Appendix P

Examples of "Weight-of-Evidence" Determinations

The following charts were developed, at the request of the EDSTAC, by one EDSTAC member and one STWG member (not an EDSTAC member). The Appendix is intended to give the reader a sense of how results could be interpreted, however, it does not include all possible examples. For instance, the document does not include an example of how one might handle positive *in vitro* results coupled with negative *in vivo* results in Tier 1, which might go into the "hold box."

T1S RESPONSE/ "Weight-Of-Evidence" TABLE. These three tables (parts 1, 2, and 3) include known or expected responses to the assays included in the proposed EDSTP T1S batttery. The list of endpoints include only those required. After th follows: + is a positive; - is negative; +-? is a possible positive response; and shaded responses are more certain than u evidence" determinations are made to determine the next steps for the chemical in question. The three possible de or (3) Other. Most chemicals truly exist, and in vivo dosage levels are provided, while a few are fictitious, generate outcomes in T1S. The substances are identified by brief descriptions.

Part 1.

T1S ASSAY	ENDPOINT	Anti-androgenic Fungicide	Estrogenic Toxic Substance	Estrogenic Pesticide	Anti-androgenic Drug	PCB Mixture	Anti-A pestici de
IN VITRO HTPS OR BENCH ASSAYS							
HTPS ER/MVLN	ER AGONIST	-	+	-+	-	-	-
ABOVE PLUS E2	ER ANTAGONIST	-	-	-	-	-	-
HTPS ER PLUS METABOLISM	METAB IS ER AGONIST	-	-	++	-	-	-
ABOVE PLUS E2	METAB IS ER ANTAGONIST	-	-	-	-	-	-
HTPS AR/CV-1	AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	AR ANTAGONIST	+	+?	-+	-	-	+
HTPS AR PLUS METABOLISM	METAB IS AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	METAB IS AR ANTAGONIST	++	-	++	-	-	-
HTPS TR	TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	TR ANTAGONIST	-	-	-	-	-	-
HTPS TR PLUS METABOLISM	METAB IS TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	METAB IS TR ANTAGONIST	-	-	-	-	-	-
IN VITRO TESTIS CULTURE	INHIBITION OF P4 OR T SYNTHESIS	-	-	-	-	-	-
IN VIVO ASSAYS							
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	+	+?	-	-	-
	UTERINE HISTOLOGY	-	+	+?	-	-	-
	BODY WEIGHT	-	-	-	-	-	-

	VAGINAL CORNIFICATION	-	+?	-	-	-	-
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT PLUS E2	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	-	-	-
	UTERINE HISTOLOGY	-	-	-	-	-	-
	BODY WEIGHT	-	-	-	-	-	-
	VAGINAL CORNIFICATION	-	-	-	-	-	-
PUBERTAL/THYROID ASSAY IN WEANLING FEMALE RAT	GROWTH	-	-	-	-	-	-
	AGE AT VAGINAL OPENING	-	++	++	-	-	-
	WEIGHT AT VAGINAL OPENING	-	++	++	-	-	-
	SERUM T4	-	-	-	-	++	-
	SERUM TSH	-	-	-	-	+?	-
	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	+?↓	-	-	
	OVARIAN WEIGHT	-	-	+?↓	-	-	-
	THYROID HISTOLOGY	-	-	-	-	+-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	LIVER↑	-	-	-	LIVER↑	LIV↑
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT	SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID	-	-	-	-	-	-
	VENTRAL PROSTATE WEIGHT	-	-	-	-	-	-
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	-	-	-	-	-
	GROWTH	-	-	-	-	+-?	-
	NONREPRODUCTIVE ORGAN WEIGHTS	LIVER ↑	-	-	-	LIVER↑	LIV ↑
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT: PLUS T	SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID	+↓	-?	+?↓	-	+-?	+
	VENTRAL PROSTATE WEIGHT	+↓	-?	+?↓	++	+-?	+
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	+↓	-?	+?↓	-	+-?	+
	GROWTH	-	-	-+	-	+-?	-
	NONREPRODUCTIVE ORGAN WEIGHTS		-	-	-		

"Weight-of-Evidence" Determination: Hold, Go to T2T, or Other		**T2T**	**T2T**	**T2T**	**Repeat T1S T2T**	**T2T**	**T2T**

PART 2. Known or expected responses of well characterized toxicants in the EDSTP T1S batttery. The list of endpoin All of the described examples would have sufficient positive responses to be triggered for T2T.

