# Extended $F_1$ One Generation Reproductive Toxicity Study

## Moving Toward a New Toxicology Testing Paradigm

# Origin

- **ILSI-HESI-ACSA effort to improve the testing requirements for agricultural chemicals. (Three Task Forces)**
  - **ADME**
  - **Systemic Toxicology**
  - **Life Stages**
- **Goal: Develop scientifically credible and viable methods for assessing the safety of crop protection chemicals more efficiently, with fewer animals and artifacts.**
  - **Conserve resources**
  - **Reduce and refine animal use**
  - **Incorporate relevant measurements**
  - **Evaluate Reproductive, CNS and Immune function.**

**ILSI: International Life Sciences Institute**
**ACSA: Technical Committee on Agricultural Chemical Safety Assessment**

# Life Stages Task Force Strategy

## How Can Testing Be Effective & Efficient (Includes measures not currently done)

- Introduce greater flexibility through a science based approach using available information and a logical "step-wise" process
- Integrate improved understanding of target dosing based on ADME
  - Dose setting
  - Life stages
- Incorporate development & reproductive endpoints as well neurological, immunological & endocrine systems

# Life Stages Task Force Recommendations

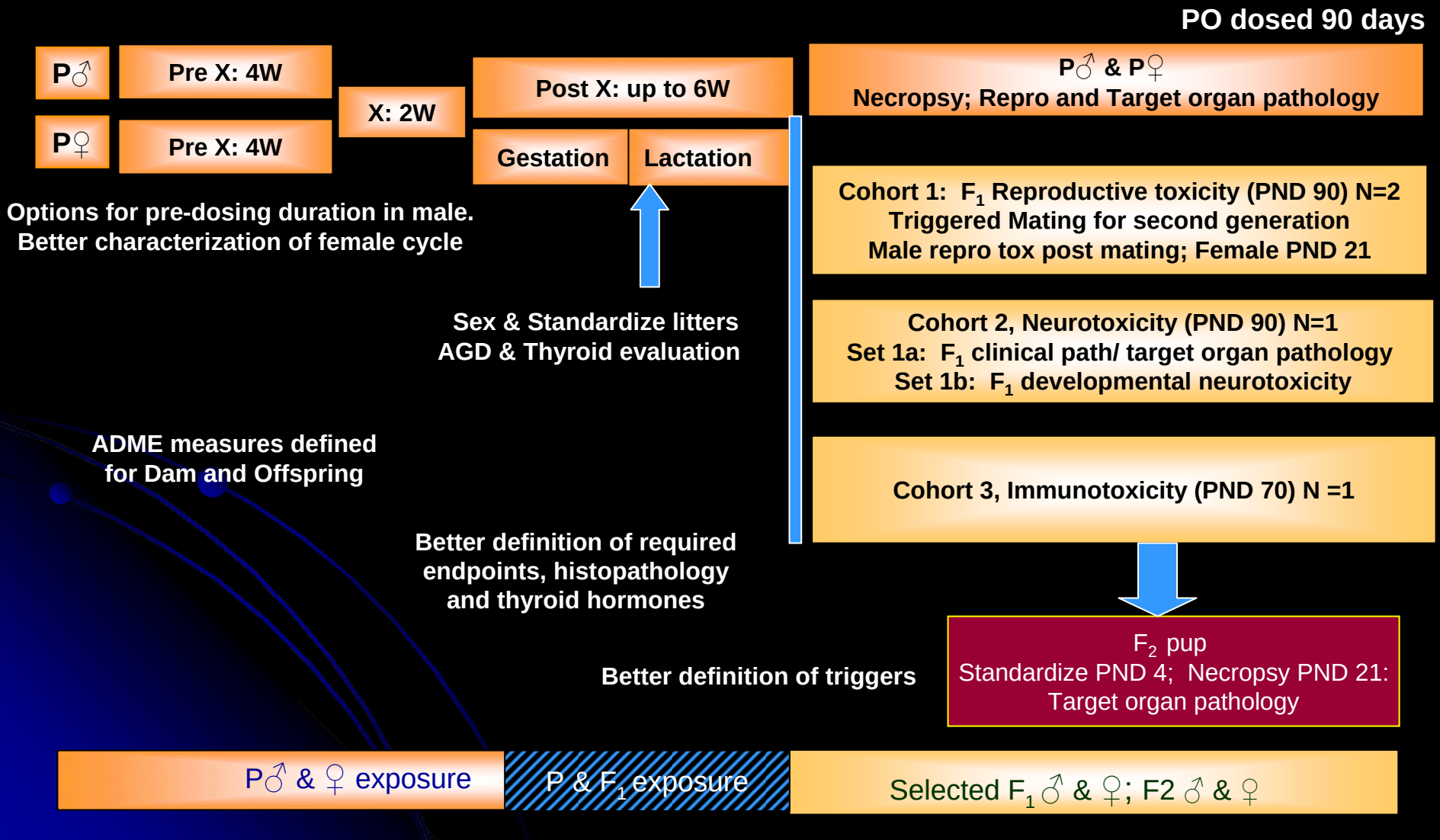
## Flexibility

- Consider all relevant information.
- Evaluate more than just reproduction and development in F1 pups (neurotox, immunotox and endocrine endpoints).
- Include key indicators (triggers for developmental effect) which, if negative, give a high level of confidence of no adverse effects
- Production of F2 generation not automatic (depends on triggers in P0, F1 and other relevant information).
  - If positive results are found, move to a more tailored testing approach follows which may include extension of testing the 2nd generation

# Life Stages Task Force Recommendations

- **New study design: Extended $F_1$ One Generation Reproductive Toxicity Test**
  - **Significant departure from the current multigeneration guideline study**
    - **$F_1$ animals subjected to a far more comprehensive evaluation than what is currently done.**
