Persistent organic pollutants (POPs) accumulated in human tissues may pose a risk for human health by interfering with the endocrine system. This study establishes a new link between actual human internal POP levels and the endocrine active dose in vitro, applying partitioning-controlled dosing from silicone to the H295R steroidogenesis assay: (1) Measured concentrations of POPs in silicone breast implants were taken from a recent study and silicone disks were loaded according to these measurements. (2) Silicone disks were transferred into H295R cell culture plates in order to control exposure of the adrenal cells by equilibrium partitioning. (3) Hormone production of the adrenal cells was measured as toxicity endpoint. 4-Nonylphenol was used for method development, and the new dosing method was compared to conventional solvent-dosing. The two dosing modes yielded similar dose-dependent hormonal responses of H295R cells. However, with the partitioning-controlled freely dissolved concentrations (C_{free}) as dose metrics, dose–response curves were left-shifted by two orders of magnitude relative to spiked concentrations. Partitioning-controlled dosing of POPs resulted in up to 2-fold increases in progestagen and corticosteroid levels at C_{free} of individual POPs in or below the femtomolar range. Silicone acted not only as source of the POPs but also as a sorption sink for lipophilic hormones, stimulating the cellular hormone production. Methodologically, the study showed that silicone can be used as reference partitioning phase to transfer in vivo exposure in humans (silicone implants) to in vitro assays (partition-controlled dosing). The main finding was that POPs at the levels at which they are found in humans can interfere with steroidogenesis in a human adrenocortical cell line.