

## Rationale for Laboratory and Chemical Selection for 15-Day Intact Adult Male Rat Assay for Inter-laboratory Validation

### Proposed design:

Laboratories	Chemicals	Chemicals
A	Ketoconazole* (steroidogenesis inhibitor)	Phenobarbital (thyroid)
B	Ketoconazole* (steroidogenesis inhibitor)	Linuron (anti-androgen)
C	Phenobarbital (thyroid)	Linuron (anti-androgen)
Lead (Dupont & Dow)	Negative Chemical (to be identified)	Ketoconazole* (steroidogenesis inhibitor)

\*Compounds only evaluated by DuPont; all other compounds have been evaluated in 2 or more laboratories.

### Rationale for Laboratory Selection

- For validation purposes, it is necessary to demonstrate that contract research laboratories or organizations (CRO) can adopt this protocol with relative ease and success (i.e., obtain expected results). At present, 6 laboratories (including 2 CRO) have conducted the adult male rat assay during pre-validation. Considering available resources, the proposal is to test 2 of the same chemicals in 3 different CRO (see design above) rather than 3 of the same chemicals in 2 different CRO and to take advantage of the results that have already been generated with these chemicals in previous studies as referenced below.

### Rationale for Chemical Selection

- Chemicals selected for this inter-laboratory phase of validation have been chosen to represent several different modes of action (MOA) that the intact male assay is expected to detect as an alternative assay for the steroidogenesis, Hershberger and pubertal female (male) assays in the Tier-1 screening battery. It is expected that the uterotrophic assay will be included in the Tier-1 screening battery; therefore, the inclusion of estrogenic/anti-estrogenic chemicals as an MOA was considered unnecessary in this validation exercise.
- The MOAs that were identified as critical for detection by the intact male assay included:
  - anti-androgen
  - thyroid-active agent
  - steroid biosynthesis inhibitor

**Ketoconazole** is a broad spectrum steroid biosynthesis inhibitor. Whereas other assays (i.e., the uterotrophic and the Hershberger assays) target chemicals operating through endocrine receptors, the adult male assay has an intact and functioning hypothalamic-pituitary-gonadal (HPG) axis and active endogenous steroid biosynthesis making it a suitable in vivo model to detect steroid biosynthesis inhibitors.

**Linuron** is a relatively weak-acting anti-androgen and is expected to challenge the sensitivity of the assay.

**Phenobarbital** is a thyroid-active agent that is considered relatively weak compared to Propylthiouracil (PTU) and, therefore, it is also expected to challenge the assay.

Each of these chemicals (Ketoconazole, Linuron and Phenobarbital) has previously been run in the adult male assay with results documented in peer reviewed publications as indicated below. Furthermore, these chemicals have been used in other potential Tier-1 screening assays (e.g., Hershberger, pubertal male and/or pubertal female assays) during validation. Hence, this selection of chemicals is expected to facilitate the future comparison among assays for deciding on which assays to include in the Tier-1 screening battery.

- Linuron:
  - Intact male assay: O'Connor *et al.*, 2002a; EPA, 2003 at RTI
  - Intact male assay (peripubertal): June/July 2005
  - Hershberger assay: Ashby *et al.*, 2004; Kang *et al.*, 2004
  
- Phenobarbital:
  - Intact male assay: O'Connor *et al.*, 1999 (ip); 2002b
  - Intact male assay (peripubertal): Oct./Nov. 2004
  - Hershberger assay: Yamada *et al.*, 2004
  - Pubertal male assay: Marty *et al.*, 2001a
  
- Ketoconazole:
  - Intact male assay: O'Connor *et al.*, 1998 (ip); 2002b
  - Pubertal male assay: Ashby and Lefevre, 2000; Marty *et al.*, 2001b
  - Pubertal female assay: Marty *et al.*, 1999
  
- Note, identification of a negative chemical may be needed to verify the specificity of the assay and is being pursued by the lead laboratories (DuPont/Dow).

## References

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