T1S ASSAY	**ENDPOINT**						
IN VITRO HTPS OR BENCH ASSAYS		**Steroidogene-sis Fungicide**	**Thyroid Hormone Analogue**	**Fungicide**	**Wood-derived Estrogen**	**Estrogenic Pesticide**	**Anti-thyroid Drug**
HTPS ER/MVLN	**ER AGONIST**	-	-	-	+-	+	-
ABOVE PLUS E2	**ER ANTAGONIST**	-	-	-	-	-	-
HTPS ER PLUS METABOLISM	**METAB IS ER AGONIST**	-	-	-	-	-	-
ABOVE PLUS E2	**METAB IS ER ANTAGONIST**	-	-	-	-	-	-
HTPS AR/CV-1	**AR AGONIST**	-	-	-	-	-	-
ABOVE PLUS DHT	**AR ANTAGONIST**	-	-	-	-	-	-
HTPS AR PLUS METABOLISM	**METAB IS AR AGONIST**	-	-	-	-	-	-
ABOVE PLUS DHT	**METAB IS AR ANTAGONIST**		-	-	-	-	-
HTPS TR	**TR AGONIST**	-	+	-	-	-	-
ABOVE PLUS T3	**TR ANTAGONIST**	-	-	-	-	-	-
HTPS TR PLUS METABOLISM	**METAB IS TR AGONIST**	-	-	-	-	-	-
ABOVE PLUS T3	**METAB IS TR ANTAGONIST**	-	-	-	-	-	-
IN VITRO TESTIS CULTURE	**INHIBITION OF P4 OR T SYNTHESIS**	++	-	-?	+??	-	-
IN VIVO ASSAYS							
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT	**UTERINE WEIGHT WITH AND WITHOUT FLUID**	-	-	-	-	+	-
	UTERINE HISTOLOGY	-	-	-	-	+	-
	BODY WEIGHT	-	-	-	-	-	-
	VAGINAL SMEAR	-	-	-	-	-	
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT PLUS E2	**UTERINE WEIGHT WITH AND WITHOUT FLUID**	-	-	-	-	-	-
	UTERINE HISTOLOGY	-	-	-	-	-	-
	BODY WEIGHT	-	-	-	-	-	-
	VAGINAL SMEAR	-	-	-	-	-	-

PUBERTAL/THYROID ASSAY IN WEANLING FEMALE RAT	**GROWTH**	-	-?	-	-	-	+-?
	AGE AT VAGINAL OPENING	+↑	-?	+↑	+?↑	+	+-?
	WEIGHT AT VAGINAL OPENING	+↑	-?	+↑	+?↑	+	-
	SERUM T4	-	+↓?	-	-	-	++
	SERUM TSH	-	+↓?	-	-	-	++
	UTERINE WEIGHT WITH AND WITHOUT FLUID	+?↓	-?	+?↓	-	-	+-?
	OVARIAN WEIGHT	+↑	-?	+↑	-	-	+?
	THYROID HISTOLOGY	-	+?↓	-	-	-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	**LIVER** ?↑	-?	**LIVER** ↑	**LIVER** ↑?	**LIVER**↑	**THYROID** ↑
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT	**SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID**	-	-	-	-	-	-
	VENTRAL PROSTATE WEIGHT	-	-	-	-	-	-
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	-	-	-	-	-
	GROWTH	-	-?	-	-	-	+-?
	NONREPRODUCTIVE ORGAN WEIGHTS	**LIVER** ?↑	-	+?↑	-	**LIVER**↑	+-?
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT: PLUS T	**SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID**	-	-	-	-	+	+-?
	VENTRAL PROSTATE WEIGHT	-	-	-	-	+	+-?
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	-	-	-	+?	+-?
	GROWTH	-	-?	-	-	-	+-?
	NONREPRODUCTIVE ORGAN WEIGHTS	**LIVER** ?↑	-	**LIVER**↑	-	**LIVER**↑	+-?
"Weight-of-Evidence" Determination: Hold, Go to T2T, or Other		**T2T**	**REPEAT T1S, T2T**	**T2T**	**MAY REPEAT T1S, T2T**	**T2T**	**T2T**