  - **Extensively peer reviewed and published "A tiered approach to life stages testing for agricultural chemical safety assessment"** [Cooper et al., (2006) Crit Rev Toxicol.;36(1):69-98. ]
  - **Post publication evaluation by U.S. experts to address further improvements in design.**
  - **May eventually replace OPPTS 870.3800 guideline and OECD 416.**

# Extended $F_1$ One Generation Reproductive Study Protocol



# Major Features of Study Design

- Incorporates use of toxicokinetic data in study design
  - TK study conducted prior to Extended $F_1$ One-Gen study usually as part of the range-finding study
- Abbreviated pre-mating period
  - 4 weeks *vs.* current 10 weeks
- Extensive hematology, clinical chemistry, urinalysis, histopathology evaluations
- Include elements of the developmental neurotoxicity and immunotoxicity studies
- Trigger production of $F_2$ generation
  - If $F_2$ generation is not triggered, the study uses ≈ 1200 fewer animals

# Advantages of Extended $F_1$ One Generation Reproductive Toxicity Study

- Inclusion of additional measures indicative of anti-androgen effects (e.g., nipple retention)
- Evaluation of special toxicities (e.g., nervous and immune system)
- Inclusion of hormonal measures (e.g., thyroid)
- Inclusion of ADME
- Reduce/refine/replace animal use
  - More efficient utilization of animals
  - Use fewer animals
- Flexible and cost effective
  - Reduce cost & time in data development
  - Reduce resources needed by EPA to review & process data

# Retrospective Analysis of Multigeneration Reproductive Toxicity Study

- **Goals**
  - **Confirm that an Extended $F_1$ 1-generation Reproductive Toxicity Study as proposed by ILSI/HESI ACSA workgroup and described in Cooper et al. (2006) would not fail to identify critical sensitive endpoints or lower NOAELs[1]**
  - **Evaluate the contribution of the second generation to hazard identification or characterization**
  - **Determine if the proposed triggers would accurately and reliably identify the need to mate the $F_1$ generation to produce an $F_2$ generation**

[1] Cooper et al., (2006) *A tiered approach to life stages testing for agricultural chemical safety assessment* **Crit Rev Toxicol.;36(1):**69-98.

