DPV loading in the matrix-type ring, in accordance with the
145 relevant theory of drug release kinetics (Malcolm et al., 2003; Siepmann and Peppas, 2011).
146 Three higher DPV loadings were selected for further investigation in this study: 100 mg, 150 mg
147 and 200 mg. Two LNG loadings – 16 mg and 32 mg – were also selected, based on previous data
148 generated as part of the project (data not published). In total, 11 different matrix-type vaginal
149 ring formulations were manufactured based on various combination loadings of DPV and LNG
150 (Table 1). Matrix-type, silicone elastomer vaginal rings (cross-sectional diameter 7.8 mm, outer
151 diameter 56.7 mm) were manufactured using a Babyplast 6/10P horizontal injection molding
152 machine fitted with a custom stainless steel ring mold assembly and a silicone dosing system.
153 Separate 50 g premixes of DPV and/or LNG in Parts A and B of the MED-4870 addition-cure
154 silicone elastomer system were prepared by adding weighed quantities of DPV and LNG into a
155 screw-cap polypropylene container followed by addition of the silicone part. The premixes were
156 then mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixer™ DAC 150 FVZ-
157 K, Hauschild, Germany) (180 s, 3000 rpm) before storing in the fridge. On the day of ring
158 manufacture, the premixes were removed from the fridge, hand-mixed (30 s) and then DAC

159 mixed (120 s, 3000 rpm). A and B premixes were combined in an overall 1:1 ratio, according to
160 the following procedure: (i) 25 g weights of each premix were alternately added to a large screw-
161 cap polypropylene container to a final weight of 100 g; (ii) this active silicone elastomer mixture
162 was hand-mixed for 30 s and then DAC mixed (30 s at 3000 rpm); (iii) this process was repeated
163 four times for each formulation to produce 400 g total of the active mix. The 400 g active mix
164 was transferred to a 500 g polypropylene SEMCO[®] injection cartridge designed for use with the
165 dosing system on the Babyplast injection molder. The ring mold assembly on the Babyplast
166 machine was heated via 2 x 200 W heater cartridges fitted to both the fixed and mobile plates.
167 Rings were manufactured by injecting the active mix into the heated ring mold assembly, under
168 the following conditions: 100 bar clamping pressure, 50 bar injection pressure, 160 °C mold
169 temperature, 60 s cure time. Rings were subsequently demolded, deflashed (where necessary)
170 and stored at ambient temperature until further testing.

171

172 **2.5. Core-type vaginal ring manufacture**

173 Two different configurations of human-sized, reservoir-type, silicone elastomer rings containing
174 DPV and LNG (Formulations L and M, Table 2) were manufactured using a three-step injection
175 molding process (Figure 2). Each step was similar to that described previously for the
176 manufacture of the matrix-type rings (Section 2.3). However, given their greater complexity, the
177 reservoir-type rings were manufactured on a laboratory-scale injection-molding machine using
178 the DDU-4320 grade of addition-cure silicone elastomer, which offers lower cure temperature,
179 lower viscosity and improved flow characteristics compared to the MED-4870 silicone
180 elastomer. Formulation L reservoir-type rings comprised a full-length DDU-4320 silicone
181 elastomer core containing both solid crystalline micronised DPV and solid crystalline non-

182 micronised LNG, each at a loading of 2% w/w. The drug-loaded core was subsequently
183 overmolded in two steps using custom molds with a drug-free DDU-4320 silicone elastomer
184 sheath (rate-controlling membrane). All mixing procedures were conducted as described for the
185 matrix-type rings. However, cure of the drug-loaded cores was performed at 90 °C for 30 s,
186 producing cores with the following dimensions: 54.9 mm outer diameter, 4.5 mm cross-sectional
187 diameter. The overmolded, non-medicated, rate-controlling membrane was cured at 90 °C for 90
188 s. The fully manufactured core rings had the following dimensions: 58.0 mm outer diameter, 7.6
189 mm cross-sectional diameter. The thickness of the non-medicated membrane was therefore $(7.6 -$
190 $4.5) / 2 = 1.55$ mm. Formulation M reservoir-type rings were manufactured in the same manner,
191 except with two separate half-length cores – one containing only 2% w/w DPV and the other
192 containing only 2% LNG (Table 2).

193

194 **2.6. *In vitro* release testing**

195 *Matrix-type rings*

196 On Day 0, matrix-type rings were placed individually into 250 mL glass bottles containing 200
197 mL 1:1 mixture of isopropanol and water and stored in an orbital shaking incubator (Unitron HT
198 Infors; 37 °C, 60 rpm, 25 mm orbital throw). After 24 ± 0.25 hr, the release medium was
199 sampled (2 mL) for subsequent HPLC analysis and the entire remaining volume replaced with a
200 fresh 100 mL of isopropanol/water mixture. This sampling and 100 mL replacement of the
201 release medium was performed daily out to Day 30, except on Fridays when, after sampling, the
202 flask was replenished with a 200 mL volume of release medium and no further replacement or
203 sampling performed until the following Monday. From Day 30 through to Day 60, twice-weekly
204 sampling and replacement of the release medium was performed on consecutive days (Days 38,

205 39, 45, 46, 52, 53, 59 and 60), with 100 mL release medium used on the first of the two
206 consecutive days and 200 mL used on the second day. Release testing was extended out to Day
207 92 for matrix-type vaginal ring formulations C and K with twice-weekly sampling (Days 66, 67,
208 73, 74, 80, 81, 87, 88, 91, 92) following the protocol described earlier. The amount of drug in
209 each sample was quantified by reverse-phase HPLC with UV detection (Section 2.6).

210

211 *Core-type rings*

212 *In vitro* release testing of reservoir-type rings over 180 days was performed in a similar manner
213 to that for matrix-type vaginal rings. Daily sampling and replacement was performed (50 mL;
214 100 mL at weekends) out to Day 30, twice-weekly sampling and replacement on consecutive
215 days (50 mL first day, 200 mL second day) out to Day 95, and twice-fortnightly sampling and
216 replacement on consecutive days (50 mL first day, 200 mL second day) out to Day 180. The
217 smaller 50 mL volume used here compared with the 100 mL volume used when testing matrix-
218 type rings is acceptable given the significantly lower drug release rates from reservoir-type rings.

219

220 **2.7. HPLC method**

221 A Waters HPLC system (Waters Corporation, Dublin, Ireland) consisting of the following
222 components was used for all HPLC analysis: 1525 Binary HPLC pump, 717 Plus Autosampler,
223 In-line Degasser AF Unit, 2487 Dual λ Absorbance Detector, 1500 Column Heater. Samples
224 were injected (25 μ L) onto a Thermo Scientific BDS Hypersil C18 column (150 mm x 4.6 mm, 3
225 μ m particle size) fitted with a guard column. The column was held at 25 °C and isocratic elution
226 was performed using a mobile phase of 55% 7.7 mM phosphate buffer (pH 3.0) and 45% HPLC-

227 grade acetonitrile (1.2 mL/min) with a run time of 9 min. DPV was detected using a wavelength
228 of 210 nm after 6.2 min, while LNG was detected after 7.7 min using a wavelength of 240 nm.

