Overview of *In Utero* through Lactational Assay as an Alternative Screening Assay in the EDSP Tier-1 Battery

Introduction

The purpose of this overview is to provide a summary of the *in utero* through lactational screening assay which was recommended by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) and supported by the Science Advisory Board and Scientific Advisory Panel (SAB/SAP) after review of the program proposed by the EDSP. Several designs for the assay will be presented as described in a Detailed Review Paper (DRP).

It is expected that this historical summation of background material along with other supplemental material provided as reference will provide the SAP with additional information to allow the committee to respond to the various charge questions. The SAP responses to the charge questions will assist the EPA in determining the Agency's need to continue, modify or suspend the present course of action in validating an *in utero* through lactational bioassay as a screening assay for use in the EDSP Tier-1 battery.

EDSTAC 1998

EDSTAC provided a final report in which it recommended that the EDSP develop, validate and standardize a combination of *in vitro* and *in vivo* assays from which several will be selected to comprise a Tier-1 Screening Battery (EDSTAC, 1998[a]). In comparison with the more refined, detailed and definitive tests in Tier 2, the EDSTAC indicated that the *in vitro* and *in vivo* screening assays in the EDSP Tier-1 battery be: 1) inexpensive, quick and easy to perform, 2) standardized and validated, 3) more sensitive than specific to minimize false negatives without an unreasonable rate of false positives, 4) capture multiple endpoints and reflect as many modes of endocrine action as possible, 5) broadly predictive across species, gender and age, 6) yield data that can be interpreted as either negative or positive for the purpose of determining the necessity and manner in which to conduct Tier-2 tests.

In regard to the mammalian *in vivo* assays, EDSTAC felt that the pubertal female rat and pubertal male and adult male rat assays proposed for consideration in the Tier-1 screening battery as primary or replacement assays would have the necessary scope to detect any currently known chemical compounds having estrogenic, androgenic and thyroidogenic (EAT) activity. However, there was concern that some chemical substances (see next section) could produce effects from prenatal exposure that would not be detected from pubertal or adult exposure. An additional concern was whether there was sufficient scientific evidence of known endocrine disruptors that could affect the prenatal stage of development without affecting the neonatal, pubertal and adult stages and whether effective doses and affected endpoints may differ among the various life stages that substances could produce effects during prenatal exposure indirectly through exposure of the dam. Despite these outstanding concerns, EDSTAC recognized the importance of *in utero* exposure to chemicals having EAT activity and recommended that the EDSP design a study and develop a protocol that could screen for effects at birth through adulthood after pre- and postnatal exposure. EDSTAC recognized that it may be difficult to design a developmental screening assay but believed it is worth the effort.

EDSTAC emphasized that any developmental screening assay be subject to validation and standardization like other *in vivo* assays and, before considering to include the assay in the Tier-1 screening battery, that its potential to replace one or more of the other recommended *in vitro* or *in vivo* assays of the battery as well as its overall impact on cost effectiveness of the battery be evaluated. In this regard, EDSTAC presented an example of an *in utero* developmental screening assay that could potentially replace the pubertal and adult rat assays (EDSTAC[b], 1998; Appendix O).

SAB/SAP 1999

A joint subcommittee of the SAB/SAP reviewed the EDSP according to the proposed statement of policy presented in FR Notice, volume 63, page 71542 (Dec 28, 1998). The assays proposed in the FR notice did not include an *in utero* assay since the fundamental design of such an assay was still being debated as to whether it would be practical as a screening assay in Tier 1 or as a more broad-based intermediate screening or testing assay between Tiers 1 and 2. Regardless, a presentation by an EPA staff scientist (Gray et al., 1999) to the SAB/SAP during a public meeting indicated that exposure of di-n-butyl phthalate (steroid synthesis disruptor) to rats produced alterations in the androgen system during development. These results indicated that there are potential critical developmental events that may not necessarily be detected in the proposed Tier-1 screening battery (false negative) which was an EDSTAC concern presented in the previous section and, therefore, convinced the committee to recognize that an *in utero* screen should be utilized as a more sensitive model for endocrine disruptor events and be considered in the Tier-1 battery (EPA-SAB/SAP, 1999). Thus, the SAB/SAP firmly agreed with EDSTAC that a screen using animals exposed *in utero* and possibly during lactation should be developed by the EDSP, even though it was not in the proposed statement of policy presented in the FR notice, and that it should be considered as a substitution assay, replacing several individual assays and not as an addition to the proposed screening battery (EPA-SAB/SAP, 1999).

