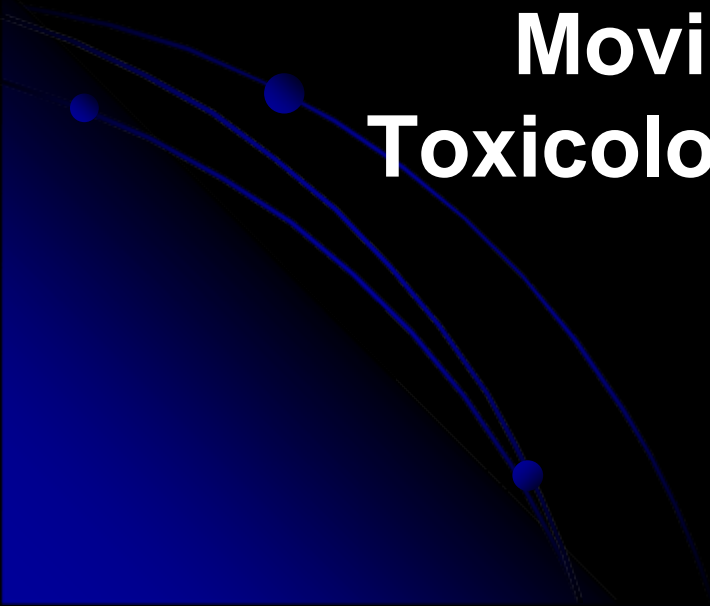


Extended F₁ One Generation Reproductive Toxicity Study

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.

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

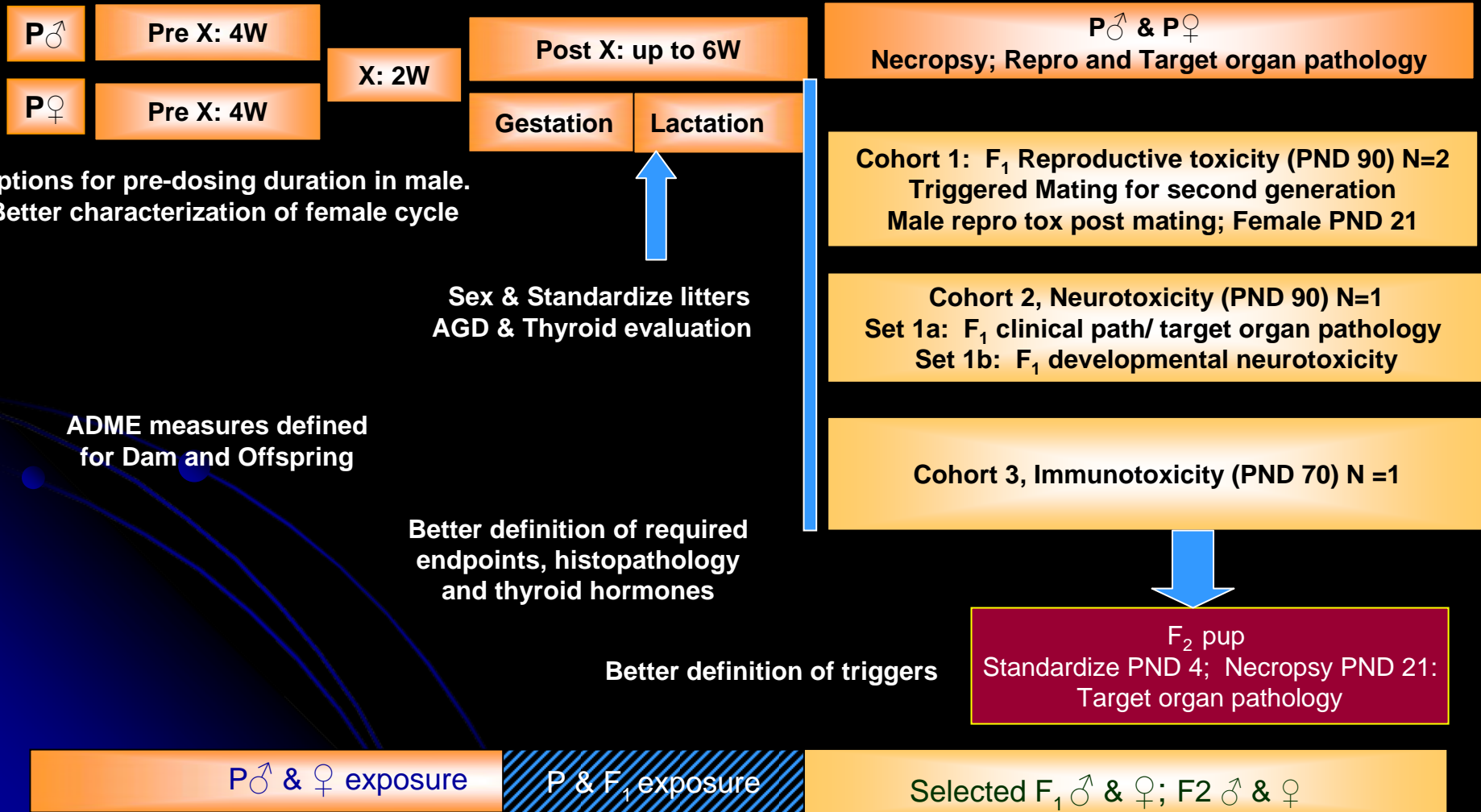
- Consider all relevant information.
- Evaluate more than just reproduction and development in F1 pups (**neurotox, immunotox and endocrine endpoints**).
- Include key indicators (**triggers for developmental effect**) which, if negative, give a high level of confidence of no adverse effects
- **Production 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 F₁ One Generation Reproductive Toxicity Test**
 - **Significant departure from the current multigeneration guideline study**
 - F₁ 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 F₁ One Generation Reproductive Study Protocol

PO dosed 90 days



Major Features of Study Design

- **Incorporates use of toxicokinetic data in study design**
 - TK study conducted prior to Extended F₁ 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 F₂ generation**
 - If F₂ generation is not triggered, the study uses \approx 1200 fewer animals

Advantages of Extended F₁ 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 F₁ 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 NOAELs¹
- 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 F₁ generation to produce an F₂ 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 F₂ Generation to Hazard Identification/characterization

- Are lower No-Observed-Adverse-Effect-Levels (NOAELs) identified in the second generation (F₂) relative to the first generation?
- Are different effects identified in F₂ generation?

Effectiveness of Triggers to Produce an F₂ Generations

- **Do triggers accurately identify the need to mate the F₁ offspring to produce an F₂ generation?**
 - **Reproductive triggers (e.g., adverse effect on fertility/fecundity of P generation, effects on sexual maturation of F₁ pups)**
 - **Offspring triggers (e.g., F₁ pup malformations, F₁ 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 F₂ 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., hypospadias, cryptorchidism, 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

- **F₂ generation has little value for establishing RfDs (ADIs) or informing FQPA SF decisions**
 - For reproductive effects, ≈2% chemicals in the F₂ LOAEL < F₁ LOAEL and F2 effects only categories
 - For offspring effects, ≈4-5% chemicals in the F₂ LOAEL < F₁ LOAEL and F2 only categories
- **F₂ generation has little value for identifying unique effects (*i.e.*, different from effects reported in the F₁)**

US EPA/OPP Retrospective Analysis: Results and Conclusions

- **If the Extended One-Generation Toxicity had been implemented,**
 - **An F₂ 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 F₂ generation
 - Currently the F₂ 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