229

230 **2.8. Statistical analyses**

231 DPV and LNG *in vitro* release was compared for each ring set using a one-way ANOVA,
232 followed by post-hoc analysis using the Tukey-Kramer multiple comparisons test. The following
233 results were compared for both drugs: Day 1 release, Day 30 release, Day 60 release, total
234 release over 60 days. Analysis was conducted using GraphPad Prism software and significance
235 was noted for a P value of less than 0.05: * = significant ($0.01 < P < 0.05$), ** = very significant
236 ($0.001 < P < 0.01$), *** = extremely significant ($P < 0.001$), ns = not significant ($P > 0.05$).

237

238 **3. Results and Discussion**

239 *DSC thermal analysis*

240 DSC analysis of the pure DPV and LNG substances showed sharp endothermic transitions at 219
241 and 238 °C, respectively, indicative of crystalline melting (Figure 3A). The additional
242 endothermic transition observed at ~100 °C in the DPV trace is due to a known polymorphic
243 transition (crystalline form I to II) (Murphy et al., 2014). For all the ring formulations tested in
244 this study, the concentrations of DPV and LNG incorporated into the silicone elastomer material
245 were so low (0.2–2.5% w/w, Tables 1 and 2) that no discernible crystalline melting endotherms
246 were observed by DSC; at the high temperature of DSC analysis, the drug loading fully dissolves
247 in the silicone elastomer (Gramaglia et al., 2005). However, evidence that DPV and LNG exist in
248 the solid crystalline state within the rings was provided using silicone elastomer samples
249 containing much higher (10% w/w) drug loadings for which the endotherms associated with

250 melting of the pure drug substances were observed at 219 and 238 °C (Figure S1, Supplementary
251 Material). Coupled with the white opaque appearance of the matrix rings (particularly those
252 containing DPV; Figure 4) and the drug-loaded cores of the reservoir rings (Table 2), the DSC
253 data strongly indicate that both drug substances are at least partially present in the solid
254 crystalline state within the silicone elastomer materials.

255
256 DSC analysis of physical mixtures of crystalline DPV and LNG revealed reduced melting
257 behaviour for both drugs (Figure 3A and 3B), a eutectic composition at 40% LNG concentration
258 (Figure 3C), and a eutectic melt temperature of ~192°C (Figure 3A and 3B). Once again, the
259 rings of this study did not contain sufficiently high concentrations of DPV and LNG to show
260 discernible DSC peaks. However, it is assumed that the same reduced melting behaviour also
261 applies to the drugs within the rings.

262

263 *In vitro release from matrix-type vaginal rings*

264 Dapivirine is an exceptionally poorly water-soluble (< 1 mcg/mL) antiretroviral drug (Murphy et
265 al., 2014). Various release media have been used for *in vitro* release testing of dapivirine-
266 releasing rings during the past twelve years of development, including simulated vaginal fluid
267 (SVF; a substantially aqueous, non-buffered medium), various buffer systems, aqueous media
268 incorporating surfactant(s), and various organic solvent/water mixtures (Fetherston et al., 2013a,
269 2013b; Malcolm et al., 2005; R. Karl Malcolm et al., 2012; Murphy et al., 2016a, 2016b, 2014;
270 Woolfson et al., 2006). SVF is unquestionably the most physiologic medium here, but it affords
271 very low *in vitro* release of dapivirine (in the order of low micrograms per day) even when
272 relatively large volumes (> 100 mL) are used, due to the poor aqueous solubility of dapivirine.

273 Moreover, *in vitro* dapivirine ring release using SVF does not correlate with release *in vivo*,
274 based on post-use residual dapivirine content data (unpublished data). (It is worth noting that the
275 daily production of human vaginal fluid is around 6 g/day, with approximately 0.5–0.75 g
276 present in the vagina at any one time (Owen and Katz, 1999).) Use of buffered aqueous release
277 media for *in vitro* release testing is not preferred since vaginal fluid has only limited buffering
278 capacity (Tevi-Bénissan et al., 1997; Wagner and Levin, 1984). Therefore, protocols for *in vitro*
279 release testing of vaginal rings containing poorly water-soluble drugs have inevitably had to
280 make use of solvent enhancement strategies to come close to measured *in vivo* release rates. Both
281 organic solvent/water mixtures and surfactant-containing aqueous media have been used and are
282 widely reported in the literature. For most of its development program, an isopropanol/water
283 mixture (1:1 volume ratio) has been used for the *in vitro* testing of the dapivirine ring, primarily
284 for the purpose of screening and comparing different formulations during preclinical
285 development. We have extensive unpublished data to confirm that this solvent mixture does not
286 cause the rings to swell and that solvent extraction is not responsible for the release of dapivirine.
287 We also have extensive data to confirm that a conventional permeation-controlled release
288 mechanism operates in this medium. Use of isopropanol/water also permits use of much lower
289 (and more practical) volumes of release media; 100 mL per day is typically used for a human-
290 sized ring, which, although still relatively large compared to vaginal fluid volumes, is
291 significantly less than the litres required when using purely aqueous media. Finally, measurement
292 of residual dapivirine content following clinical use and testing in sheep of the 25 mg dapivirine
293 ring for 28 days indicates that the total amount of dapivirine released (~4 mg) is broadly similar
294 to that measured following *in vitro* release testing using 1:1 isopropanol/water over the same
295 time period (Fetherston et al., 2013a; Holt et al., 2015; Nel et al., 2016a; Spence et al., 2016). For

296 these reasons, a 1:1 isopropanol/water mixture was selected as the *in vitro* release medium in this
297 study. The solubility of DPV in different isopropanol/water mixtures has been reported
298 previously (Woolfson et al., 2010).

299
300 All of the matrix-type rings containing DPV (Formulations A–C and F–K; Table 1) were white
301 and opaque in appearance (Figure 4), consistent with uniform distribution of the white
302 micronised DPV particles throughout the silicone elastomer matrix. By comparison, the 16 and
303 32 mg LNG rings (Rings D and E, Table 1) were partially transparent (Figure 4), with the non-
304 micronised LNG particles clearly visible within the matrix as discrete particles upon close
305 inspection. Ring weights for all matrix-type ring formulations were close to 8 g (Table 1).

306
307 A validated HPLC-UV method was developed for quantification of *in vitro* release of DPV and
308 LNG from the vaginal ring formulation. Full details – including representative chromatogram,
309 baseline quality, precision, recovery, resolution and linearity – are provided in the
310 Supplementary Material (Figures S3 and S4, Tables S4, S5, S6, S7 and S8). Graphs depicting
311 DPV and LNG release from the matrix-type rings over the 60-day test period are presented in
312 Figures 5 and 6, respectively, while summary release data are presented in Supplementary
313 Material (Tables S1 and S2). For all ring formulations containing DPV, DPV release showed a
314 burst release on Day 1 (ranging between 4132 and 6038 µg, depending upon initial DPV loading
315 within the ring) followed by steadily declining daily release quantities with time (Figure 5A). By
316 Day 30, daily DPV release was within the range 407–634 µg, and by day 60 284–454 µg (Table
317 2). In accordance with theory (Malcolm et al., 2003; Siepmann and Peppas, 2011) and based on
318 previously reported *in vitro* release data for 25 mg DPV-only rings under similar experimental

319 conditions (Fetherston et al., 2013a), cumulative DPV release on Day 30 showed an approximate
320 two-fold increase for every four-fold increase in DPV loading.

