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Lack of Adverse Effects on Fertility of Female CD-1 Mice Exposed to Repetitive Intravaginal Gel–Microemulsion Formulation of a Dual-function Anti-HIV Agent: Aryl Phosphate Derivative of Bromo-methoxy-zidovudine (Compound WHI-07)

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5-bromo-6-methoxy-5,6-dihydro-3′-azidothymidine-5′-(p-bromophenyl) methoxyalaninyl phosphate **(WHI-07), a novel bromo-methoxy-substituted aryl phosphate derivative of zidovudine (ZDV), is a potent dual-function contraceptive agent with anti-HIV activity. Its potential for reproductive toxicity was assessed in a series of experiments using CD-1 mice under the conditions of its intended use as an intravaginal microbicide. Female CD-1 mice were exposed intravaginally to a gel–microemulsion formulation containing 0%, 0.5%, 1.0% or 2.0% WHI-07 for up to 13 weeks. On a molar basis, these concentrations represent 1400–5700 times its *in vitro* spermicidal IC_{50} and 1.4–5.7 ($\times 10^6$) times its *in vitro* anti-HIV IC_{50}. We examined the effects of intravaginally administered WHI-07 on: ovulation efficiency; *in vivo* fertilization and early embryonic, fetal development; and reproductive outcome, including neonatal survival and pup development. Compound WHI-07 was administered intravaginally during superovulation, organogenesis and prior to mating for 5 and 10 consecutive days and for 13 weeks, respectively. Mice were evaluated for ovulation efficiency and fertilization rate and cleavage 14 and 40 h after human chorionic gonadotropin (hCG) injection, respectively. Pregnant mice were administered 2% WHI-07 intravaginally during gestation days (GD) 6–15 and measures of teratogenicity were evaluated on GD 17. For short-term toxicity study, mice were given intravaginal treatment of gel–microemulsion containing 0%, 0.5%, 1.0% and 2.0% WHI-07 for 13 weeks and then mated with untreated males to evaluate potential reproductive and developmental effects. Repeated intravaginal exposure of mice to 2% WHI-07 had no adverse effects on ovulation response, mean number of eggs recovered or the percentage of eggs fertilized or cleaved. No evidence of reproductive toxicity, fetal toxicity or teratogenicity was found following repetitive intravaginal application of 2% WHI-07 during the period of organogenesis. Furthermore, repeated intravaginal exposure of mice to 0.5–2.0% WHI-07 for 13 weeks had no adverse effect on the subsequent reproductive capability, perinatal outcome or growth and development of the offspring. Compound WHI-07 shows unique clinical potential as a safe, dual-function vaginal contraceptive for curbing mucosal and perinatal HIV transmission.**

INTRODUCTION

Sexual transmission of human immunodeficiency virus, type 1 (HIV-1), the causative agent of acquired immune deficiency syndrome (AIDS), continues to be the predominant mode of the epidemic spread of HIV/AIDS.[1] The emergence of AIDS as a disease spread through sexual intercourse has prompted the search for new, effective, safe and female-controlled vaginal microbicides for curbing mucosal and perinatal viral transmission.[2] Microbicides would provide protection by inactivating viruses or preventing viruses from replicating either in semen or the infected host cells that line the vaginal wall.

In a systematic effort to develop a microbicidal contraceptive potentially capable of preventing HIV transmission as well as providing fertility control, we previously identified novel dual-function aryl phosphate derivatives of 3′-azido-3′-deoxythymidine (zidovudine, ZDV) that exhibit potent anti-HIV and spermicidal activities.[3,4] The

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50% inhibitory concentrations (IC_{50} values) for the *in vitro* anti-HIV and spermicidal activity of the lead compound *5-bromo-6-methoxy-5,6-dihydro-3′-azidothymidine-5′-(p-bromophenyl) methoxyalaninyl phosphate* (WHI-07) were 439-fold and 13.5-fold lower, respectively, than those for the detergent-based virucidal spermicide nonoxynol-9 (N-9). At spermicidal concentrations, WHI-07 was non-cytotoxic to normal human female genital tract epithelial cells.[4,5] Unlike the intravaginally applied N-9, repeated intravaginal application of WHI-07 via a gel–microemulsion formulation did not damage the vaginal epithelium or cause local inflammation in the rabbit vaginal irritation test.[4] Furthermore, repeated intravaginal exposure of $B_6C_3F_1$ mice to increasing concentrations of WHI-07 for 13 weeks had no adverse effects on survival, growth, metabolism or organ functioning.[6] The dual-function properties of WHI-07 and its lack of cytotoxic and inflammatory effects make it an attractive lead compound for further development as a topical anti-HIV and spermicidal agent.

