**Title**: Comparison of plasma etonogestrel concentrations sampled from the contralateral-to-implant and ipsilateral-to-implant arms of contraceptive implant users

**Authors:**

Alida M Gertz1,2,3

Ian J Bishop4

Boikhutso Simon3

Kwana Lechiile1

Opelo Badubi3

Aamirah Mussa3

Carolyn L Westhoff4$

Chelsea Morroni1,3,5$\*

$These authors contributed equally to the manuscript

**\*Corresponding author:** Chelsea Morroni, email: chelsea.morroni@lstmed.ac.uk, tel: +267 765 24112

Postal address: Botswana Harvard Partnership, Private Bag BO 320, Gaborone, Botswana.

1. Botswana-University of Pennsylvania Partnership, University of Botswana Main Campus

244G - Room 103, Gaborone, Botswana

2. Afya Bora Consortium Fellowship Program

3. Botswana Harvard Partnership, Private Bag BO 320, Gaborone, Botswana

4. Columbia University Medical Center, New York, USA, 622 West 168th Street

New York, NY 10032, United States.

5. Liverpool School of Tropical Medicine, Pembroke Pl, Liverpool L3 5QA, United Kingdom

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**ABSTRACT**

**Objective:** To compare plasma etonogestrel concentrations sampled from the contralateral- versus ipsilateral-to-implant arm.

**Study Design:** Sub-analysis of a cross-sectional study in Botswana in 33 participants who provided contralateral and ipsilateral blood samples.

**Results:** Plasma etonogestrel concentrations in contralateral and ipsilateral specimens were highly correlated (correlation coefficient=0.99; p<0.0001).Bland-Altman analysis of agreement showed that etonogestrel levels were on average 5.9 pg/mL higher (2.1%) in ipsilateral compared to contralateral specimens (95% confidence interval: -4.1, 15.9 pg/mL).

**Conclusions:** We found no meaningful differences in plasma etonogestrel concentrations between samples taken from the contralateral- versus ipsilateral-to implant arm.

**Keywords:**

Contraceptive implant; etonogestrel; pharmacokinetics; ipsilateral arm; contralateral arm

**Implications**

Our data suggest that etonogestrel plasma concentrations are unlikely to be meaningfully different between samples drawn from the ipsilateral- versus the contralateral-to-implant arms in etonogestrel contraceptive implant users.

**1. INTRODUCTION**

In contraceptive implant pharmacokinetic studies, blood specimens are conventionally sampled from the arm contralateral to where the implant is located. Data from two studies of the six-rod levonorgestrel implant in the 1980s suggested that samples drawn from the ipsilateral-to-implant arm (arm where implant is located) had two-to-three times higher hormone concentrations compared to samples drawn from the contralateral-to-implant arm[[1,2]](https://paperpile.com/c/Me28Jr/N9Vi+XfpT). Subsequent pharmacokinetic studies have specified that samples be drawn from the contralateral-arm[[3–5]](https://paperpile.com/c/Me28Jr/lb2n+v0R1+SP46). Single-rod etonogestrel and two-rod levonorgestrel implants have now replaced the six-rod levonorgestrel implant globally. The contraceptive implant is ideal for pharmacokinetic and drug-drug interaction studies given no compliance concerns and its steady-drug release rates[[6]](https://paperpile.com/c/Me28Jr/Eav7). For this reason, many studies examine implant hormone concentrations and drug-drug interactions. Knowing whether adherence to this practice of contralateral-arm sampling is still necessary may, therefore, be useful.

In a recent Botswana-based study examining plasma etonogestrel concentrations in implant users[[5]](https://paperpile.com/c/Me28Jr/SP46), samples from two participants were accidentally drawn from the ipsilateral arm. This prompted us to investigate agreement between plasma etonogestrel concentrations sampled from the contralateral- versus ipsilateral-to-implant arms by obtaining paired samples in a subset of participants. We sought to evaluate whether there are meaningful differences in ipsilateral versus contralateral plasma etonogestrel concentrations.