321
322 Day 60 DPV release values for these matrix-type rings were significantly higher than both the
323 predetermined minimum acceptable value of 200 μg and the 136 $\mu\text{g}/\text{day}$ mean release rate
324 reported previously for DPV release from a reservoir-type silicone elastomer ring (Malcolm et
325 al., 2005). The cumulative release versus root time graph (Figure 5B) more clearly illustrates the
326 impact of initial DPV loading upon release. Increasing the DPV loading produced a significant
327 increase in the DPV release rate ($P < 0.001$ for all relevant comparisons). However, the
328 additional presence of LNG in rings having a fixed DPV loading did not significantly influence
329 DPV release. For rings containing 100 mg DPV and 0, 16 or 32 mg LNG (formulations A, F and
330 G), there was no significant difference in DPV release for any of the comparisons made ($P >$
331 0.05), with the exception of 60-day cumulative release for formulations A and F ($P < 0.01$). The
332 total release of DPV from formulations A and F was 36.7 and 36.1 mg, respectively (Table S1 in
333 the Supplementary Material), a difference unlikely to manifest itself *in vivo*. The same is true for
334 rings containing 150 mg DPV (B, G and H) and 200 mg DPV (C, J and K). Very low percentage
335 RSD values for the daily release data were observed, indicating that ring manufacture and *in*
336 *vitro* release are highly reproducible. All cumulative DPV release versus root time profiles were
337 linear (Figure 5B), with coefficient of variation (R^2) values very close to unity (Table S1 in the
338 Supplementary Material), indicating a permeation-controlled release mechanism for DPV from
339 these rings (Malcolm et al., 2003). Based on the DPV *in vitro* release data generated, each
340 formulation tested has potential as a 60-day product.

341

342 *In vitro* LNG release from the matrix-type rings is rather more nuanced than that for DPV. In
343 general, the daily LNG release versus time profiles are also indicative of matrix-type kinetics
344 with highest release occurring on Day 1, followed by declining daily release over time (Figure
345 6A). In general LNG release from the rings fall into four distinct groups in order of increasing
346 LNG release: 16 mg LNG ring < 32 mg LNG ring < 16 mg LNG + DPV ring < 32 mg LNG +
347 DPV ring. Release from the LNG-only rings D and E was clearly much lower than that for
348 combination rings having the same initial LNG loading (Figure 6A) and shows significant
349 deviation from root time kinetics based on linear regression modelling (Table S2, Supplementary
350 Material). This suggests either a release-enhancing effect in the presence of DPV or a lack of
351 LNG availability / inhibition of LNG release in the absence of DPV. The non-linear cumulative
352 release versus square root time profiles for the LNG-only rings (Rings D and E, Figure 6B)
353 further suggest that only a fraction of the initial LNG loading is capable of being released from
354 the rings; at Day 60, only 2.0 and 5.2 μg LNG were released from Rings D and E, respectively
355 (Table S1 in the Supplementary Material).

356

357 We have recently reported that a hydrosilylation reaction occurs between LNG and the hydride-
358 functionalised polydimethylsiloxane component of addition-cure silicone elastomer system
359 leading to irreversible covalent binding of LNG with the silicone and ultimately reduced LNG
360 release (Murphy et al., 2016b). This binding phenomenon is almost certainly occurring in both
361 the LNG-only and the LNG+DPV rings of this study. However, it is clearly not the only
362 mechanism affecting LNG release, since LNG release is very significantly increased by the
363 presence of DPV when LNG-only rings are compared to DPV+LNG rings with equivalent LNG
364 loading (Figure 6, Table S2 in the Supplementary Material) ($P < 0.001$ for all comparisons).

365 Each of the combination rings released significant quantities of LNG on Day 60 (23–91 μg),
366 culminating in total release of 31–36% of the nominal LNG loading over the course of the
367 release experiment (Table S2 in the Supplementary Material). There are several possible
368 explanations for the enhanced release of LNG in the presence of DPV. The presence of DPV in
369 the silicone elastomer may modify the silicone elastomer environment so as to enhance the
370 solubility of LNG in the elastomer, resulting in a corresponding increase in release. This
371 phenomenon has been reported previously for *in vitro* release of DPV from a silicone elastomer
372 ring when maraviroc (MVC) is incorporated as a second microbicide agent (Fetherston et al.,
373 2013a), and is attributed to 'pore-forming' theory first postulated for drug/excipient loaded
374 silicone elastomers back in the 1980s (Carelli et al., 1989; Di Colo, 1992; Golomb et al.,
375 1990). Additionally, and supported by the DSC experiments previously discussed in this study
376 for powder mixtures of DPV and LNG, DPV and LNG might form a solid state eutectic-type
377 mixture within the silicone elastomer matrix, as reported previously in other combination drug
378 delivery systems, including vaginal rings (Liu et al., 2006; Stott et al., 1998; van Laarhoven et
379 al., 2002). The reduced melting temperature for each drug component in the eutectic would result
380 in its increased solubility in the silicone elastomer and increased drug release. Finally,
381 incorporation of DPV in the rings will lead to competition for the solubility sites in the silicone
382 elastomer which will reduce LNG solubility in the elastomer leading to reduced exposure to and
383 reaction with the hydrosilane groups in the silicone elastomer formulation (Murphy et al.,
384 2016b). Given the complexity of the system, it is very difficult to determine the relative
385 contribution of these various mechanisms to the enhanced LNG release in the presence of DPV.
386

387 Based on previous unpublished data from preliminary studies on matrix-type vaginal rings
388 containing both DPV and LNG, the LNG loadings for rings in this study (16 and 32 mg) were
389 selected to target Day 60 LNG release values of 35 and 70 μg . For rings F, H and J, each
390 containing 16 mg LNG, LNG release on Day 60 was in the range 23–29 μg (Table S2 in the
391 Supplementary Material), slightly below the target value (Section 2.2). For Rings G, I and K,
392 each containing 32 mg LNG, Day 60 release ranged between 84 and 91 μg (Table S2 in the
393 Supplementary Material), significantly above the 70 μg target (Section 2.2).

394
395 Two matrix-type ring formulations – Ring C containing 200 mg DPV and Ring K containing 200
396 mg DPV and 32 mg LNG – were selected for extended *in vitro* release testing in order to
397 determine the feasibility of a matrix-type ring as a 3-month product. Both ring formulations
398 provided similar DPV release on Day 92 (301 and 299 μg ; formulations C and K, respectively),
399 significantly in excess of the 200 μg minimum daily release rate (Section 2.2). For formulation
400 K, LNG release was 46 μg on Day 92, above the lower target of 35 μg (Section 2.2).

401
402 Based on these data, the matrix-type DPV and LNG ring may be suitable for extended use over 3
403 months. By adjusting the initial loadings of DPV and LNG within the matrix ring the *in vitro*
404 release behaviour of both drugs could be further modified. One of the difficulties with this
405 approach, and a consequence of the kinetic model used to describe drug release from matrix-type
406 rings, is that any changes in loading to affect drug release near the end of the intended use period
407 have a disproportionate effect on the initial burst release of the drug, which may have
408 implications for drug product safety. This issue is considered more pertinent to the LNG
409 component within the matrix ring. Future clinical development of this matrix-type MPT ring

410 should seek to evaluate the relationships between drug loading, pharmacokinetic /
411 pharmacodynamic behaviour, and product safety.