Compound WHI-07 is a derivative of the nucleoside analog ZDV that was designed to bypass the thymidine kinase-dependent activation of ZDV in seminal cells as well as genital tract epithelial cells of the cervicovaginal region.[7] The aryl phosphate derivatives of bromomethoxy-ZDV have been shown previously to be active against ZDV-resistant HIV isolates and inhibit HIV replication in thymidine kinase-deficient cells.[8] Because WHI-07 is non-cytotoxic and is not absorbed systemically when given intravaginally, it is unlikely to have adverse effects on fertility. However, because oral administration of the parent compound ZDV has been shown to be toxic to early murine embryos,[9,10] it was prudent to test the potential effects of this dual-function ZDV derivative on reproduction/fertility, embryogenesis and viability and development of the fetus. It is anticipated that, under the conditions of its intended use as a intravaginal microbicide, both pregnant and non-pregnant women may be exposed to WHI-07 on a short-term repeated use. Therefore, it was necessary to determine the effects of repeated intravaginal exposure of WHI-07 on ovulation efficiency, *in vivo* fertilization, prenatal embryonic and fetal development and peri/postnatal outcome, including neonatal survival and pup development. These studies were performed in outbred CD-1 mice.

MATERIALS AND METHODS

Chemical synthesis and characterization of WHI-07

Compound WHI-07 was synthesized by phosphorochloridate chemistry in four steps, according to our earlier published procedure.[4,11] In brief, *p*-bromophenol was treated with phosphorus oxychloride to produce its phosphorodichloridate derivative. After purification, the phosphorodichloridate was condensed with methoxyalaninyl ester at low temperature (−78 °C) in methylene chloride to furnish the *p*-bromophenyl alanine phosphochloridate. Condensation of this compound with ZDV in anhydrous tetrahydrofuran gave the aryl phosphoramidate derivative of ZDV (*3′-azidothymidine alaninyl phosphodichloridate*), which was modified further by reacting with a solution of bromine in anhydrous methanol to produce the target aryl phosphate derivative of ZDV (WHI-07; Fig. 1).

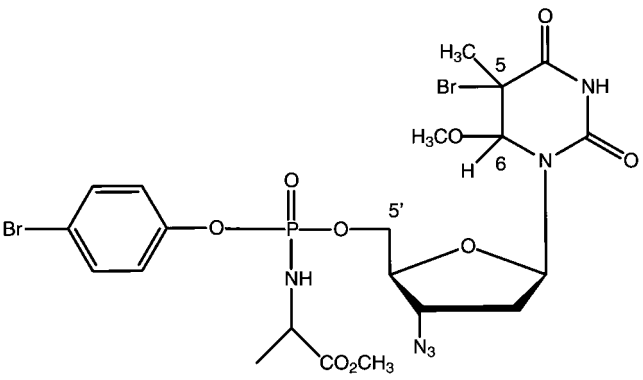

Figure 1. The chemical structure of WHI-07: 5-bromo-6-methoxy-5,6-dihydro-3′-azidothymidine-5′-(*p*-bromophenyl) methoxyalaninyl phosphate.

The pure WHI-07 (*5-bromo-6-methoxy-5,6-dihydro-3′-azidothymidine-5′-(p-bromophenyl) methoxyalaninyl phosphate*) was obtained as white flakes following silica-gel column chromatography of crude WHI-07 using chloroform–methanol (95 : 5, v/v) as the solvent. The purity was >98%, as determined by proton (^1H), carbon (^{13}C) and phosphorus (^{31}P) nuclear magnetic resonance spectra and Fourier transform infrared spectra.