DRP 2001

A DRP was prepared for the EDSP (EPA-DRP, 2002) in which it surveyed and investigated the status of various protocols that are currently in use, or that have been proposed for use to identify and study chemicals that act as potential EDCs after exposure *in utero* and through lactation. From these results, it was expected that the EDSP would choose a design according to its understanding of the role of this assay in the program.

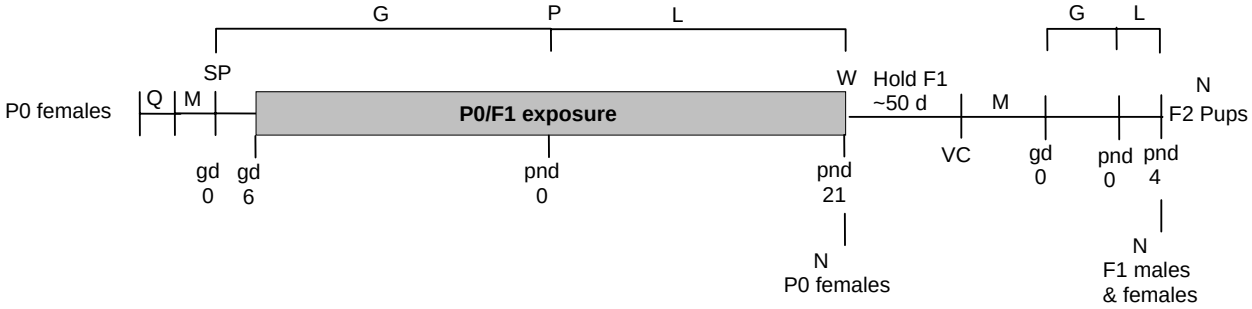
The DRP consisted of a literature review on the evaluation of endocrine-influenced endpoints after exposure to known endocrine active chemicals (EACs) and included: 1) searching the literature databases, 2) contacting individuals to obtain information on unpublished research, and 3) evaluating the literature and personal communications. From this exercise, candidate study designs were evaluated, Protocols A, B and C where Protocol A primarily served as a model for Protocols B and C.

The following is a general overview of the study designs (Protocols A, B and C) emphasizing key stages of development for treatment and observation. They are presented with the expectation to provide a fundamental understanding of the various designs to facilitate a broad comparison among the designs for discussion. A general description of the similarities and differences of each design are given; details can be found in the DRP (EPA-DRP, 2002).

Protocol A: FDA Segment III Protocol as Modified by ICH

The FDA Segment III Protocol is a current guideline for food additives and pharmaceutical compounds that utilizes *in utero* through lactation exposure and postnatal evaluation (gd 15 through pnd 21 - weaning) that primarily served as a model for developing Protocols B and C. Protocol A or Segment III protocol was designed to be run with a Segment I protocol (dosing prior to mating, during mating, and until gd 6 - implantation) and a Segment II protocol (dosing the pregnant female from implantation on gd 6 through 15 - end of major embryo organogenesis). With all three protocols, various life stages are evaluated from mating of parents (P0), through development of the first generation (F1) and production of the second generation (F2). In considering the Segment III protocol as an assessment tool for postnatal consequences after exposure *in utero* and during lactation, the International Commission of Harmonization (ICH) modified the protocol to start exposure to the dam on gd 6 as illustrated (Diagram 1):

Diagram 1. FDA Segment III Protocol as Modified by ICH



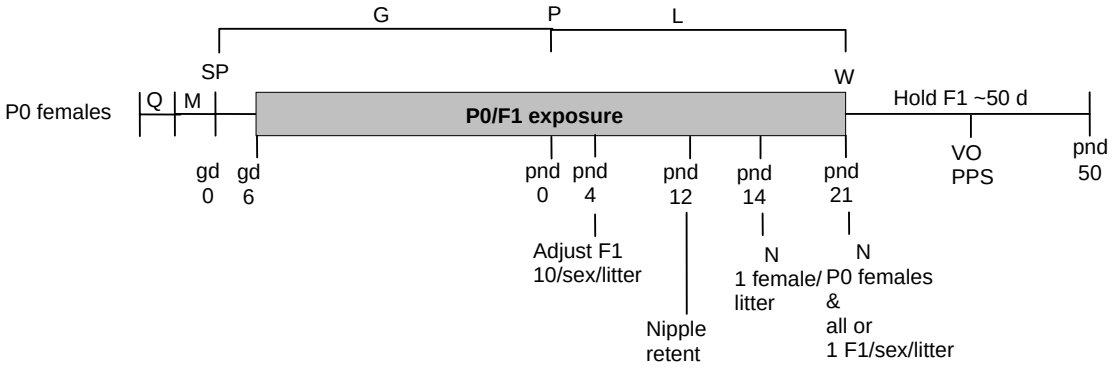
Key:

Q	=	quarantine (approximately 1 week)
M	=	mating (approximately 1 week for F0 and approximately 2 weeks for F1)
SP	=	sperm positive
G	=	gestation (approximately 21-23 days)
gd	=	gestational day
P	=	parturition
pnd	=	postnatal day
L	=	lactation (approximately 21 days)
W	=	wean
N	=	necropsy
VC	=	vaginal cyclicity evaluation of F1 prior to mating

Protocol B: *In Utero* through Lactational Protocol

The *In Utero* through Lactational Protocol is a modification of the ICH-modified FDA Segment III Protocol which was developed by Dr. Gray from EPA and Dr. Tyl from RTI. A major modification involved excluding the production of a second generation (F2) of animals and included additional endpoints during lactation and post-weaning to assess potential EACs as illustrated (Diagram 2).

Diagram 2. *In Utero* through Lactational Protocol



Key:

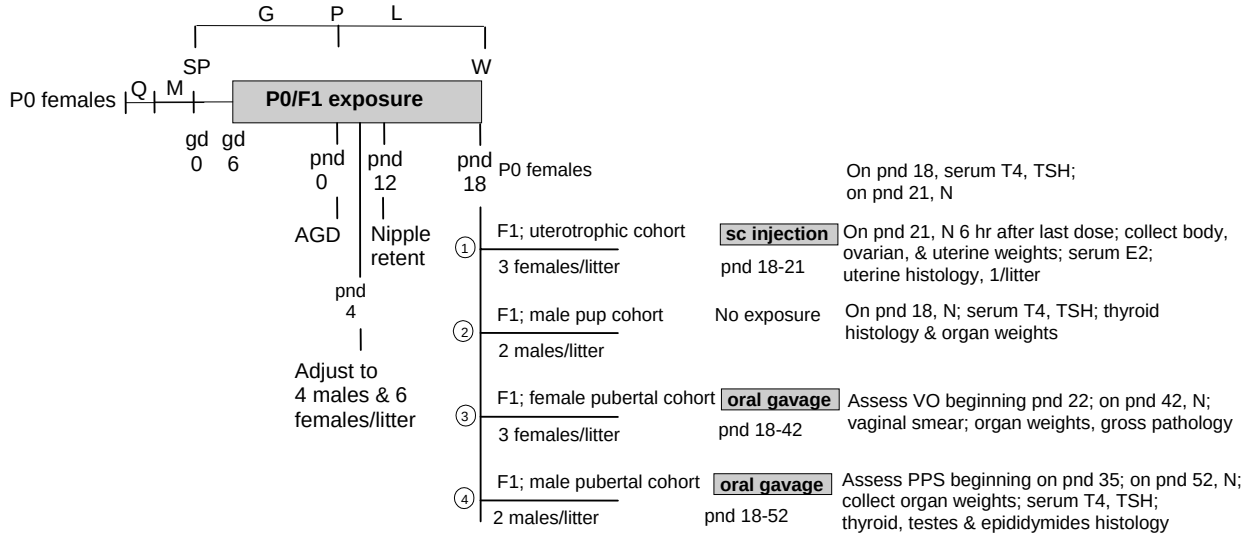
Q	=	quarantine (approximately 1 week)
M	=	mating (approximately 1 week for F0 and approximately 2 weeks for F1)
SP	=	sperm positive
G	=	gestation (approximately 21-23 days)
gd	=	gestational day
P	=	parturition
pnd	=	postnatal day
L	=	lactation (approximately 21 days)
W	=	wean
S	=	standardize litters on pnd 4 to 10
N	=	necropsy
VO	=	vaginal opening evaluation of F1 beginning on pnd 22
PPS	=	preputial separation evaluation of F1 beginning on pnd 30

Endocrine sensitive endpoints were added as follows: 1) anogenital distance, 2) preputial separation, 3) vaginal opening, 4) retained nipples in the males, 5) weight of reproductive organs, 6) vaginal cytology, 7) uterine histology, 8) thyroid weight and histology and 9) serum estradiol, TSH and T4 concentrations.

Protocol C: *In Utero* through Lactational Protocol with Direct Exposure to F1

The *In Utero* through Lactational Protocol was designed by a committee of nine scientists led by Dr Kavlock and modified to include separate cohorts of F1 male and female animals that could be directly exposed from weaning through puberty. It was expected that this design could replace other mammalian *in vivo* assays in the Tier-1 screening battery as illustrated (Diagram 3).