412

413 *In vitro release from reservoir-type vaginal rings*

414 Daily and cumulative *in vitro* release versus time graphs for reservoir-type vaginal ring
415 formulations L and M over 180 days are presented in Figure 7. Daily DPV release from ring
416 formulation L (Table 2; comprising a full length core loaded with 51.2 mg each of DPV and
417 LNG) ranged from 131 µg on Day 1 through to 75 µg on Day 180, representing a 42% decline
418 (Figure 7A; Table S3 in the Supplementary Material). By comparison, the dual half-core ring
419 configuration (ring formulation M; Table 2) provided Day 1 release of 61 µg and Day 180
420 release of 37 µg, exactly half the values for the full-length reservoir-type ring formulation L
421 (Figure 7A; Table S3 in the Supplementary Material). This linear relationship between daily
422 release and length of drug-loaded core is in accordance with Crank's equation (Woolfson et al.,
423 2003, 1999). After 180 days, total cumulative DPV release was 17.0 and 9.1 mg for Rings L and
424 M (Figure 7C, Table S3 in the Supplementary Material), respectively, equivalent to 33.2% and
425 35.7% of initial DPV loading, respectively (Table S3 in the Supplementary Material).

426

427 Ring formulations L and M (Table 2; comprising a full length core loaded with 51.2 mg each of
428 DPV and LNG) showed distinct lag effects in the graphs of daily LNG release versus time
429 (Figure 7C). Both rings show negligible release on Day 1 (Table S3 in the Supplementary
430 Material), and maximum daily release is only achieved on Day 15 for Ring L (149.9 µg) and Day
431 25 for Ring M (57.2 µg). This behaviour is clearly very different from that of DPV (Figure 7A).
432 Lag effects are commonly observed in reservoir-type rings when insufficient time has passed

433 between ring manufacture and release testing or clinical use to permit equilibration of dissolved
434 drug between core and sheath components; this effect is exacerbated at low curing temperatures.
435 However, this explanation does not account for the very substantial lag effects observed for LNG
436 in the rings of this study. Rather, as postulated previously for the unusual release characteristics
437 observed for the LNG-only matrix-type rings (Rings D and E; Figure 6), the lag effect here is
438 most likely attributed to a hydrosilylation reaction between the ethynyl functional group in
439 dissolved LNG molecules and excess silane groups in the silicone elastomer system leading to
440 irreversible chemical binding (Murphy et al., 2016b). LNG release rates steadily increased
441 during the initial release period (Figure 7B), suggesting that LNG binding within the non-
442 medicated silicone elastomer rate-controlling sheath predominates until all of the excess silane
443 groups have reacted. Thereafter, solubilised LNG molecules diffused through the sheath layer
444 uninhibited resulting in the expected zero-order kinetic profile (Figure 7B). After 180 days, total
445 cumulative LNG release was 21.5 and 8.6 mg for Rings L and M (Figure 7D, Table S3 in the
446 Supplementary Material), respectively, equivalent to 42.0% and 33.4% of initial LNG loading,
447 respectively (Table S3 in the Supplementary Material). That Ring M comprising the half-length
448 LNG core provides LNG release characteristics that are slightly lower than expected compared
449 to Ring L comprising the full-length core DPV+LNG core (Table S3 in the Supplementary
450 Material) is attributed to LNG binding in the non-medicated silicone elastomer sheath layer. This
451 represents a confounding factor to accurate modelling of LNG release and underlines the need
452 for experimental determination of drug release.

453

454 *Comment on stability of DPV and LNG*

455 Although pharmaceutical stability data are not presented in this manuscript, both DPV and LNG
456 generally show good long-term stability in silicone elastomer rings. DPV Ring-004, containing
457 25 mg DPV in an addition-cure silicone elastomer, has recently completed Phase III clinical
458 testing and shows long-term stability performance over its 36-month shelf life (Devlin et al.,
459 2013). Stability performance for a combination microbicide ring device containing DPV and
460 MRV has been published previously (Fetherston et al., 2013a). Stability data for LNG-only and
461 DPV+LNG rings are currently unpublished, but are planned for inclusion in a future publication.

462

463 **4. Conclusions**

464 Extending the duration of DPV release over the current 28-day 25 mg DPV-only vaginal ring
465 and developing a MPT ring combining DPV with a contraceptive agent are important next steps
466 in the development of practical and effective HIV microbicide products. The data presented here
467 highlights the feasibility of pursuing either a 60-day matrix-type ring or a 90-day reservoir-type
468 ring for simultaneous release of DPV and LNG as a viable MPT strategy.

469

470

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476

477 **Transparency declarations**

478 The authors declare no conflicts of interest.

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683

684

685 **FIGURE CAPTIONS**

686

687 **Figure 1.** Chemical structures for dapivirine (A) and levonorgestrel (B).

688

689 **Figure 2.** Three stages of manufacture of a reservoir-type vaginal ring: (A) core; (B) half-
690 overmolded core; (C) final fully overmolded ring device; (D) cross sectional view of ring. In
691 these representative photos, both the sheath layer and core consist of blank silicone elastomer.
692 However, a red dye has been incorporated into the silicone elastomer of the core for illustration
693 purposes only. Note that the core (A) was cut prior to overmolding to compensate for shrinkage
694 upon cooling.

695

696 **Figure 3.** A – Representative DSC traces showing thermal behaviour of DPV, LNG and their
697 mixtures. The traces are presented in concentration order, with 100% LNG at the top of the
698 figure and then each subsequent trace representing a 10% interval. In addition to a crystalline
699 melt, DPV also shows a polymorphic transition $\sim 100^{\circ}\text{C}$. B – Eutectic phase diagram for DPV
700 and LNG constructed from crystalline melt data from A. C – Estimation of eutectic composition
701 (dashed line) from heat of fusion vs LNG concentration plot.

702

703 **Figure 4.** Representative photographs of each ring formulation, presented according to DPV and
704 LNG loading (images not to scale). Letters in the centre of each photograph denote the
705 formulation code (Table 1). Rings D and E appear are semi-transparent due to their low drug
706 loading.

707

708 **Figure 5.** Mean daily release versus time (A) and cumulative release versus root time (B)
709 profiles for release of DPV from MED-4870 matrix-type vaginal rings containing DPV (100, 150
710 and 200 mg per ring), with or without LNG (0, 16 and 32 mg per ring), over 60 days. Error bars
711 in graph A represent \pm standard deviation of six replicates; error bars were often smaller than the
712 plot symbols.

713
714 **Figure 6.** Mean daily release versus time (A) and cumulative release versus root time (B)
715 profiles for release of LNG from MED-4870 matrix rings containing LNG (16 and 32 mg per
716 ring), with or without DPV (0, 100, 150 and 200 mg per ring), over 60 days. Error bars in graph
717 A represent \pm standard deviation of six replicates; error bars were often smaller than the plot
718 symbols.

719
720 **Figure 7.** Mean daily and cumulative release versus time profiles reservoir-type vaginal rings L
721 and M containing DPV and LNG. Each data point in the daily release graphs represents the mean
722 \pm standard deviation of 6 replicates.

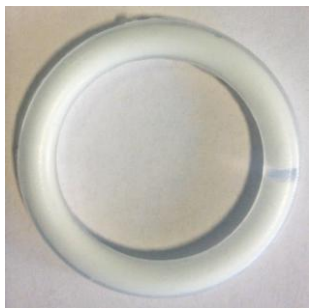
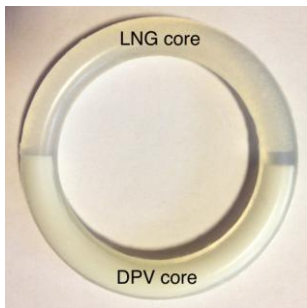
1 **Table 1.** Description of the various matrix-type vaginal ring formulations containing DPV and
 2 LNG.