Gel–microemulsion formulation of WHI-07

Owing to the lipophilic nature of WHI-07, a microemulsion-based formulation strategy was developed.[4] A microemulsion-based system with high solubilizing capacity for WHI-07 was identified through systemic mapping of ternary phase diagrams and drug solubilization study. Various polymeric gels were screened to produce a gel with desirable viscosity. Polymer suspensions of carrageenan were selected as additives to the microemulsion-based system to obtain a gel with desirable viscosity containing 0.5–2.0% WHI-07 with high thickening capability and compatibility with microemulsion. These polymers did not cause drug precipitation or alter the microemulsion particle size. The gel–microemulsion was found to be very stable at ambient temperature. Particle size determination was made using a Nicomp Model 380 laser diode source (Particle Sizing Systems, Santa Barbara, CA). Measurements of drug concentrations were carried out by HP 1100 series HPLC (Hewlett Packard Instruments, Wilmington, DE) and a Beckman DU7500 UV–visible spectrophotometer (Beckman Instruments, Fullerton, CA). Viscosity measurements were made using the Brookfield viscometer (Brookfield Engineering Laboratories, Spoughton, MA). Compound WHI-07 was dissolved in a submicron particle size (30–80 nm) gel–microemulsion formulation at concentrations of 0.5, 1.0 and 2.0%.

Animals

Sexually mature, virgin female CD-1 mice of 6 weeks of age and proven fertile male CD-1 mice of 3 months of age were purchased from Charles River Laboratories (Wilmington, DE). Animals were allowed to acclimatize to housing conditions (temperature 22 ± 2 °C, humidity 50 ± 10%) under a 12-h light/dark cycle for 3–4 weeks prior to use. They were assigned to groups of five per

cage, in polycarbonate cages, and provided with corncob as bedding (Harlan Teklad, Madison WI). All assigned mice were identified with specific metal ear tags and ear notches. Tapwater and laboratory diet (Teklad LM-485; Harlan Teklad) were available *ad libitum*. Animal studies were approved by the Parker Hughes Institute Animal Care and Use Committee and all animal care procedures were conducted according to the current National Institutes of Health guidelines.

Evaluation of the effects of intravaginal administration of WHI-07 on induced ovulation

For ovulation efficiency studies, 69 sexually mature female CD-1 mice (in subgroups of 5 or 13) were superovulated by an intraperitoneal (i.p.) injection of 7.5 IU of pregnant mare serum gonadotropin (Gestyl; Diosynth B.B., Oss, The Netherlands) followed by an i.p. injection of 7.5 IU of human chorionic gonadotropin (hCG; Steris Laboratories, Phoenix, AZ) 48 h later. They were given 50 µl of intravaginal gel–microemulsion containing 0% (placebo control) or 2.0% WHI-07, respectively, 3 days prior to and during 2 days of superovulation. The intravaginal treatment was performed inside a micro-isolator. Fourteen hours after hCG injection, the females were euthanized by CO_2 inhalation and the oviducts were excised and the tubal eggs were obtained by puncturing the ampulla under a dissecting microscope. Ovulation efficiency was determined by counting the number of ovulated eggs in the ampulla of oviducts of each ovulated female as well as the total eggs recovered per treatment group 14 h after hCG injection. Scoring was done using an Olympus SZH10 stereo microscope (Olympus Corporation, Lake Success, NY). Five independent experiments were performed to evaluate the effects of intravaginal administration of WHI-07 on ovulation induction.

Evaluation of the effects of intravaginal administration of WHI-07 on *in vivo* fertilization

In experiments designed to test the potential effect of daily intravaginal administration of WHI-07 on *in vivo* fertilization and embryonic development, CD-1 female mice (in subgroups of 10) were given 50 µl of intravaginal gel–microemulsion with and without 2.0% WHI-07 3 days before and during 2 days of superovulation. After hCG injection they were mated overnight with fertile untreated males. The placebo controls received gel–microemulsion alone and the untreated control group received no gel–microemulsion. Forty hours after hCG injection, plug-positive females were euthanized by CO_2 inhalation and their oviducts were removed. Eggs and embryos were obtained by flushing the oviducts with human tubal fluid (HTF; Conception Technologies, San Diego, CA). The total number of eggs and embryos that were recovered from the oviducts were enumerated using a dissection microscope and the cleavage stage was recorded. Fertility was based upon embryos and unfertilized eggs recovered 40 h after hCG administration. The eggs and embryos from control and treated mice were washed in microdroplets of HTF medium supplemented with 3 mg ml^{-1} human serum albumin (HSA) and incubated for 24 h in a temperature- and gas-phase-controlled incubator (37 °C, 95% O_2, 5% CO_2) and their cleavage stage was recorded. Embryos were scored as 2-cell or ≥4-cell. Scoring was done using an Olympus IX50 inverted microscope (Olympus Corporation). Three independent experiments were performed to evaluate the effects of intravaginal administration of WHI-07 on *in vivo* fertilization.

