APPLIED PHARMACOLOGY
AND TOXICOLOGY, INC.

Consulting & Research Services

MAY 23, 2003

I. INTRODUCTION & BACKGROUND

Applied Pharmacology & Toxicology, Inc. (APT) is pleased to release the results of its Cost Estimate Survey for a subset of the endocrine screening assays that comprise the EDSTAC recommended (and alternatives) Tier 1 battery. The assays comprising the survey include: estrogen receptor binding assay, androgen receptor binding assay, steroidogenesis assay, aromatase assay, uterotrophic assay (three protocols), Hershberger assay, intact male assay, pubertal male assay, and the pubertal female assay. These eleven assays are currently in various stages of validation by the OECD and EPA's Office of Science Coordination and Policy.

The purpose of this survey was to obtain up-to-date cost estimates for conducting the eleven assays under Good Laboratory Practices (GLP) in commercial testing facilities and providing standard reports to a sponsor. The survey was funded by the American Chemistry Council and was carried out in consultation with scientists in member companies, academic institutions, and the U.S. EPA. To conduct the survey, APT obtained estimates from laboratories competent to conduct the specified screening assays. Three industry toxicology laboratories, six contract laboratories and two academic institutions contributed cost estimates for the survey. A list of the participating laboratories is provided in Table 1.

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II. SURVEY DESIGN

Protocols

Protocols for nine screening assays were collected from OECD documents, from Detailed Review Papers (DRPs) developed by EPA's Endocrine Methods Validation Subcommittee (EDMVS),[1] and from industry laboratories that have conducted the assays in parallel with the EDMVS process. Those protocols were reviewed to ensure that the detailed requirements for each assay were included in the protocols used in the survey. Where protocols differed in substantive ways (e.g., number of dose groups or animals per dose group), the version currently under consideration by the EDMVS was used; otherwise, the protocol contained in the latest DRP draft was given preference.

This cost survey stipulated that assays be conducting according to the requirements of Good Laboratory Practices as specified in Subpart J §160.185 of FIFRA or §792.185 of TSCA. However, laboratories were *not* asked to estimate costs for the analytical work generally required to verify chemical identity, purity, or dosage concentrations because the level of effort required varies widely depending upon the chemical.

Under the OECD program, three protocols for the uterotrophic assay were evaluated (administration by oral gavage to ovariectomized (OVX) adult rats; administration by oral gavage to immature female rats; administration by subcutaneous injection in immature female rats); therefore, all three protocols were included in the survey. It should be noted that these protocols do not include a test group to screen for anti-estrogenic activity. Presumably, testing for anti-estrogenic activity would require one additional test group to be administered a high dose of the test material plus the positive control (ethynylestradiol). This would increase the number of groups by approximately one-sixth.

Table 2 lists the numbers of animals, treatment groups and endpoints measured in each assay.

A standardized format was developed for the survey protocols and the details of each assay adapted to the format. The protocols were incorporated into a single portable document format (Adobe Acrobat PDF) together with a cover letter describing the survey process. A separate cost estimate response form was developed in Microsoft Excel. These two documents comprised the survey. Appendix A contains the survey and detailed protocols provided to the laboratories for developing cost estimates.

Initially, approximately thirty-nine laboratories were contacted by telephone regarding their ability and willingness to participate in the survey. During those calls, the purpose and format of the survey was explained and an invitation to participate extended. On February 21, 2003, surveys were sent

[1] http://www.epa.gov/scipoly/oscpendo/index.htm

electronically or by Priority Mail to the twenty-two laboratories that initially indicated a willingness to review the survey.

Survey responses were obtained under the assurance of confidentiality so that individual cost estimates would not be attributable to specific laboratories. This precludes use of the survey results to compare laboratories by cost and helps to ensure that responses to the survey reflect actual price projections.

All laboratories were contacted to verify receipt of the survey and to solicit questions or comments regarding the survey and providing cost estimates. The study director discussed specific technical questions and comments with individual respondents during the period of protocol review and estimate formulation. Cost estimate responses were accepted through March 30, 2003.

III. RESULTS

Individual cost estimates along with maximum, minimum, median and mean values for each assay are listed in Table 3. Figure 1 presents statistical calculations and graphical analysis of the distribution of estimates for each assay generated by JMP Statistical Discover software (SAS Institute, version 3.2.2).

Of particular interest is the potential cost per chemical for conducting the subset of Tier 1 screening assays surveyed here. Using either the mean or median value for the four *in vitro* assays, the Hershberger assay, one of the uterotrophic assays, and one assay from among the male pubertal, female pubertal, or intact male assay yields a total projected cost of more than $100,000 (Table 4).