# Contribution of $F_2$ Generation to Hazard Identification/characterization

- Are lower No-Observed-Adverse-Effect-Levels (NOAELs) identified in the second generation ($F_2$) relative to the first generation?
- Are different effects identified in $F_2$ generation?

# Effectiveness of Triggers to Produce an $F_2$ Generations

- Do triggers accurately identify the need to mate the $F_1$ offspring to produce an $F_2$ generation?
  - Reproductive triggers (*e.g.*, adverse effect on fertility/fecundity of P generation, effects on sexual maturation of $F_1$ pups)
  - Offspring triggers (*e.g.*, $F_1$ pup malformations, $F_1$ pup weight decreases in the absence of maternal body weight decreases)
  - Results are consistent with those reported RIVM and Canada/PMRA

| List of potential endpoints considered for triggering an $F_2$ generation*. | |
|---|---|
| **Reproductive Endpoint** | **Offspring Endpoint** |
| $P_1$ Estrous Cycle Evaluation | ↓Maternal (P) bw same dose as ↓$F_1$ pup bw |
| $P_1$ Fertility (# implantations, pregnancy rate, gestational interval) | ↓ lactation index (PND4-21) |
| $F_1$ Litter parameters (litter size, litter weight, sex ratio) | $F_1$ pup mortality |
| $F_1$ Developmental landmarks (AGD, nipple retention, puberty onset, PPS, VO) | $F_1$ pup malformations (*eg*.., hypospadia, cryptocordysm, one eye, large head) |
| $F_1$ Estrous Cycle Evaluation | ↓$F_1$ pup viability index (PND0-4) |
| $P_1$ Reproductive Organ Weights | ↓$F_1$ live birth index |
| $P_1$ Reproductive Organ Histopathology | ↓$F_1$ pup bw only |
| $P_1$ Andrology (sperm parameters) | |
| $P_1$ Qualitative Ovarian Assessment | |
| $F_1$ Reproductive Organ Weights | |
| $F_1$ Reproductive Organ Histopathology | |
| $F_1$ Andrology (sperm parameters) | |
| $F_1$ Qualitative Ovarian Assessment | |

# US EPA/OPP Retrospective Analysis: Results and Conclusions

- $F_2$ generation has little value for establishing RfDs (ADIs) or informing FQPA SF decisions
  - For reproductive effects, ≈2% chemicals in the $F_2$ LOAEL < $F_1$ LOAEL and F2 effects only categories
  - For offspring effects, ≈4-5% chemicals in the $F_2$ LOAEL < $F_1$ LOAEL and F2 only categories
- $F_2$ generation has little value for identifying unique effects (*i.e.,* different from effects reported in the $F_1$)

# US EPA/OPP Retrospective Analysis: Results and Conclusions

- **If the Extended One-Generation Toxicity had been implemented,**
  - **An $F_2$ generation would have been triggered for approximately 43% of the chemicals**
  - **100,000 animals would have been saved**

# Ongoing Activities

- Draft guideline being considered for adoption by OECD
- Merging retrospective analyses conducted by the Netherlands, Canada, and the US

# Outstanding Issues

- DNT and DIT modules
  - Will these modules be mandatory or optional?
    - Will they be mandatory for all chemicals including industrial chemicals, cosmetics
- Refining the triggers to produce an $F_2$ generation
  - Currently the $F_2$ is triggered 43% of the time
- Sample size
  - Number of animals for reproductive toxicity cohort

# Future Activities

- New guideline will be discussed at OECD's WNT (Working Group of the National Coordinators of the Test Guidelines Program) meeting in March 2009
- Expert group will reconvene in October 2009 to discuss remaining technical issues including refined triggers and merged retrospective analyses
- Proposed new guideline will be presented to the SAP on Nov. 2009
- OECD will consider adoption of new guideline (including refined triggers) during 2010 WNT meeting