Evaluation of the effects of intravaginal administration of WHI-07 during the period of organogenesis on fetal development

Because early stages of embryonic development are variably sensitive to the embryotoxic effects of nucleoside analogs,[12–14] we tested the potential toxicity of intravaginally applied 2% WHI-07 given during the period of organogenesis in pregnant mice. Sexually mature female CD-1 mice (in groups of 10) were superovulated as described above and caged overnight with proven breeder males (1 : 1). Plug-positive mice were removed and given daily intravaginal administration of 50 µl of 2% WHI-07 in gel–microemulsion or vehicle alone from gestation day (GD) 6 to 15 (the period of organogenesis). Mice were sacrificed on GD 17. The uterine contents were examined to determine the number of live and dead fetuses. All fetuses were weighed and examined externally for gross anomalies. Three independent experiments were performed to evaluate the effects of intravaginal administration of WHI-07 during the period of organogenesis on fetal development.

Evaluation of the effects of 13-week intravaginal WHI-07 administration on subsequent fertility of female mice

Eighty female CD-1 mice were allocated to four groups of 20. They were given 50 µl of intravaginal gel–microemulsion containing 0, 0.5, 1.0 or 2.0% WHI-07, respectively. The treatment period was 5 days per week for 13 consecutive weeks. The gel–microemulsion formulation of WHI-07 was prepared weekly and the intravaginal treatment was performed inside a micro-isolator. All animals were individually observed daily for signs of toxic effects. Body weights were obtained before exposure (day 0), weekly during exposure and after completion of treatment. Immediately after cessation of the 13-week intravaginal administration, mice in control and treatment groups were caged (1 : 2) with untreated males of the same strain. The females were allowed to complete a pregnancy (21 ± 2 days). Litter size, neonatal weight and condition of each offspring were determined. The pups were allowed to remain with the dam until day 5. The number of pups per litter and the mean weight of the pups surviving on day 5 were used to measure perinatal effects.[15]

Statistical analysis

Statistical significance of the treated group mean with that of the control group was analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using GraphPad Prism software (San Diego, CA). The data on egg recovery was analyzed by Student's *t*-test (two-tailed). Pregnancy rates were analyzed by using Fishers' exact test. Differences were considered statistically significant if $P < 0.05$.

RESULTS

Intravaginally administered WHI-07 had no adverse effect on ovulation efficiency

To test whether repetitive intravaginal administration of WHI-07 has any adverse effect on the ovulation efficiency, the number of eggs were enumerated from placebo control and WHI-07-treated superovulated mice. Table 1 summarizes the ovulation rates of placebo control and 2% WHI-07-treated CD-1 mice. In five independent experiments, the ovulation response was similar when PMSG/hCG-primed mice were given an intravaginal gel–microemulsion with and without 2% WHI-07 before and during superovulation for five consecutive days. All mice from the control and WHI-07-treated groups ovulated. Although a slight decrease in the total number of eggs recovered was observed for the 2% WHI-07 treatment group, this decrease was not statistically significant (1139 vs. 1045; $P = 0.17$, Student's t-test). Also, the difference in the mean number of eggs recovered from the ampullae of their oviducts was not statistically significant (33.5 vs. 29.8; $P > 0.05$, ANOVA + Dunnett test).

