Notes for Appendix 9 - Multi-dose study - TherImmune 2 - summary table

Histopathology

Thyroid

Preserved tissues were transferred to PAI, Frederick, Maryland. The following tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by the study pathologist: thyroid, testes, epididymides, and gross lesions from all males, as well as the ovaries, uterus, cervix, thyroid and gross lesions from all females. The methods are described in the Pathology Report, presented in Appendix 17.

Data from the treated F1 animals were analyzed by Analytical Sciences Inc. (Durham, North Carolina).

All endpoints (age and weight at vaginal opening/preputial separation, body weights, estrous cycle length, and organ weights at necropsy) with the exception of a few related to vaginal cytology (number of cycling females and number of females with regular cycles) were analyzed using ANOVA or ANCOVA to determine if there was a dose effect. For endpoints that were analyzed with an ANOVA, Levene's test was used to assess whether the assumption of equal variance across dose groups was tenable. For those endpoints where the Levene's test was rejected at the 0.1 level, no p-values for the overall ANOVA are reported.

Fitting the ANCOVA models, appropriate statistical tests were carried to ascertain the validity of a constant slope for each endpoint. The hypothesis of constant slope was rejected only in a handful of cases (ovaries, uterus and cervix without fluid for ethynil estradiol and pituary for vinclozolin). However, caution should be used in interpreting the results from the ANCOVA model given that the covariate (terminal body weight) could be impacted by the dose treatment.

Animals in each treated group were compared with animals in the control group using appropriate multiple comparison procedures. The Dunnett's (1955) test was used for all endpoints that were analyzed with an ANCOVA model or an ANOVA models in which the assumption of homogeneity of variance was not rejected. When the Levene 's test was rejected at the 0.1 level, Dunn's test (nonparametric) for multiple comparisons with a control was used. For number of cycling females and number of females with regular cycles, multiple comparisons with the control were performed using an unadjusted chi-square test.

As this was a blinded study, the test article information was disclosed after the processing and histological evaluation of the tissues was completed.

The majority of the gross lesions concerned the accessory sex glands of the males, and dilation of the kidneys of both males and females, which corresponded to hydronephrosis of variable degree. Hydronephrosis was observed in both males and females throughout

all groups, except in the 25 mg/kg/day Flutamide-treated males and 50 mg/kg/day Methoxychlor-treated females, and considered an incidental finding.

Microscopic findings observed at each group in both males and females were incidental and expected in animals of similar age and environment, except for 50 mg/kg/day Flutamide treated males. Although 5 animals were microscopically normal in this group, changes in the remaining 10 males included luminal dilation of the testicular tubules, degeneration of the germinal epithelium and edema. Four of these males were also observed with sperm granulomas in the epididymis.

Ovary

Preserved tissues were transferred to PAI, Frederick, Maryland. The following tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by the study pathologist: thyroid, testes, epididymides, and gross lesions from all males, as well as the ovaries, uterus, cervix, thyroid and gross lesions from all females. The methods are described in the Pathology Report, presented in Appendix 17.

Data from the treated F1 animals were analyzed by Analytical Sciences Inc. (Durham, North Carolina).

All endpoints (age and weight at vaginal opening/preputial separation, body weights, estrous cycle length, and organ weights at necropsy) with the exception of a few related to vaginal cytology (number of cycling females and number of females with regular cycles) were analyzed using ANOVA or ANCOVA to determine if there was a dose effect. For endpoints that were analyzed with an ANOVA, Levene's test was used to assess whether the assumption of equal variance across dose groups was tenable. For those endpoints where the Levene's test was rejected at the 0.1 level, no p-values for the overall ANOVA are reported.

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Microscopic findings observed at each group in both males and females were incidental and expected in animals of similar age and environment, except for 50 mg/kg/day Flutamide treated males. Although 5 animals were microscopically normal in this group, changes in the remaining 10 males included luminal dilation of the testicular tubules, degeneration of the germinal epithelium and edema. Four of these males were also observed with sperm granulomas in the epididymis.

Uterus

Preserved tissues were transferred to PAI, Frederick, Maryland. The following tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by the study pathologist: thyroid, testes, epididymides, and gross lesions from all males, as well as the ovaries, uterus, cervix, thyroid and gross lesions from all females. The methods are described in the Pathology Report, presented in Appendix 17.

Data from the treated F1 animals were analyzed by Analytical Sciences Inc. (Durham, North Carolina).

All endpoints (age and weight at vaginal opening/preputial separation, body weights, estrous cycle length, and organ weights at necropsy) with the exception of a few related to vaginal cytology (number of cycling females and number of females with regular cycles) were analyzed using ANOVA or ANCOVA to determine if there was a dose effect. For endpoints that were analyzed with an ANOVA, Levene's test was used to assess whether the assumption of equal variance across dose groups was tenable. For those endpoints where the Levene's test was rejected at the 0.1 level, no p-values for the overall ANOVA are reported.

Fitting the ANCOVA models, appropriate statistical tests were carried to ascertain the validity of a constant slope for each endpoint. The hypothesis of constant slope was rejected only in a handful of cases (ovaries, uterus and cervix without fluid for ethynil estradiol and pituary for vinclozolin). However, caution should be used in interpreting the results from the ANCOVA model given that the covariate (terminal body weight) could be impacted by the dose treatment.

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As this was a blinded study, the test article information was disclosed after the processing and histological evaluation of the tissues was completed.

The majority of the gross lesions concerned the accessory sex glands of the males, and dilation of the kidneys of both males and females, which corresponded to hydronephrosis of variable degree. Hydronephrosis was observed in both males and females throughout all groups, except in the 25 mg/kg/day Flutamide-treated males and 50 mg/kg/day Methoxychlor-treated females, and considered an incidental finding.

Microscopic findings observed at each group in both males and females were incidental and expected in animals of similar age and environment, except for 50 mg/kg/day Flutamide treated males. Although 5 animals were microscopically normal in this group, changes in the remaining 10 males included luminal dilation of the testicular tubules, degeneration of the germinal epithelium and edema. Four of these males were also observed with sperm granulomas in the epididymis.