Diagram 3. *In Utero* through Lactational Protocol with Direct Exposure to F1



Key:

Q	=	quarantine (approximately 1 week)
M	=	mating (approximately 1 week for F0 and approximately 2 weeks for F1)
SP	=	sperm positive
G	=	gestation (approximately 21-23 days)
gd	=	gestational day
P	=	parturition
pnd	=	postnatal day
AGD	=	anogenital distance on pnd 0
L	=	lactation (approximately 21 days)
RN	=	retained nipples evaluation of male F1 on pnd 12
W	=	weaning
N	=	necropsy
E2	=	estradiol
T4	=	thyroxin
TSH	=	thyroid stimulating hormone
VO	=	vaginal opening evaluation of F1 beginning on pnd 22
PPS	=	preputial separation evaluation of F1 beginning on pnd 30

EPA Choice of an *In Utero* through Lactational Protocol 2001

From the three protocols, the DRP recommended development of Protocol B, which is a modification of the ICH-modified FDA Segment III Protocol and seemed comparable to that proposed by EDSTAC (EDSTAC[b], 1998; Appendix O). Advantages over other protocols, especially relative to Protocol C, included simplicity, an improved chance of identifying potential EACs, fewer overall number of animals used to identify EACs, ensures that each animal is used to its fullest extent, and aids in the selection of endpoints and appropriate number of F1 animals used per litter for the Tier-2 multigeneration tests. This study design was expected to maximize the data collected from the minimum number of animals. Other than the use of a minimal number of animals, no disadvantages were indicated.

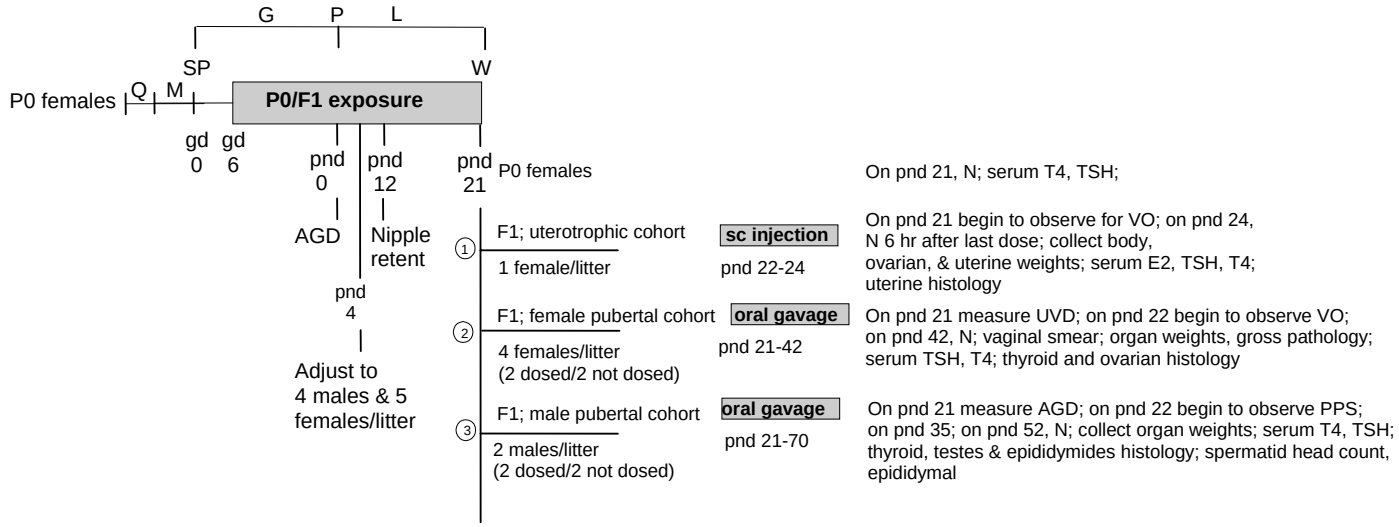
Despite the DRP recommendation, the EPA chose Protocol C, the more comprehensive study design. It was thought at the time that this protocol could potentially replace a number of assays in the Tier-1 screening battery (e.g., *in vitro* steroidogenesis and aromatase assays; *in vivo* Hershberger and uterotrophic and either or both pubertal assays and adult male assay) as recommended by the EDSTAC and supported by the SAB/SAP, be considered as a follow-up test with more endocrine-specific endpoints which were apparently limited after revising the current two-generation reproductive toxicity test in 1998, and to potentially augment the current developmental toxicity testing protocol. Moreover, this substitution could allow for cost savings and a reduction in the number of animals used in Tier-1 screening.