3

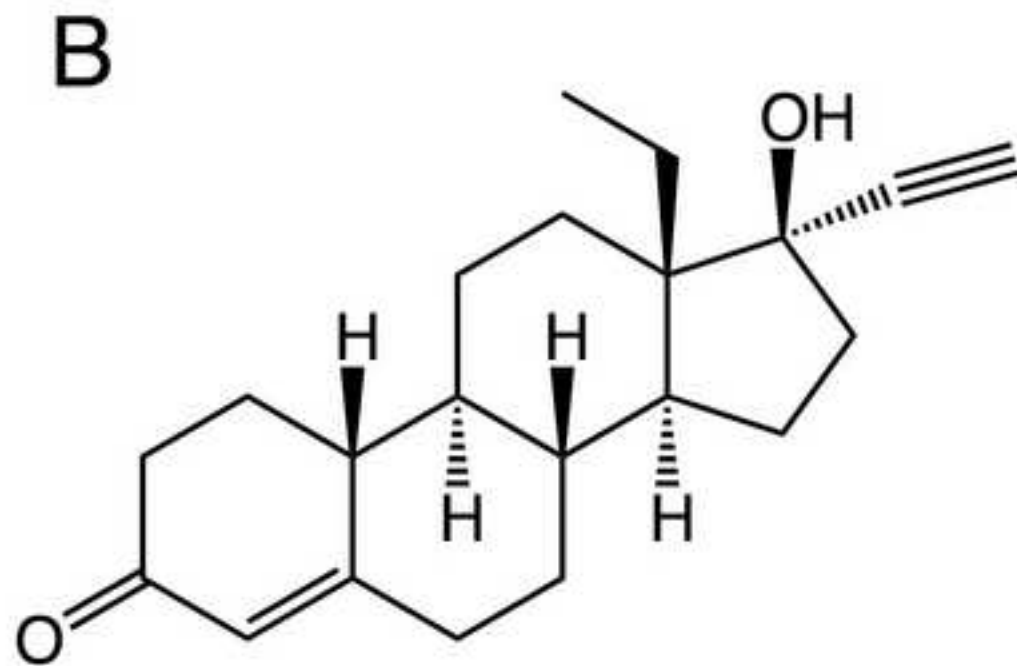
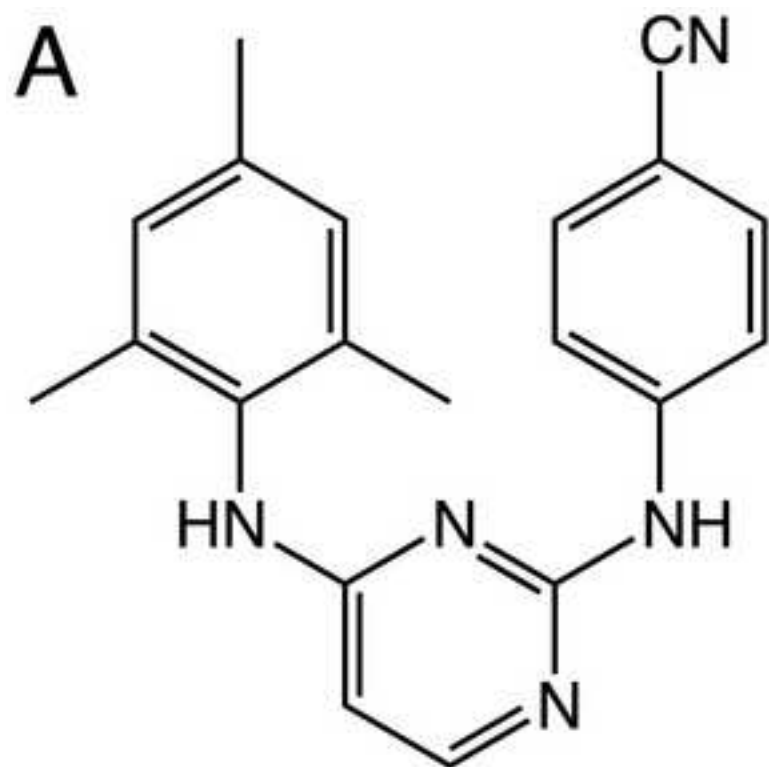
| Formulation | Target DPV loading | | Target LNG loading | | Mean ring mass (g) (\pm SD; n=6) |
|-------------|--------------------|-------|--------------------|-------|-------------------------------------|
| | mg/ring | % w/w | mg/ring | % w/w | |
| A | 100 | 1.25 | – | – | 7.99 (\pm 0.01) |
| B | 150 | 1.88 | – | – | 7.98 (\pm 0.01) |
| C | 200 | 2.50 | – | – | 8.01 (\pm 0.01) |
| D | – | – | 16 | 0.20 | 7.98 (\pm 0.01) |
| E | – | – | 32 | 0.40 | 7.95 (\pm 0.01) |
| F | 100 | 1.25 | 16 | 0.20 | 7.97 (\pm 0.00) |
| G | 100 | 1.25 | 32 | 0.40 | 8.00 (\pm 0.01) |
| H | 150 | 1.88 | 16 | 0.20 | 8.00 (\pm 0.00) |
| I | 150 | 1.88 | 32 | 0.40 | 8.00 (\pm 0.00) |
| J | 200 | 2.50 | 16 | 0.20 | 7.97 (\pm 0.01) |
| K | 200 | 2.50 | 32 | 0.40 | 8.06 (\pm 0.01) |

4

1 **Table 2.** Description of core-type vaginal rings containing DPV and LNG. Values in brackets
 2 represent standard deviations (n=6).

| | Ring Formulation L | Ring Formulation M |
|------------------------------------|---|---|
| Ring type | core-type (reservoir) | core-type (reservoir) |
| Core | single full-length core loaded with both DPV (2% w/w) and LNG (2% w/w) | two half-length cores, one loaded with DPV (2% w/w), the other loaded with LNG (2% w/w) |
| Sheath | non-medicated DDU-4320 1.55 mm thick | non-medicated DDU-4320 1.55 mm thick |
| Representative image* |  |  |
| Mean ring mass (g) | 7.45 (\pm 0.02) | 7.46 (\pm 0.02) |
| Mean core mass (g) | | |
| Core 1 | 2.56 (\pm 0.01) | 1.27 (\pm 0.02) (DAP) |
| Core 2 | – | 1.28 (\pm 0.01) (LNG) |
| Mean sheath mass (g) | 4.89 (\pm 0.02) | 4.91 (\pm 0.02) |
| Mean theoretical drug loading (mg) | | |
| DPV | 51.2 (\pm 0.3) | 25.5 (\pm 0.3) |
| LNG | 51.2 (\pm 0.3) | 25.6 (\pm 0.3) |