**2. METHODS**

We conducted a cross-sectional study with a single time-point sample (details in [[5]](https://paperpile.com/c/Me28Jr/SP46)). Eligible women used the etonogestrel implant for 3-12 months. We based the sample size on the only published similar studies [[1,2]](https://paperpile.com/c/Me28Jr/N9Vi+XfpT). After written informed consent, participants provided a blood sample from both arms. The phlebotomist palpated the implant prior to sample collection. Like previous researchers, we did not record where the tourniquet was placed and for how long, and did not record the sequence of blood draws. Alvarez, et al[[2]](https://paperpile.com/c/Me28Jr/XfpT) had reported that applying the tourniquet for variable periods before blood sampling did not influence plasma levonorgestrel concentrations. Blood pressure measurements had not been taken. Blood samples were collected into K-EDTA plasma tubes, stored on ice (4°C), transported to the University of Botswana/UPenn Laboratory, and centrifuged at 3400 revolutions/minute for 10-minutes to separate plasma within 24-hours of collection. Plasma aliquots were stored at -80°C and shipped on dry ice to the Columbia University Biomarkers Core Laboratory. Technicians were blinded to participant identity and laterality of the samples and analysed in batches consisting of both contralateral- and ipsilateral-to-arm samples to avoid any variation due to machine calibration. The laboratory measured etonogestrel in plasma by liquid chromatography-mass spectrometry after liquid/liquid extraction using D-8 Progesterone as the internal standard[[7]](https://paperpile.com/c/Me28Jr/H1VR). We calculated the level of agreement between contralateral and ipsilateral specimen etonogestrel plasma concentrations using Bland Altman Plot analysis[[8]](https://paperpile.com/c/Me28Jr/VERH) using STATA 16 (College Station, Texas).

The University of Botswana, Health Research and Development Division of the Botswana Ministry of Health and Wellness, Princess Marina Hospital, and Columbia University Irving Medical Center institutional review boards approved the study. This study followed the Declaration of Helsinki recommendations and was registered at clinicaltrials.gov (NCT03336346).

**3. RESULTS**

Thirty-three participants provided paired contralateral-ipsilateral samples for this analysis: 12 HIV-negative women, 18 using dolutegravir-based ART, and three using efavirenz-based ART. Median implant use duration was 203 days (interquartile range (IQR): 110-274), and median BMI was 23.7 kg/m2 (IQR 20.3-27.7). The median etonogestrel concentration measured from the contralateral-arm sample was 283.7 pg/mL (range: 104.9-649.7), and from the ipsilateral-arm was 281.0 pg/mL (range: 98.5-607.3).

Figure 1 shows level of agreement between plasma etonogestrel concentrations in samples drawn from the ipsilateral arm and the contralateral arm. The Spearman’s correlation coefficient between plasma etonogestrel concentrations for contralateral and ipsilateral-arm specimens was 0.99 (p<0.001), indicating very high correlation. Bland-Altman analysis comparing ipsilateral to contralateral arms showed that on average, etonogestrel levels were 5.9 pg/mL higher (2.1%) in ipsilateral-arm specimens compared to contralateral-arm specimens with a 95% confidence interval of -4.1-15.9 pg/mL (1B). The limits of agreement (which represent +/- 1.96 standard deviations on either side of the mean difference and thus include 95% of differences) ranged from 50.9 pg/mL lower to 62.6 pg/mL higher. The differences between sample arms did not vary in any systematic way over the concentration range (Pitman's Test of difference in variance: r=0.03, p=0.86) or with length of implant use (linear regression coefficient=0.05; 95% confidence interval -0.057 to 0.16; p-value 0.34).

**4. DISCUSSION**

We found no meaningful difference in plasma etonogestrel concentrations in blood specimens taken at the same time from participants’ contralateral-to-implant and ipsilateral-to-implant arms. Plasma etonogestrel concentrations were on average about 6 pg/mL higher in the ipsilateral-arm compared to the contralateral-arm, with a 95% confidence interval that included zero.

We found no studies assessing whether etonogestrel concentrations differ in the contralateral- versus ipsilateral-arm. Our findings differ from older studies on this topic. In a 1981 study of the six-rod levonorgestrel implant by Croxatto, et al. [1], in which eight participants provided multiple samples from both contralateral and ipsilateral-arms, it was found that ipsilateral samples contained two-to-three times more levonorgestrel than contralateral samples. In 1983, Alvarez, et al.[[2]](https://paperpile.com/c/Me28Jr/XfpT) also reported two-to-three times higher ipsilateral compared to contralateral levonorgestrel concentrations in 17 users of the levonorgestrel six-rod implant and 11 participants using either the four or six-covered silastic rods.

The present and previous implants differ considerably. Factors that could affect pharmacokinetic properties and account for differences seen in our study include different hormones, different doses (levonorgestrel 216mg in six-rod implants versus etonogestrel 68mg in single-rod implants), different implant sites, and different membrane materials, which control release rates of the hormonal ingredient. Etonogestrel implants are rods in which etonogestrel is embedded within an ethylene vinyl acetate (EVA) matrix, and the rod is then wrapped with an EVA membrane[[9]](https://paperpile.com/c/Me28Jr/hhHR). The older six-capsule levonorgestrel implants contained levonorgestrel crystals that were encased in silastic tubing (polydimethylsiloxane and methylvinyl siloxane copolymer)[[9,10]](https://paperpile.com/c/Me28Jr/hhHR+JWps). Also, in the earlier studies, implants had been placed in the anterior aspect of the forearm, not in the upper-arm[[1,2]](https://paperpile.com/c/Me28Jr/N9Vi+XfpT). In our study, we sampled participants between 3-12 months of implant use whereas Croxatto et al. [1] sampled between 11-58 months; Alvarez, et al. [2] did not report duration of implant use. Exactly how these factors might explain our different findings with regards to contralateral and ipsilateral concentrations is not clear, but implant site may be the key difference. Similar data regarding the two-rod levonorgestrel implant are lacking.