Endocrine Disruptor Methods Validation Subcommittee (EDMVS) 2001

The EPA went before the EDMVS in December 2001 to present the Agency's choice for Protocol C. According to the minutes of the meeting (EPA-EDMVS, 2001), several members expressed the opinion that Protocol C was beyond Tier 1, uses too many animals to be considered a screen and whether it is necessary to have a Tier-1 screen with exposure at an extremely sensitive life stage. Another member also commented that it is difficult to know where the *in utero* through lactational assay fits in the EDSP before having the details and results of the various other screening assays. Nonetheless, the EPA proposed to run Protocol C and bring the results to a federal advisory committee such as the SAP to review and discuss the assay's future role in the EDSP - the EDMVS agreed.

Through the primary contractor, RTI was selected as the subcontract laboratory to run the study (WA 2-23) using the positive test compound Methoxychlor in slightly modified version of Protocol C as illustrated in Diagram 4.

Diagram 4. *In Utero*/Lactational Exposure Testing Protocol C with Methoxychlor



Key:

Q	=	quarantine (approximately 1 week)
M	=	mating (approximately 1 week for F0 and approximately 2 weeks for F1)
SP	=	sperm positive
G	=	gestation (approximately 21-23 days)
gd	=	gestational day
P	=	parturition
pnd	=	postnatal day
AGD	=	anogenital distance on pnd 0
L	=	lactation (approximately 21 days)
W	=	weaning
N	=	necropsy
E2	=	estradiol
T4	=	thyroxin
TSH	=	thyroid stimulating hormone
VO	=	vaginal opening evaluation of F1 beginning on pnd 22
UVD	=	urethral vaginal distance
PPS	=	preputial separation evaluation of F1 beginning on pnd 30

RTI Study Results 2003

A report of the results using Protocol C with Methoxychlor from the contract research laboratory (RTI) has been finalized and and is included in the materials submitted to the SAP. In addition to the biological results, an RTI scientist presentation will address the transferability and practicability of the assay from a contract research laboratory perspective.

Research Considerations Regarding Chemical Exposure *In Utero* and During Lactation

Outstanding EDSTAC concerns are that some chemical substances could produce effects from prenatal exposure that would not be detected from pubertal or adult exposure and whether there was sufficient scientific evidence of known endocrine disruptors that could affect the prenatal stage of development without affecting the neonatal, pubertal and adult stages. Considering the current results of the *in utero* through lactational assay with methoxychlor, an initial attempt was made to compile key results from the uterotrophic, pubertal female and pubertal male cohorts following subcutaneous expose to methoxychlor beginning on pnd 21 and to make a cursory comparison with the results from OECD/EPA sponsored utertrophic assay and EPA sponsored pubertal female and male assays and adult male assay following methoxychlor treatment.

Uterotrophic: The results of exposure to methoxychlor in the uterotrophic cohort of the *in utero* through lactational assay could not be readily compared with the other assays because of the relatively high final uterine weight values observed in the control cohort. Nonetheless, a synopsis of the results of the OECD/EPA sponsored uterotrophic assay indicated that final uterine weights were significantly increased relative to control values at 50, 100, 120, 250, 300, 500 and 800 mg/k/d of methoxychlor across multiple laboratories using various protocols (i.e., intact vs ovariectomized animals and oral vs subcutaneous administration). Final uterine weight was also higher at 20 mg/k/d following oral gavage but not necessarily after subcutaneous administration

Pubertal Female: Several pubertal female assays have been done with methoxychlor at various dose levels in several different contract research laboratories (Table 1). The results were qualitatively compared respective to comparable dose levels across studies relative to respective dose levels in the *in utero* through lactational assay.

Table 1. Pubertal females exposed to methoxychlor at various dose levels relative to the pubertal female cohort in the *in utero* through lactational assay. The results were assessed qualitatively regardless of absolute (abl) or relative (rel) final organ weight and histology of either the ovary or uterus from several different laboratories.