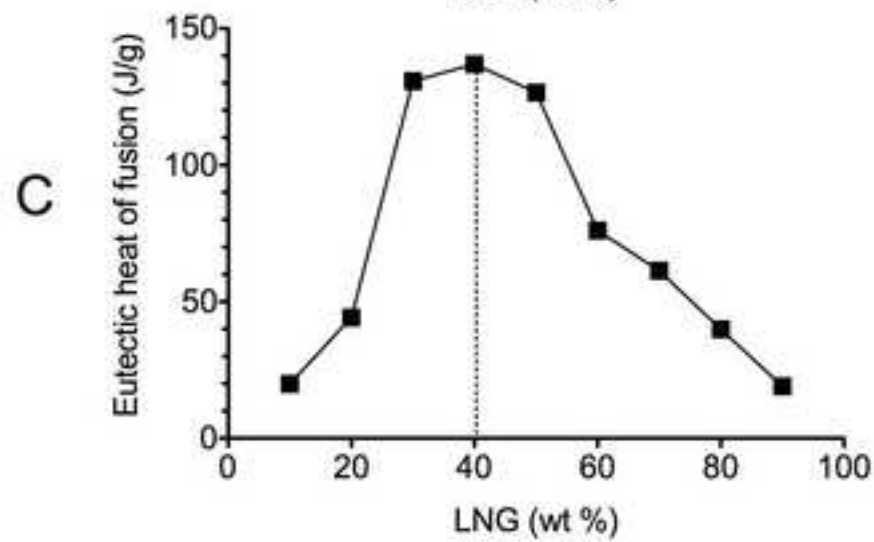
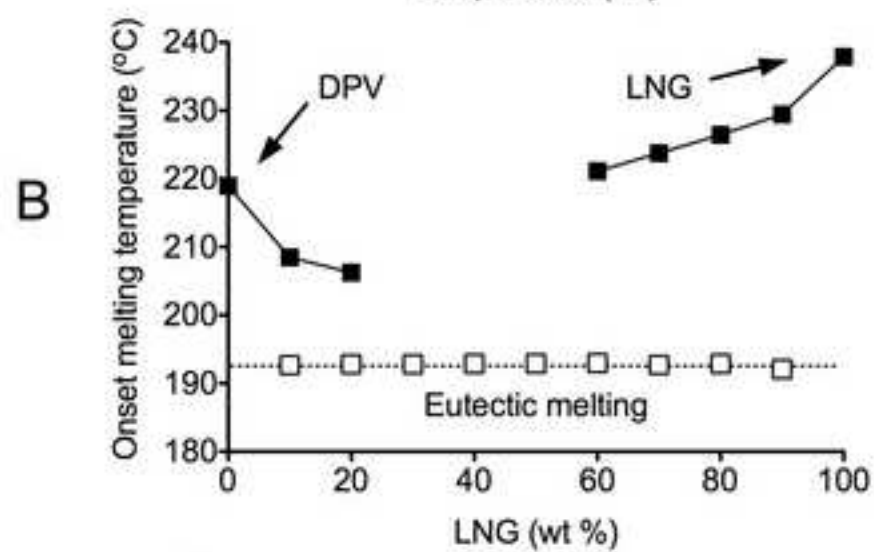
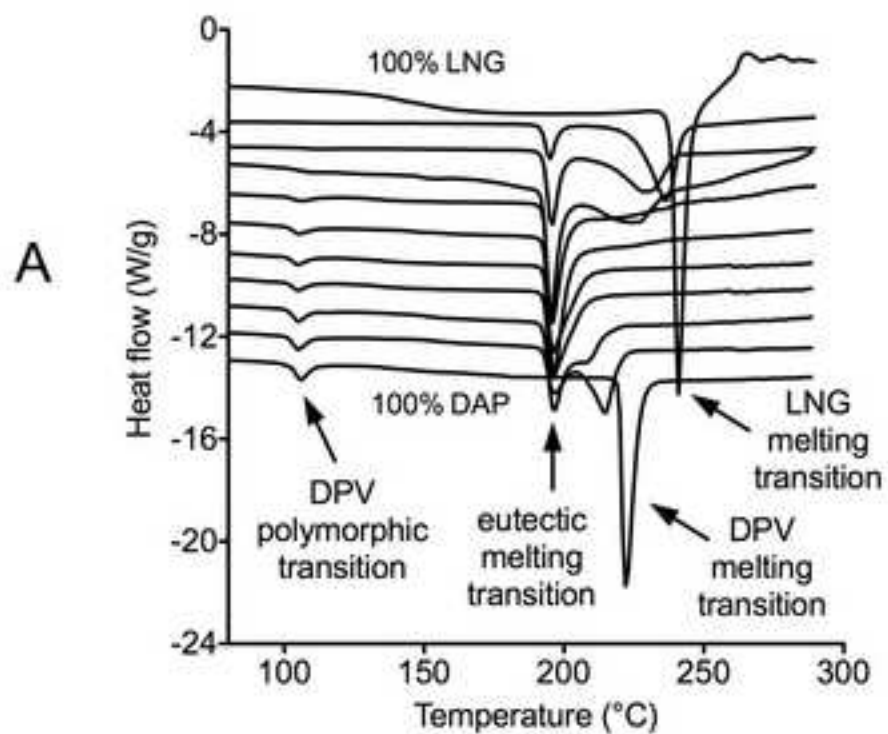
3
 4 * Note the visible gap between the two ends of the core in Ring Formulation L due to the cut made in the
 5 core prior to overmolding. For Ring Formulation M, the two separate half-length cores are clearly visible
 6 in this image; the white core is the DPV-loaded segment (white appearance due to the use of micronized
 7 DPV), while the more transparent core is the LNG-loaded segment (LNG was not micronized; small
 8 particles of LNG were clearly visible in the silicone elastomer, although these may not be evident from
 9 the image in the table.)



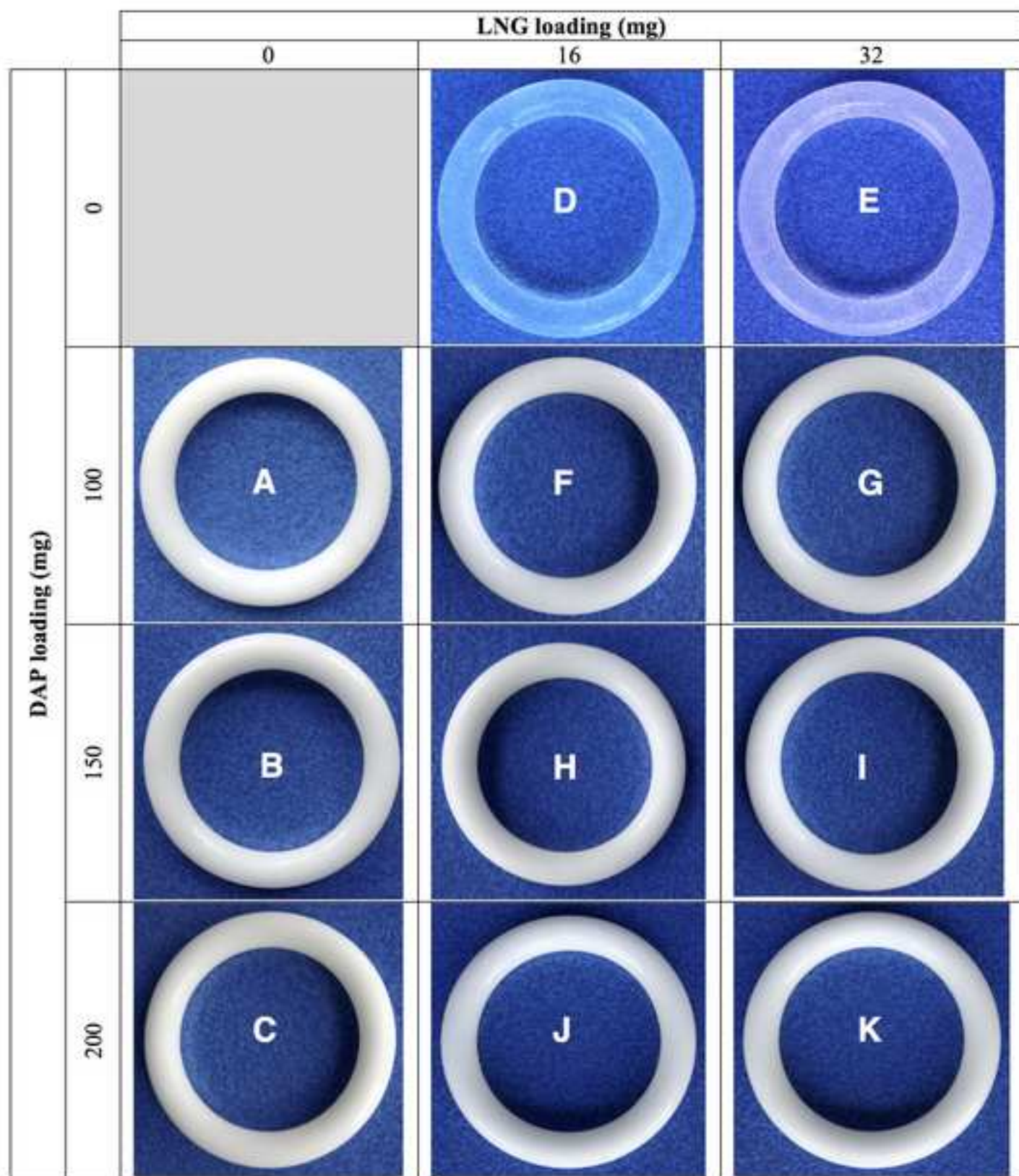
Figure(s)