Overall, these exploratory data suggest that with the single-rod etonogestrel contraceptive implant, when drawing samples from the ipsilateral or contralateral arms, it is unlikely that there will be appreciable differences in the measured plasma etonogestrel concentration for an individual.

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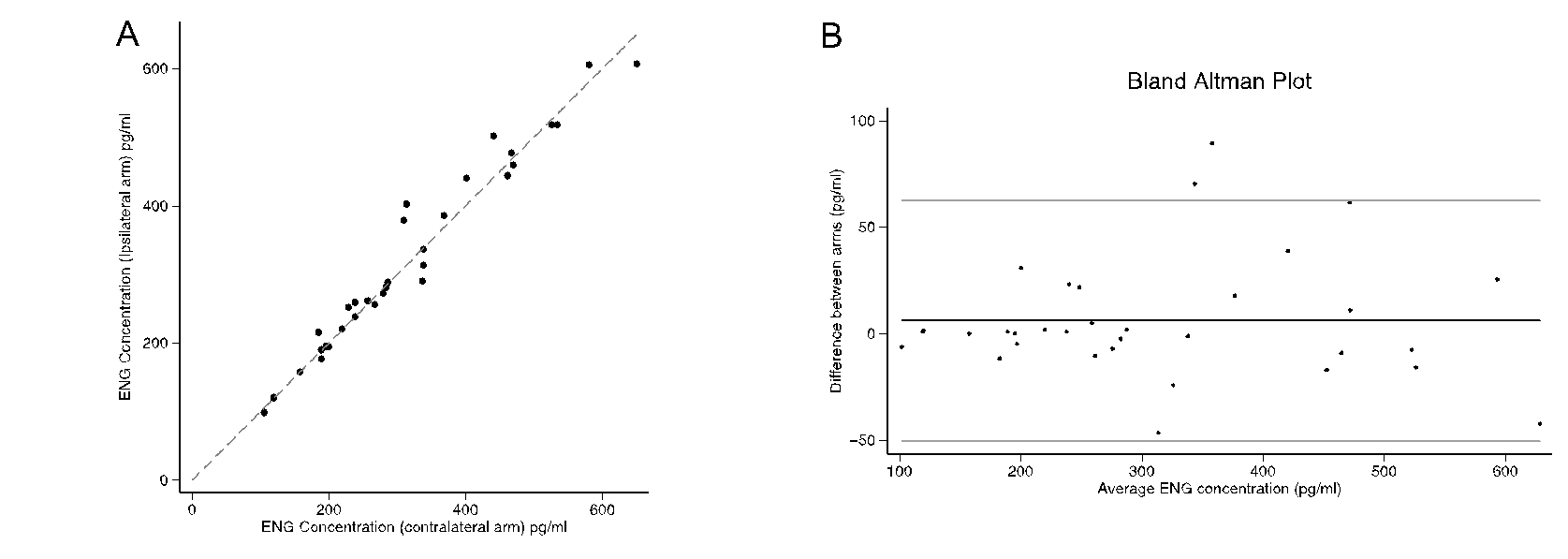
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**Figure Legend**

**Figure 1a:** Plot of plasma etonogestrel concentrations (pg/mL) in contralateral versus ipsilateral arm samples in a sub-analysis of 33 participants in a pharmacokinetic study of the etonogestrel implant in Botswana. Each point on the plot represents an individual participant. The dotted line on the plot represents the hypothetical line of equality, where the ipsilateral concentration is exactly equal to the contralateral concentration for each participant. ENG, etonogestrel.

**Figure 1b:** A Bland-Altman Plot for difference in plasma etonogestrel concentrations (pg/mL) comparing ipsilateral-to-implant to contralateral-to-implant samples in 33 participants in a pharmacokinetic study of the etonogestrel implant in Botswana.The Bland-Altman Plot is a plot of the difference between ipsilateral-to-implant and contralateral-to-implant etonogestrel concentrations against their mean. The mean difference was -5.9 (95% CI -15.9 to 4.1 pg/mL). The limits of agreement (i.e., -1.96sd to +1.96sd) are shown by the grey lines and range from -50.9 to 62.6 pg/mL. ENG, etonogestrel.