In Utero/Lactational Pubertal Female Cohort		**25 mg/k/d**	**50 mg/k/d**	**100 mg/k/d**
*VO (hastened)		↓	↓	↓
Organ wt (abl/rel)				
Ovaries		-	↓	↓
Uterus		-	-	-
Histlogy (ovary/uterus)		-	+	+
Pubertal Female Assay				**100 mg/k/d**
Vaginal opening				↓
Organ wt (abl/rel)				
Ovaries				↓ (1 of 2 labs)
Uterus				-
Histlogy (ovary/uterus)				+
Pubertal Female Assay	**12.5 mg/kg/d**	**25 mg/k/d**	**50 mg/k/d**	
Vaginal opening	↓	↓	↓	
Organ wt (abl/rel)				
Ovaries	-	-	↓	
Uterus	-	-	-	
Histlogy (ovary/uterus)	-	-	-	
Pubertal Female Assay	**12.5 mg/kg/d**		**50 mg/k/d**	
Vaginal opening	-		↓ (3 of 3 labs)	
Organ wt (abl/rel)				
Ovaries	-		↓ (1 of 3 labs)	
Uterus	-		-	
Histlogy (ovary/uterus)	-		-	

↑↓ indicates significant ($P<0.05$) increase or decrease relative to controls.
+ indicates a positive effect of treatment.
- indicates no significant difference from controls.
*VO, vaginal opening.

Pubertal Male and Adult Male: Methoxychlor is not typically the positive test chemical of choice to run in the pubertal or adult male assay for validation of these assays. Nonetheless, methoxychlor at various dose levels have been done in different contract research laboratories (Table 2). The results were qualitatively compared respective to comparable dose levels across studies relative to respective dose levels in the *in utero* through lactational assay.

Table 2. Pubertal and adult males exposed to methoxychlor at various dose levels relative to the pubertal male cohort in the *in utero* through lactational assay. The results were assessed qualitatively regardless of absolute (abl) or relative (rel) final organ weight and histology of either the testis or epididymus (epi) from several different laboratories.

In Utero/Lactational Pubertal Male Cohort		**25 mg/k/d**		**50 mg/k/d**	**100 mg/k/d**
*PPS (delayed)		-		↑	↑
Organ wt (abl/rel)					
Testes		↓		↓	↓
Prostate		-		↓	↓
*SVCG		↓		↓	↓
*LABC		-		↓	↓
Cowpers gland				↓	↓
Histology (testis/epi)		-		-	-
Pubertal Male Assay		**25 mg/k/d**		**50 mg/k/d**	
PPS (delayed)		-		-	
Organ wt (abl/rel)					
Testes		-		-	
Prostate		-		-	
*SVCG		-		↓	
*LABC		-		-	
Cowpers		-		-	
Histology (testis/epi)		-		-	
Adult Male Assay	**12.5 mg/k/d**	**25 mg/k/d**	**37.5 mg/k/d**	**50 mg/k/d**	
Organ wt (abl/rel)					
Testes	-	-	↑	↑	
Prostate	-	-	↓	↓	
*SVCG	-	↓	↓	↓	
Histology (testis/epi)	-	-	-	+	

↑↓ indicates significant ($P<0.05$) increase or decrease relative to controls.
+ indicates a positive effect of treatment.
- indicates no significant difference from controls
*PPS, preputial separation, SVCG, seminal vesicles with coagulating gland and LABC,

Except, perhaps, for the pubertal male assay, the effects of methoxychlor at doses less than 100 mg/k/d seem to be readily detected by the uterotrophic, pubertal female and adult male assays proposed for the EDSP Tier-1 screening battery. Apart from this cursory assessment of

EPA/OECD sponsored studies with methoxychlor, there are likely other studies that have used comparable bioassays with different test chemicals that may further clarify the concerns that EDSTAC had regarding chemical substances that could produce effects from prenatal exposure that would not be detected from pubertal or adult exposure and whether there was sufficient scientific evidence of known endocrine disruptors that could affect the prenatal stage of development without affecting the neonatal, pubertal and adult stages. In addition, this may be an area of ongoing research or basis for initiating research to determine the relevance of an *in utero* through lactational assay as an alternative or additive screening tool in the EDSP Tier-1 battery.