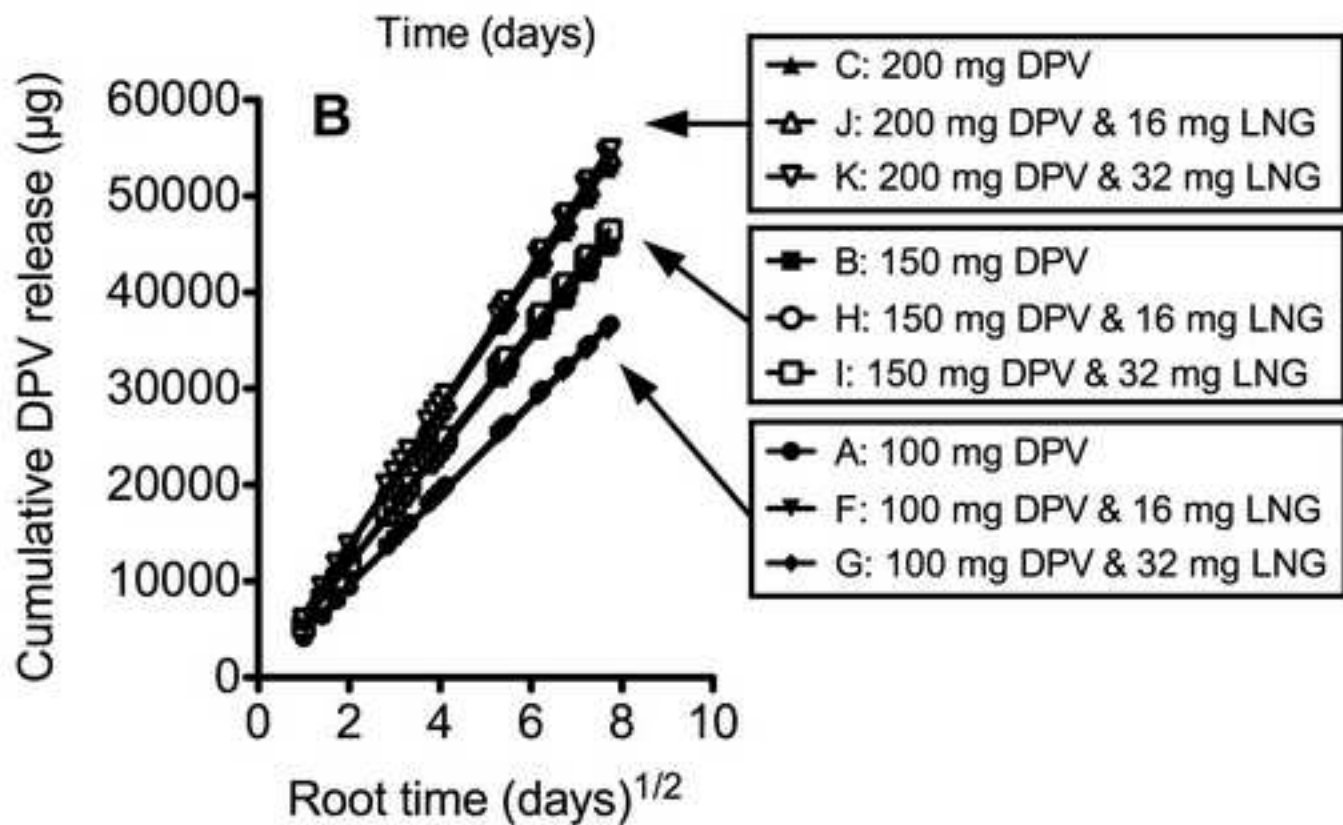
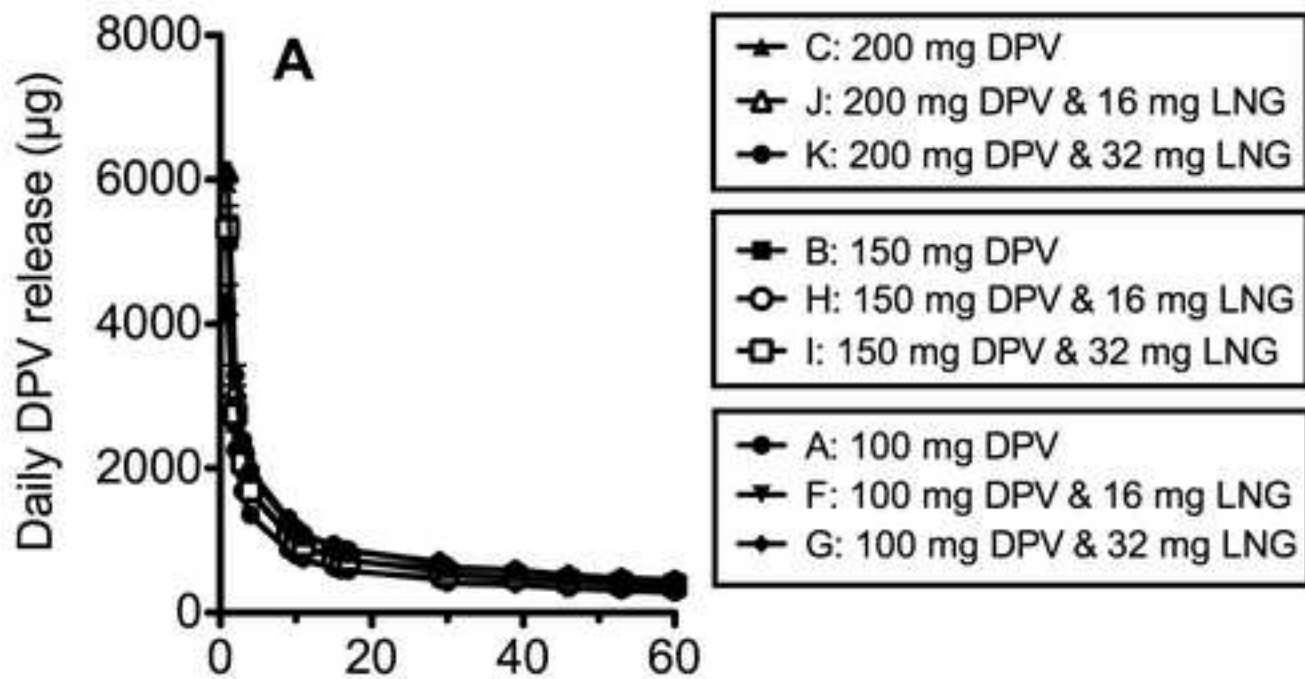
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