Intravaginally administered WHI-07 had no adverse effect on *in vivo* fertilization and subsequent cleavage

To test whether repetitive intravaginal administration of WHI-07 has any adverse effects on *in vivo* fertilization, the number of eggs and embryos were enumerated from superovulated, untreated, vehicle only and WHI-07-treated superovulated female mice that had been mated with untreated males. Table 2 summarizes the effects of repeated intravaginal application of 2% WHI-07 on *in vivo* fertilization and the recovery of total embryos and further development of the 2-cell embryos to 4-cell or greater stage embryos. In three independent experiments, fertilization of eggs was similar when PMSG/hCG-primed mice were mated after daily exposure to 2.0% WHI-07 before and during superovulation for five consecutive days. There was no significant difference in the total number of eggs recovered and the proportion of eggs fertilized or cleaved between untreated control or placebo control and 2.0% WHI-07-treated test groups ($P > 0.05$, Fisher's exact test). The average of 61% (333/542) fertilized eggs obtained with mice after intravaginal treatment with gel–microemulsion was similar to the 53% (375/710) average obtained with mice treated with 2% WHI-07 gel–microemulsion intravaginally ($P = 0.31$, Fisher's exact test).

Table 1—Summary of effects of intravaginal administration of 2% WHI-07 on ovulation response in PMSG/hCG-primed CD-1 mice

Treatment	Ovulation response[a] (%)	Total eggs recovered	Eggs recovered per mouse (mean ± SD)
Placebo	34/34 (100)	1139[b]	33.5[c] ± 18.2
WHI-07 (2%)	35/35 (100)	1045[b]	29.8[c] ± 21.5

[a] Mice with eggs in the ampullae 14 h after hCG injection.
[b] Totals were not different ($P = 0.17$, Student's t-test).
[c] Means were not different ($P > 0.05$; ANOVA + Dunnett's test).

Table 2—Summary of effects of intravaginal administration of 2% WHI-07 on ***in vivo*** fertilization and subsequent cleavage in PMSG/hCG-primed CD-1 mice

Treatment	No. of females	No. of eggs or embryos				Percentage of eggs fertilized[a]
		Eggs	2-cell	>4-cell	Total	
None	29	278	353	33	664	58
Placebo	28	209	288	45	542	61[b]
WHI-07 (2%)	29	335	329	46	710	53[b,c]

[a] The rate of fertilization was determined 40 h after hCG injection.
[b] Not significantly different from control by Fisher's exact test, $P > 0.05$.
[c] Not significantly different from placebo control by Fisher's exact test, $P > 0.05$.

Intravaginal exposure to WHI-07 during pregnancy had no adverse effect on reproduction and fertility

To test whether repetitive intravaginal administration of WHI-07 during pregnancy has any adverse effect on the developing fetus, the reproductive outcome of superovulated female mice that had been mated with males and exposed to vehicle alone or WHI-07 during the period of major organogenesis was assessed. Table 3 summarizes the reproductive parameters of pregnant mice on gestation day 17 following intravaginal administration of gel–microemulsion with and without 2% WHI-07 during GD 6–15. In three independent experiments, there was no significant group differences in the mean litter size (11 ± 9 vs. 10.3 ± 6.8; $P > 0.05$), mean fetal weight (0.58 ± 0.19 vs. 0.55 ± 0.17; $P > 0.05$) and percentage of live fetuses (93.4% vs. 94.5%; $P > 0.05$) in mice administered gel–microemulsion alone or gel–microemulsion containing 2% WHI-07. Intravaginal administration of 2% WHI-07 during the period of major organogenesis did not result in significant increase in external malformations in offspring when compared with the placebo control group Table 3. A low incidence of external anomalies (short

Table 3—Summary of effects of intravaginal administration of 2% WHI-07 on gestation days 6–15 of pregnancy on litter parameters in PMSG/hCG-primed CD-1 mice

Parameter	Placebo ($n = 14$)[a]	WHI-07 (2%)[b] ($n = 16$)[a]
Total litter size[b] (n)	154	165
Mean litter size (median)	11.0 ± 9.0 (11)[c,d]	10.3 ± 6.8 (10)[c,d]
Mean fetal weight (g)[b]	0.58 ± 0.19[d]	0.55 ± 0.17[d]
Viable fetus (%)	93.4	94.5
Fetal external malformation (%)	1.2	0.6
Fetal skeletal malformation (%)	5.3	4.8

[a] Number of plug-positive females.
[b] At necropsy on gestation day 17 of pregnancy.
[c] Mean per female ± SD.
[d] Means were not different ($P > 0.05$; ANOVA + Dunnett's test).

limbs) and fetal skeletal malformations (absence of ribs and vertebral column) was found in both the placebo control and the 2% WHI-07-treated mice.

