Notes on the Design of Bioequivalence Study: Levonorgestrel implant

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Unit – Medicines Assessment Team (PQT/MED) are issued to aid manufacturers with the development of their product dossier. Deviations from the approach suggested below can be considered acceptable if justified by sound scientific evidence.

The current notes should be read and followed in line with the general guidelines of submission of documentation for WHO prequalification. In particular, please consult the "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability". In: *Fifty-seventh Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations*. Geneva, World Health Organization, 2024. WHO Technical Report Series, No. 1052, Annex 8.

Below, additional specific guidance is provided on the invited prolonged release 2-rod hormone releasing implant containing levonorgestrel.

Pharmacokinetics of levonorgestrel

Levonorgestrel is delivered directly into interstitial fluids from the subdermal implants. However, the bioavailability of levonorgestrel after insertion of the implants compared with intravenous administration is not known. After placement of the implants, maximum levonorgestrel concentrations are reached in about 2 to 3 days, with the mean ± standard deviation being 772 ± 414 pg/mL at 2 days. After the initial phase, mean levonorgestrel concentrations slowly decline to approximately 435 ± 172 pg/mL at 1 month, 357 ± 155 pg/mL at 6 months, and 280 ± 123 pg/mL at 3 years. Concentrations at 4 and at 5 years are similar to those at 3 years.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of levonorgestrel implants, the following guidance with regard to the study design should be taken into account:

Design: A single-dose parallel design is recommended.

Dose: As the EoI includes two-rod hormone releasing implant of levonorgestrel, each rod containing 75 mg (150 mg in total), the bioequivalence study should be conducted with this product.

Fasted/fed: N/A.

Subjects: Healthy adult female subjects should be recruited. It is not necessary to include patients in the bioequivalence study.

Parent or metabolite data for assessment of bioequivalence: The parent drug is considered to best reflect the biopharmaceutical quality of the product. The data for the parent compound should be used to assess bioequivalence of levonorgestrel.

Sample size: Levonorgestrel C_{max} inter-subject variability seems to be around 40 – 50%. These data may facilitate the calculation of a sufficient sample size for a single dose parallel bioequivalence study.

Washout: N/A.

Blood sampling: The blood sampling should be intensive for the first days after administration to properly characterize the C_{max} of levonorgestrel. For example, samples can be taken pre-dose and 6.0, 12, 24, 36, 48, 54, 60, 66, 72, 84, 96, 108, 120 144 and 168 hours after injection as well as 1, 3, and 6 months after injection and 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 years after injection.

Analytical considerations: Information currently available indicates that it is possible to measure levonorgestrel in human plasma using LC-MS/MS analytical methodology (e.g., 25 pg/ml). The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the C_{max} in most profiles of each formulation (test or comparator). The bioanalytical method for each analyte should be validated in the presence of the other analyte (See Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information on bioanalytical recommendations).

Statistical considerations: The data for levonorgestrel should meet the following bioequivalence standards in a single-dose parallel design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}t}$ of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean $C\tau$ ($C_{5\ years}$) of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean $AUC_{0\text{-}1\ year}$, $AUC_{1\text{-}2\ years}$, $AUC_{2\text{-}3\ years}$, $AUC_{3\text{-}4\ years}$ and $AUC_{4\text{-}5\ years}$ should be submitted as supportive information.