Consideration of Replacing the Current Two-Generation Reproductive Toxicity Test

The EDSTAC recognized that endpoints sensitive to detect EAT activity and related neurobehavioral deficits were not thoroughly represented in the current standard two-generation mammalian reproductive toxicity test proposed for the Tier-2 battery (OPPTS, 1998) and, therefore, recommended additional, relevant endpoints for inclusion and validation in the two-generation reproductive toxicity test or any alternative Tier-2 tests the EDSP may develop (EDSTAC, 1998). Similarly, a SAB/SAP review report recognized that the standard two-generation reproductive toxicity test guideline included few endpoints that could critically detect effects on the thyroid (SAB/SAP, 1999). Since the EDSTAC and SAB/SAP recommendations, it has also been suggested (Gray & Foster, 2004) that the number of pups/sex/litter at necropsy as weanlings or adults may be biologically and statistically insufficient to detect endocrine disruptor activity using the standard two-generation reproductive toxicity test. The current standard two-generation mammalian reproductive toxicity test that the EDSP proposed for its Tier-2 battery, therefore, seemed deficient in its current form. Hence, the EPA's basis for developing and validating an *in utero* through lactational assay using Protocol C was not only to determine its potential in Tier 1 but also in Tier 2, perhaps, as a supplemental assay that could potentially be considered as a follow-up test with more endocrine-specific endpoints and augment the current developmental toxicity testing protocol.

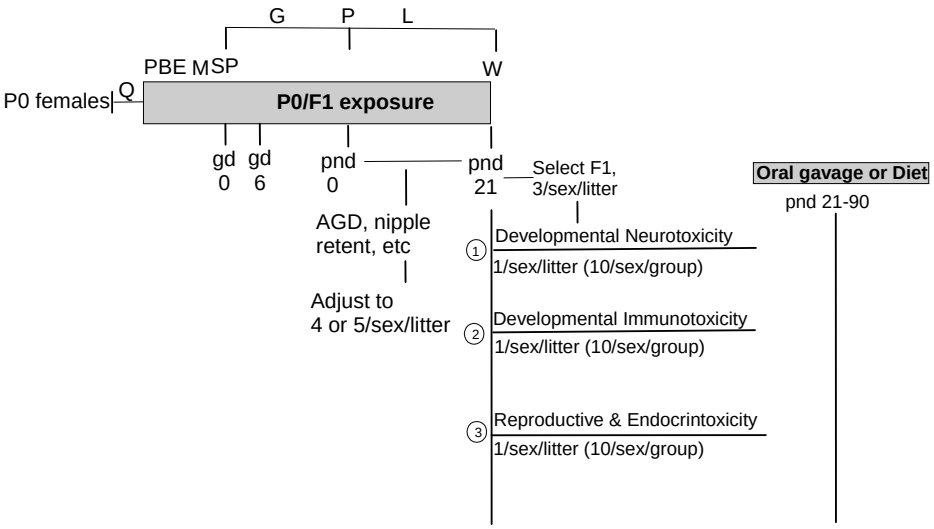
In 2000, the Health and Environmental Sciences Institute (HESI) of the International Life Sciences Institute (ILSI) formed the Agricultural Chemical Safety Assessment (ACSA) Technical Committee, which included individuals from the EPA, to design a toxicity testing paradigm that would incorporate the current understanding of pesticide toxicity and exposure. The result was a "new" paradigm having three main components: ADME (Absorption, Distribution, Metabolism and Excretion), Systemic Toxicity and Life-Stages. The basis and description of the ADME (Barton et al., 2006), Systemic Toxicity (Doe et al., 2006) and Life-Stages (Cooper et al., 2006) approaches as well as an overall review of the "new" paradigm (Carmichael et al., 2006) are thoroughly presented and discussed in respective reports.

The conceptual approach of the Life-Stages developmental and reproductive toxicity test (Diagram 5) is expected to provide information on the integrity and performance of male and female gonadal function, estrous cycle, mating behavior, conception, gestation, parturition, nursing behavior, lactation, weaning, and the growth and development of the offspring to adulthood, including neurological and immunological information. Many of the endpoints (e.g., retention of nipples/areolae, thyroid weight, histology and hormones, and neurobehavioral measurements) proposed for the Life-Stages test (Cooper et al., 2006) are comparable to those

recommended to the EPA by the EDSTAC (EDSTAC, 1998). In addition, the protocol design effectively utilizes more pups/sex/litter at necropsy, especially as adults, to increase the statistical power of analyses and the versatility of the test allows it to be extended to adulthood or a second generation if historical information or early observations within the test trigger a design change. Hence, endocrine-specific endpoints and protocol designs issues critical to detect endocrine effects that are apparently limited in the current two-generation reproductive toxicity test proposed for the EDSP Tier 2 are included in Life-Stages developmental and reproductive toxicity test as well as neurological and immunological information that may also be affected by EACs.