Thirteen-week intravaginal administration of WHI-07 had no adverse impact on subsequent fertility

To test whether repetitive intravaginal application of WHI-07 has any adverse effect on subsequent fertility, 22-week-old female CD-1 mice treated with increasing concentrations of WHI-07 for 13 weeks were mated with 15-week-old untreated CD-1 males and allowed to complete a pregnancy. Mean body weight gain and final mean body weight of mouse groups exposed intravaginally to gel–microemulsion containing 0%, 0.5%, 1.0% or 2.0% WHI-07 for 13 consecutive weeks were similar (Fig. 2). There were no deaths during exposure or following the 13-week post-exposure in any group of mice. One animal in the 0.5% WHI-07 group was removed from the study after 9 weeks of intravaginal treatment due to injury caused during the intravaginal application. All the remaining animals were clinically healthy at the end of the study. Table 4 summarizes the fertility parameters for CD-1 mice given intravaginally increasing concentrations of WHI-07 for 13 weeks prior to mating. When gel–microemulsion control and WHI-07-treated female CD-1 mice were evaluated for their fertility immediately after cessation of the 13-week intravaginal administration, neither concentration of WHI-07 had a statistically significant effect on fertility parameters. The 13-week treatment of WHI-07 at concentrations higher than 1400–5700 times its *in vitro* spermicidal EC_{50} and 1.4–5.7($\times 10^6$) times its *in vitro* anti-HIV IC_{50} had no statistically significant effect on subsequent fertility (89–100% fertile), median litter size ($n = 10–13$), neonatal survival (88.5–100%), pup morphology or development (Table 4). The mean neonatal and pup weights from mice exposed to 0.5%, 1.0% and 2.0% WHI-07 were not lower than that of the placebo control group at birth and on lactation day 5.

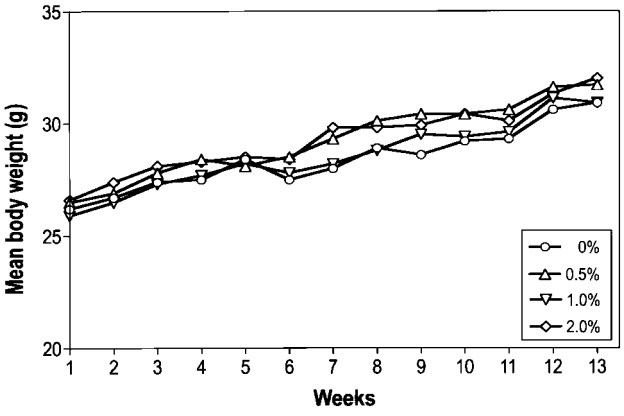

Figure 2. Mean body weights of female CD-1 mice exposed to a gel–microemulsion formulation containing 0%, 0.5%, 1.0% or 2.0% WHI-07 intravaginally for 5 days a week for 13 consecutive weeks. Animals were weighed weekly and the growth was plotted as the mean weight per cohort (19 or 20) per week.

DISCUSSION

Fertility studies were performed under different conditions to determine the reproductive toxicity potential of repetitive intravaginal administration of the dual-function bromo-methoxy-substituted aryl phosphate derivative of ZDV, compound WHI-07. The intravaginal effect of WHI-07 was studied by varying the concentration, treatment on specific days and/or length of treatment period during and before pregnancy. For topical studies, we used a standardized gel microemulsion of WHI-07 that exhibits suitable spreading, retention and compatibility with the vaginal mucosa. Our results demonstrated that mouse ovulation response, *in vivo* fertilization, early embryonic and fetal development were not adversely affected by repeated intravaginal administration of WHI-07 (2.0%). Furthermore, repeated intravaginal exposure of mice to 0.5–2.0% WHI-07 for 13 weeks had no adverse effect on the subsequent reproductive capability, perinatal outcome, growth and development of the offspring. The concentrations of WHI-07 used in the present study were 1400–5700 times higher than its spermicidal IC_{50} and 1.4–5.7($\times 10^6$) times higher than its anti-HIV IC_{50}. Collectively, these findings demonstrate that repetitive intravaginal administration of WHI-07 to yield effective spermicidal and antiviral concentrations does not adversely affect the reproductive performance in mice.