**Figure 1.**

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**Supplemental Table**. Contralateral and ipsilateral arm etonogestrel plasma concentrations (pg/mL) in all 33 participants in a pharmacokinetic study of the etonogestrel implant in Botswana.BMI, body mass index; ART, antiretroviral therapy (DTG, dolutegravir-based ART; EFV, efavirenz-based ART NEG, HIV-negative); ENG, etonogestrel.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Participant | Age | BMI | ART Regimen | Days of implant use | Contralateral ENG plasma concentration (pg/ml) | Ipsilateral ENG plasma concentration (pg/ml) | Mean ENG plasma concentration  (pg/ml) | Difference in contralateral and ipsilateral ENG plasma concentration  (pg/ml) |
| 1 | 27 | 27.5 | DTG | 139 | 337.02 | 290.46 | 313.74 | -46.56 |
| 2 | 40 | 27.6 | DTG | 214 | 649.72 | 607.28 | 628.5 | -42.44 |
| 3 | 38 | 24.5 | DTG | 174 | 337.94 | 313.60 | 325.77 | -24.34 |
| 4 | 34 | 27.7 | NEG | 286 | 460.95 | 443.92 | 452.43 | -17.03 |
| 5 | 22 | 21.7 | DTG | 98 | 533.84 | 517.92 | 525.88 | -15.92 |
| 6 | 40 | 31.6 | NEG | 354 | 188.71 | 176.84 | 182.77 | -11.87 |
| 7 | 39 | 40.5 | NEG | 91 | 266.79 | 256.30 | 261.54 | -10.49 |
| 8 | 34 | 19.6 | NEG | 96 | 468.94 | 459.87 | 464.40 | -9.07 |
| 9 | 27 | 32.0 | DTG | 190 | 526.24 | 518.53 | 522.38 | -7.71 |
| 10 | 35 | 20.1 | DTG | 364 | 279.33 | 272.21 | 275.77 | -7.12 |
| 11 | 20 | 26.1 | EFV | 264 | 104.89 | 98.54 | 101.71 | -6.35 |
| 12 | 30 | 18.8 | DTG | 99 | 199.49 | 194.71 | 197.1 | -4.78 |
| 13 | 30 | 23.2 | NEG | 203 | 283.72 | 281.01 | 282.36 | -2.71 |
| 14 | 29 | 28.3 | DTG | 198 | 338.37 | 337.15 | 337.76 | -1.22 |
| 15 | 36 | 25.7 | NEG | 147 | 157.73 | 157.61 | 157.67 | -0.12 |
| 16 | 31 | 26.1 | NEG | 304 | 195.15 | 195.20 | 195.17 | 0.05 |
| 17 | 35 | 23.7 | EFV | 272 | 118.67 | 119.57 | 119.12 | 0.90 |
| 18 | 31 | 23.4 | DTG | 361 | 188.96 | 189.93 | 189.44 | 0.97 |
| 19 | 19 | 18.5 | DTG | 121 | 237.71 | 238.69 | 238.2 | 0.98 |
| 20 | 27 | 17.7 | EFV | 152 | 119.10 | 120.43 | 119.76 | 1.33 |
| 21 | 22 | 20.4 | NEG | 242 | 219.02 | 220.78 | 219.9 | 1.76 |
| 22 | 35 | 29.7 | DTG | 98 | 286.33 | 288.12 | 287.22 | 1.79 |
| 23 | 23 | 21.5 | NEG | 265 | 256.46 | 261.27 | 258.86 | 4.81 |
| 24 | 26 | 18.7 | DTG | 93 | 466.67 | 477.51 | 472.09 | 10.84 |
| 25 | 24 | 29.1 | DTG | 231 | 367.77 | 385.36 | 376.56 | 17.59 |
| 26 | 27 | 23.5 | NEG | 326 | 237.72 | 259.33 | 248.52 | 21.61 |
| 27 | 19 | 18.3 | NEG | 230 | 228.36 | 251.48 | 239.92 | 23.12 |
| 28 | 35 | 26.5 | DTG | 195 | 580.51 | 605.84 | 593.17 | 25.33 |
| 29 | 24 | 20.9 | DTG | 276 | 184.86 | 215.75 | 200.30 | 30.89 |
| 30 | 19 | 20.2 | DTG | 97 | 401.26 | 440.00 | 420.63 | 38.74 |
| 31 | 32 | 27.4 | NEG | 122 | 440.91 | 502.52 | 471.71 | 61.61 |
| 32 | 44 | 30.1 | DTG | 332 | 308.67 | 378.98 | 343.82 | 70.31 |
| 33 | 40 | 23.7 | DTG | 230 | 313.35 | 402.72 | 358.03 | 89.37 |