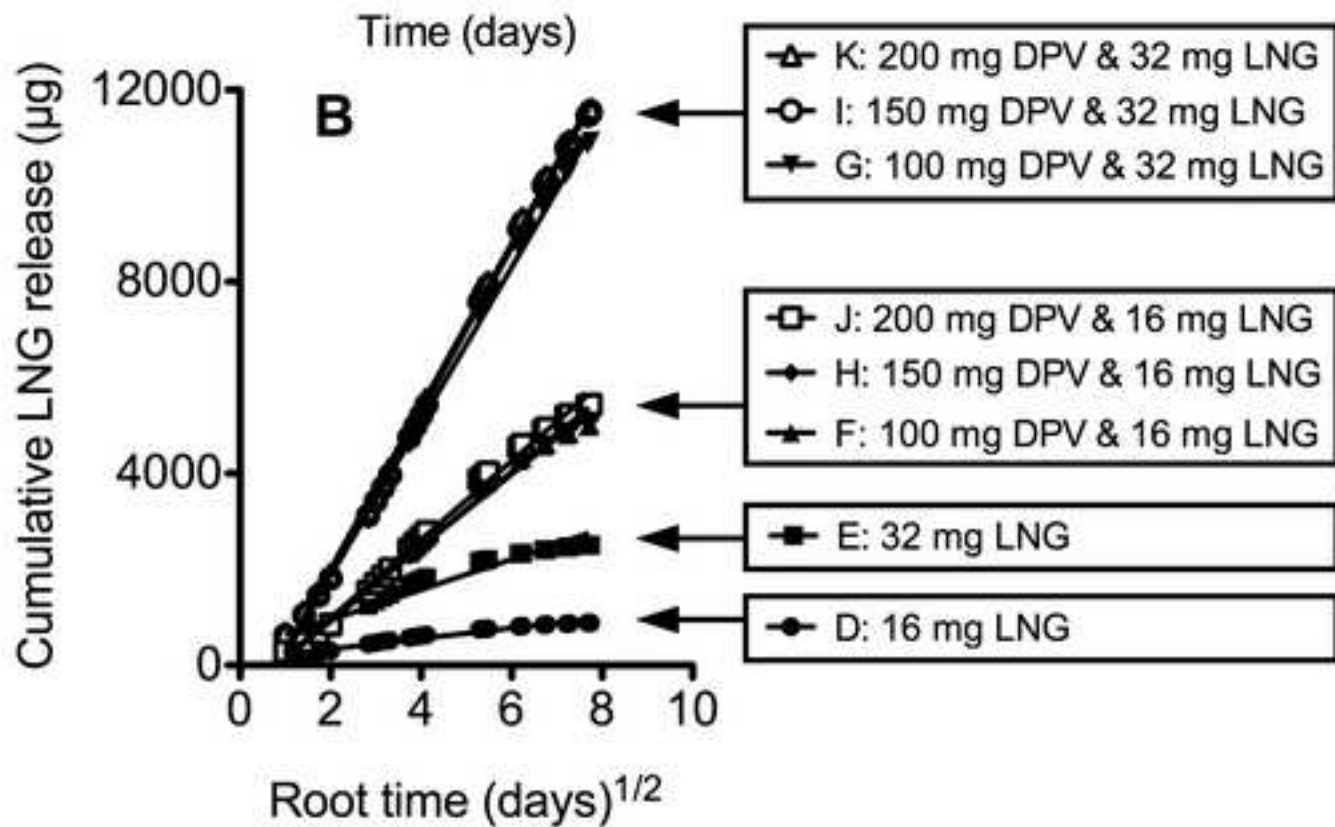
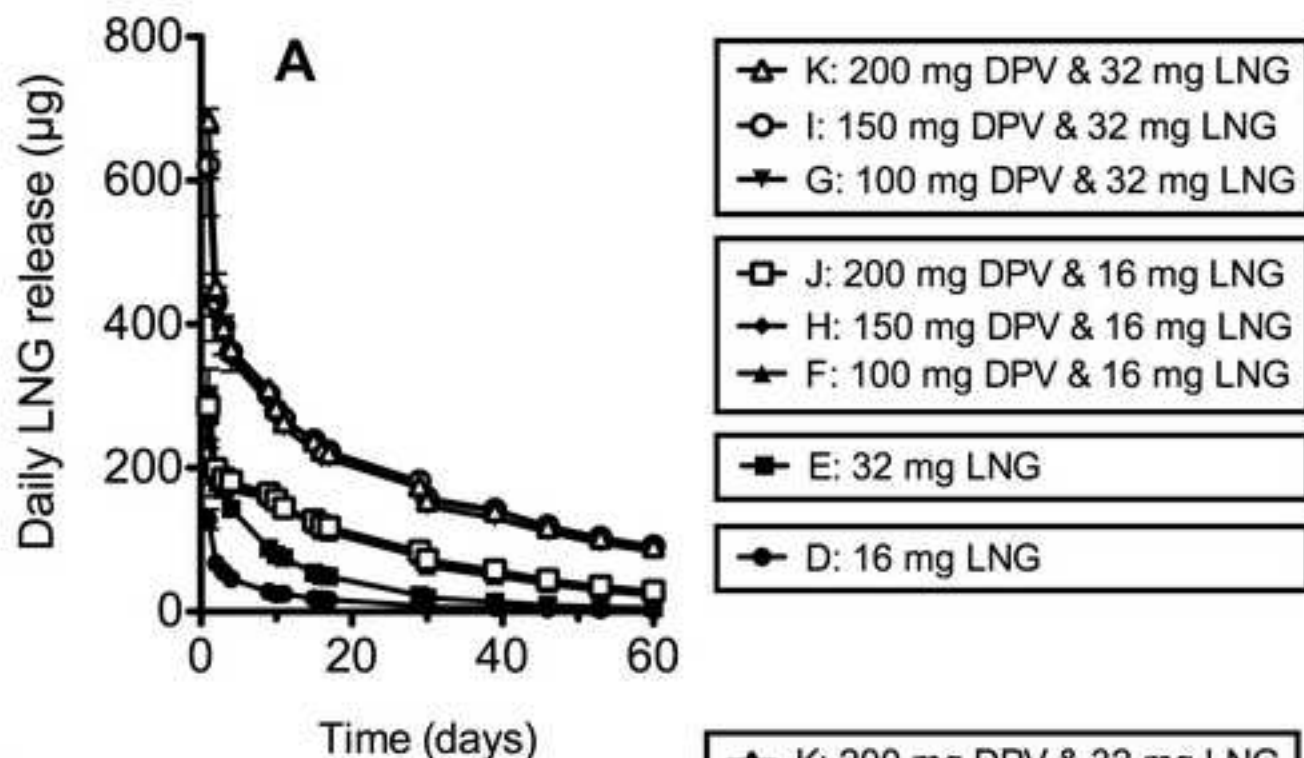
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