In previous studies, WHI-07 was shown to lack the capacity to be absorbed through the vaginal epithelium.[4] In quantitative tissue adsorption and retention studies using a validated HPLC with a detection limit of 25 pmol, neither WHI-07 nor its metabolites (alaninyl-ZDV monophosphate, ZDV monophosphate, and ZDV) were detectable in serum of rabbits treated intravaginally with

Table 4—Fertility parameters for CD-1 mice given 0.5–2.0% WHI-07 intravaginally for 13 weeks

	WHI-07 concentration (%)			
Parameter	0	0.5	1.0	2.0%
No. of mice per group	20	19	20	20
Pregnancy rate (%)	20/20 (100)	17/19 (89.4)[a]	18/20 (90)[a]	20/20 (100)[a]
Mean litter size (median)	11.1 ± 2.4 (11)	12.8 ± 2.2[b] (13)	11.1 ± 2.2[b] (11)	9.6 ± 2.9[b] (10)
Mean neonatal weight (g)	1.58 ± 0.15	1.60 ± 0.14	1.71 ± 0.25	1.70 ± 0.16
Total litter size (*n*)	222	218	200	192
Total litter size on lactation day 5 (*n*)	207	208	200	170
Neonatal survival on lactation day 5 (%)	93.2	95.4[a]	100[a]	88.5[a]
Mean neonatal weight on lactation day 5 (g)	3.15 ± 0.43	3.20 ± 0.50	3.48 ± 0.52	3.56 ± 0.42

[a] Not significant ($P > 0.05$; Fisher's exact test).
[b] Means were not different from control ($P > 0.05$; ANOVA + Dunnett's test).

WHI-07.[4] Furthermore, repeated intravaginal application of 0.5–2.0% WHI-07 for 13 weeks did not result in systemic toxicity and no specific target organs were identified.[6] No statistically significant treatment-related effects on survival, growth, hematological, and clinical chemistries, absolute or relative organ weights or histopathology were noted.[6] These studies demonstrated that WHI-07 has low potential to produce systemic toxicity after intravaginal application, and WHI-07 is not toxic to the female genital tract. In the present study, no evidence of fetal toxicity or teratogenicity was observed when pregnant mice were treated intravaginally with 2% WHI-07 during the period of major organogenesis. The low incidence of external anomalies or skeletal malformations observed in this study was evident in both gel–microemulsion control and WHI-07-exposed superovulated mice. Based on our preclinical studies performed in mice and rabbits, intravaginal application of WHI-07 when used as a topical dual-function microbicide during pregnancy is unlikely to cause adverse systemic or local side-effects.

Currently, ZDV is being used clinically during pregnancy.[16] Oral administration of ZDV has been evaluated for adverse effect on reproduction and fetal development in animal test species.[17] In both the reproduction/fertility study and a peri- and postnatal study in rats, liveborn offspring showed no adverse effects on survival, growth or developmental measurements. In humans, oral ZDV therapy during pregnancy has been shown to reduce perinatal HIV transmission from an infected woman to her infant by nearly 70%.[18] Epidemiological data have since confirmed the efficacy of ZDV for reduction of perinatal transmission and have extended this efficacy to children of women with advanced disease, low CD4+ T-lymphocyte counts and prior ZDV therapy. Children exposed to ZDV *in utero*, during labor and delivery and also as newborns for 6 weeks showed no adverse health effects when followed up for as long as 5.6 years.[18]

In conclusion, repeated intravaginal administration of up to 2.0% WHI-07 via a gel–microemulsion formulation given to CD-1 mice during ovulation, in *in vivo* fertilization or before or during pregnancy had no adverse effect on fertility. Compound WHI-07 was not teratogenic in mice. Repeated intravaginal exposure of mice to WHI-07 for 13 weeks had no adverse effects on subsequent reproductive performance, neonatal survival or pup development. Compound WHI-07 shows unique clinical potential as a safe dual-function vaginal contraceptive for curbing mucosal and perinatal HIV transmission.

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