PART 3. Known or expected responses of well characterized toxicants in the EDSTP T1S batttery. The list of endpoin

T1S ASSAY	ENDPOINT						
IN VITRO HTPS OR BENCH ASSAYS		**Neurotoxic Pesticide**	**Plasticizer**	**Anti-androgenic Fungicide**	**Anti-estrogenic Drug**	**Herbicide**	**Xeno-estrogen**
HTPS ER/MVLN	ER AGONIST	+-	+-	-	-	-	++
ABOVE PLUS E2	ER ANTAGONIST	-	-	-	-+	-	-
HTPS ER PLUS METABOLISM	METAB IS ER AGONIST	-	-	-	+	-	-
ABOVE PLUS E2	METAB IS ER ANTAGONIST	-	-	-	+++	-	-
HTPS AR/CV-1	AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	AR ANTAGONIST	-	-	-	-	-	-?
HTPS AR PLUS METABOLISM	METAB IS AR AGONIST	-	-	-	-	-	-
ABOVE PLUS DHT	METAB IS AR ANTAGONIST	-	-	++	-?	-	-
HTPS TR	TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	TR ANTAGONIST	-	-	-	-	-	-
HTPS TR PLUS METABOLISM	METAB IS TR AGONIST	-	-	-	-	-	-
ABOVE PLUS T3	METAB IS TR ANTAGONIST	-	-	-	-	-	-
IN VITRO TESTIS CULTURE	INHIBITION OF P4 OR T SYNTHESIS	-	-?	-	-	-	-
IN VIVO ASSAYS							
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	+-	-	++
	UTERINE HISTOLOGY	-	-	-	-+	-	++
	BODY WEIGHT	+	-	-	-	-	-
	VAGINAL CORNIFICATION	-	-	-	+-	-	+
3 DAY UTEROTROPHIC IN ADULT OVX'D RAT PLUS E2	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	++	-	-
	UTERINE HISTOLOGY	-	-	-	++	-	-
	BODY WEIGHT	+	-	-	-	-	-
	VAGINAL CORNIFICATION	-	-	-	++	-	-

PUBERTAL/THYROID ASSAY IN WEANLING FEMALE RAT	**GROWTH**	+	-	-	-	-	-
	AGE AT VAGINAL OPENING	-	-	-	+?	+↓?	-
	WEIGHT AT VAGINAL OPENING	-	-	-	+?	+↓?	-
	SERUM T4	-	-	-	-	-	-
	SERUM TSH	-	-	-	-	-	-
	UTERINE WEIGHT WITH AND WITHOUT FLUID	-	-	-	+	-	-
	OVARIAN WEIGHT	-	-	-	+	-	-
	THYROID HISTOLOGY	-	-	-	-	-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	+	-	-	-	-	-
							-
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT	**SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID**	-	-	-	-	-	-
	VENTRAL PROSTATE WEIGHT	-	-	-	-	-	-
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	-	-	-	-	-
	GROWTH	+	-	-	-	-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	+	-	-	-	-	-
7-10 DAY HERSHBERGER-TYPE ASSAY IN CASTRATED MALE RAT: PLUS T	**SEMINAL VESICLE WEIGHT, WITH AND WITHOUT FLUID**	-	+?	++	-	-	-
	VENTRAL PROSTATE WEIGHT	-	+?	++	-	-	-
	LEVATOR ANI PLUS BULBOCAVERNOSUS WEIGHT	-	+?	+	-	-	-
	GROWTH	+	-	-	-	-	-
	NONREPRODUCTIVE ORGAN WEIGHTS	+	-	-	-	-	-
"Weight-of-Evidence" Determination: Hold, Go to T2T, or Other		**REPEAT T1S, Hold**[1]	**T2T**	**T2T**	**T2T**	**REPEAT T1S, T2T**	**Other**[2]

[Footnote (1) Of the described examples, the neurotoxic pesticide would go to the "hold box" or be studied further before it would go to T2T. Footnote (2) The xenoestrogen is only estrogenic in vitro and by injection, but was not estrogenic by oral treatment. The uterotrophic assay should be repeated with oral dosing to determine if the xenoestrogen was estrogenic by the oral route before it went to T2T.]