**Appendix C2 – *In Utero* Through Lactational**

| *In Utero* Through Lactational | |
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| Purpose | Determine developmental and reproductive consequences of exposure to chemicals that affect the estrogen, androgen and thyroid hormonal systems during development *in utero*, during lactation and after weaning until puberty. |
| Design | Protocol C:<br>F0 females gavaged orally from gd 6 - pnd 21 (weaning).<br>F1 offspring divided into two female and two male cohorts after weaning: 1) immediate necropsy of males, 2) Uterotrophic cohort: subcutaneous injection (1/litter) from pnd 22-24, 3) Pubertal female cohort: oral gavage (4/litter, 2 dosed/2 not dosed) from pnd 21 – 42, and 4) Pubertal male cohort: oral gavage (4/litter, 2dosed and 2 not dosed) from pnd 21 – 70.<br><br>F0 females and F1 offspring dosed at 3 dose levels plus a vehicle control. |
| Endpoints | Maternal:<br>During in-life, body weights, feed consumption and clinical observations are taken.<br>At necropsy (pnd 21), final body, liver and thyroid weights, count of uterine implantation sites, serum T4/TSH and thyroid histology are collected and analyzed.<br><br>Offspring:<br>Body weights on pnd 0, 4, 7, 14, 18, 21, 22, 24, (necropsy uterotrophic), 42 (necropsy female pubertal) or 70 (necropsy male pubertal).<br>Anogenital distance on pnd 21 and necropsy.<br>Uterotrophic (dosed from pnd 22-24): Ovarian and uterine weight, uterine histology and serum T4 and TSH concentrations.<br>Female pubertal (dosed from pnd 22 – 42): acquisition of vaginal patency, weights of reproductive organs and thyroid, histology on ovaries, uterus and thyroid and serum T4 and TSH concentrations.<br>Male pubertal (dosed from pnd 22 – 70): acquisition of preputial separation, retained nipples and areolae, weights of reproductive organs and thyroid, histology on testis, epididymis, and thyroid, serum T4 and TSH concentrations. |

| *In Utero* Through Lactational | |
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| Interpretation | No data interpretation criteria were adopted by the EPA. |
| Main peer review comments | The EPA presented the alternative *in utero* through lactational (IUL) rat screening assay to the FIFRA SAP in February 2007 to consider whether the IUL assay, as represented by Protocol C and tested with methoxychlor, was suitable as an alternative Tier 1 screening assay and whether the assay validation process should continue using Protocol C or some other protocol (e.g., A or B).<br><br>In general, the SAP considered Protocol C too complex for a Tier 1 screen and not in accord with the EDSTAC criteria of a Tier 1 assay. Although it was felt that Protocol B or a modification of it could be validated as a simpler screen, there was concern that none of the proposed protocols (A, B, C) could be standardized and validated to find utility as a routine Tier 1 screen. |
| Strengths | Evaluates the effects of chemical exposure *in utero*, during lactation and after weaning until puberty.<br><br>Pre- and postnatal endpoints are appropriate and sensitive to endocrine disrupting activates. |
| Limitations | • Complex<br>• Long<br>• Costly |