The protocol for the Life-Stages developmental and reproductive test has recently been reviewed within the EPA by an expert work group to consider modifications or revisions to the initial protocol for optimization and has been introduced to the OECD in conjunction with submitting a project submission form to consider the Life-Stages developmental and reproductive test as a potential test guideline. Thus, the Life-Stages developmental and reproductive test beign considered for Tier 2 may eventually obviate the need for a comprehensive endocrine-specific *in utero* through lactational assay in Tier 1. However, if it is still warranted to consider *in utero* and lactational exposure in Tier 1, perhaps a less comprehensive or complex *in utero* through lactational screening assay can be proposed and developed that would comply with the initial objective and criteria of a screening assay as recommend by the EDSTAC.

Diagram 5. Life-Stages Developmental and Reproductive Toxicity Test



Key:

Q	=	quarantine (approximately 1 week)
PBE	=	pre-breed exposure (approximately 2 or 10 weeks)
M	=	mating (approximately 1 week for F0 and approximately 2 weeks for F1)
SP	=	sperm positive
G	=	gestation (approximately 21-23 days)
gd	=	gestational day
P	=	parturition
pnd	=	postnatal day
AGD	=	anogenital distance on pnd 0
L	=	lactation (approximately 21 days)
W	=	weaning
N	=	necropsy

Key additions with respect to potential endocrine disruptors:

Reproductive (developmental and target organs), neurological and immunological endpoints

Thyroid specific endpoints

More F1 pups/sex/litter/dose level retained for necropsy at adulthood

References

Barton, HA, Pastoor, TP, Baetcke, K, Chambers, JE, Diliberto, J, Doerrer, NG, Driver, JH, Hastings, CE, Iyengar, S, Krieger, R, Stahl, B, and Timchalk, C. 2006. The acquisition and application of absorption, distribution, metabolism, and excretion (ADME) data in agricultural chemical safety assessments. *Crit Rev Toxicol,* 36:9-35.

Carmichael, NG, Barton, HA, Boobis, AR, Cooper, RL, Dellarco, VL, Doerrer, NG, Fenner-Crisp, PA, Doe, JE, Lamb, JC, and Pastoor, TP. 2006. Agricultural chemical safety assessment: a multi-sector approach to the modernization of human safety requirements. *Crit Rev Toxicol,* 36:1-7.

Cooper, RL, Lamb, JC, Barlow, SM, Bentley, K, Brady, AM, Doerrer, NG, Eisenbrandt, DL, Fenner-Crisp, PA, Hines, RN, Irvine, L, Kimmel, CA, Koeter, H, Li, AA, Makris, SL, Sheets, L, Speijers, GJA, and Whitby, K. 2006. A tiered approach to life stages testing for agricultural chemical safety assessment. *Crit Rev Toxicol,* 36:69-98.

Doe, JE, Boobis, AR, Blacker, A, Dellarco, VL, Doerrer, NG, Franklin, C, Goodman, JI, Kronenberg, JM, Lewis, R, McConnell, EE, Mercier, T, Moretto, A, Nolan, C, Padilla, S, Phang, W, Solecki, R, Tilbury, L, van Ravenswaay, B, and Wolf, DC. 2006. A tiered approach to systemic toxicity testing for agricultural chemical safety assessment. *Crit Rev Toxicol,* 36:37-68.

EDSTAC (1998[a]). Endocrine Disruptor Screening and Testing Advisory Committee, Final Report, Volume I.

EDSTAC (1998[b]). Endocrine Disruptor Screening and Testing Advisory Committee, Final Report, Technical Appendices and Public Comments (Apendices F – U), Volume II.

EPA-SAB/SAP, (1999). Review of the Endocrine Disruptor Screening Program by a Joint Subcommittee of the Science Advisory Board and Scientific Advisory Panel. EPA-SAB-EC-99-013.

EPA-DRP, (2002). Detailed Review Paper on *In utero*/Lactational Protocol. WA 2-8.

EPA-EDMVS, (2001). Draft Meeting Summary. Endocrine Disruptor Methods Validation Subcommittee, Second Meeting, December 10-12, 2001.

EPA (1998) Office of Prevention, Pesticides and Toxic Substances (OPPTS), Health Effects Test Guideline, OPPTS 970.3800, Reproduction and Fertility Effects, EPA712-C-98-208.

EPA (2003) Draft Final Report: Validation of the *InUtero*/Lactational Exposure Screening Protocol with Methoxychlor. WA 2-23.

Gray, EL and Foster, PMD. 2003. Significance of experimental studies for assessing adverse effects of endocrine-disrupting chemicals. Pure Appl Chem. 75:2125-2141.

Gray LE, Wolf C, Lamhright C, Mann P, Price M, Cooper RL and Ostby J. 1999. Administration of potentially anitandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p'-DDE and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169 and ethane

dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicology and Industrial Health (15) ½:94-118.