Inclusion of a dose group to screen for anti-estrogenic activity in the uterotrophic assay would add approximately $2700 to the cost. Analytical chemistry, if necessary, would add to these cost projections as well. Screening several test compounds concurrently rather than separately, as was assumed for this survey, might reduce costs somewhat (e.g., through use of a single set of controls), however, logistical problems inherent in conducting large studies would limit potential savings.

The estimates for each assay showed considerable variability. Several possible reasons for the variability seem plausible. First, the laboratories most experienced with these assays may have achieved economies of operation that other laboratories have yet to develop. Differences in the way overhead costs are calculated by industry, contract and academic laboratories may also contribute variability to the estimates. Based on APT's knowledge of the individual laboratories, it would appear that those laboratories capable of conducting an entire protocol in-house generally projected lower costs. This seems reasonable considering that some protocols require relatively expensive hormone analyses and histopathology. While some laboratories gave consistently higher- or lower-than-average estimates, no laboratory consistently provided the maximum or the minimum estimate.

The total cost projection for this subset of Tier 1 screening assays is less than half that projected for the entire Tier 1 battery based on a survey conducted by APT in 1998 (EDSTAC Final Report, Appendix S[2]). These cost differences can be accounted for on the basis of:

- Tier 1 assays in frogs and fish that were included in the 1998 survey are not included in the subset of assays surveyed here;
- The current survey requires the measurement of fewer endpoints in the mammalian screening assays than was required in the 1998 protocols;
- An estimated range for analytical costs was included in the 1998 survey, but was omitted in the current survey.

IV. ACKNOWLEDGEMENTS

APT is grateful to the participating laboratories for their willingness to participate in this survey and for their hard work in generating professional cost estimates. We especially wish to thank Gary Timm (U.S.EPA), Sue Marty (Dow Chemical), Mike Kaplan and John O'Conner (DuPont Haskell Laboratory), Willie Owens (Proctor & Gamble) and Rick Becker (American Chemistry Council) for providing assay protocols and advice. Those who are informed by the results of this survey should appreciate the significant time and resources the participants devoted to generating cost estimates. Any shortcomings in the results of the survey are solely the responsibility of APT and should not be attributed to the participating laboratories, the American Chemistry Council or the U.S. EPA.

Christopher J. Borgert, Ph.D. May 23, 2003
President & Principal Scientist, APT, Inc.

[2] http://www.epa.gov/scipoly/oscpendo/history/finalrpt.htm (see link to Appendix S). The survey conducted in 1998 also obtained cost estimates for Tier 2 tests.

Table 1: Participating Laboratories

BioQual Inc.
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Calvert Laboratories, Inc.
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Carol D. Sloan

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Lori B. Kaiser, Ph.D. Lkaiser@genelogic.com

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TABLE 2: Numbers of Animals and Treatment Groups Specified

Assay		Number of Animals	Treatment Groups or Incubations	Endpoints Measured
1.	Estrogen Receptor Binding	*	14 tubes	Radioligand displacement
2.	Androgen Receptor Binding	*	14 tubes	Radioligand displacement
3.	Steroidogenesis (Minced Testis)	10**	48 wells	Enzyme activity
4.	Human Aromatase	**	21 wells	Enzyme activity
5.	Uterotrophic Adult, OVX	36	6 groups	Body Weights, Uterine Weights, Histopathology
6.	Uterotrophic Immature, Oral	36	6 groups	Body Weights, Uterine Weights, Histopathology
7.	Uterotrophic, Immature, SC	36	6 groups	Body Weights, Uterine Weights, Histopathology
8.	Hershberger	54***	9 groups	Feed Consumption, Body Weight, Organ Weights, Gross Pathology
9.	Female Pubertal Onset	60	4 groups	Feed Consumption, Body Weight, Organ Weights, Gross Pathology, Vaginal Opening, Vaginal Lavage, Histology, Hormone Analysis
10.	Male Pubertal Onset	45	3 groups	Feed Consumption, Body Weight, Organ Weights, Gross Pathology, Histology, Preputial Separation, Hormone Analysis
11.	Intact Male Endocrine Screen	75	5 groups	Body Weights, Feed Consumption, Hormone Analysis, Organ Weights, Histopathology

* A small, unspecified number of rats are required.
**Only tissue or cells is required
*** Hershberger protocol includes groups to assess anti-androgenic activity

Table 3: Cost Estimate Data by Laboratory and Assay*

Lab*	ER Binding	AR Binding	Steroidogen	Aromatase	Uterotrophic OVX/Oral	Uterotrphic Immat/Oral	Uterotrophic Immat/SC	Hershberger	Female Pubertal	Male Pubertal	Intact Male
A	.	.	.	.	$25,000	$25,000	$25,000	$25,000	$45,500	$49,500	$63,000
B	$7,500	$8,500	.	.	$20,000	$18,000	$18,000	$25,000	.	.	.
C	.	.	.	.	$15,000	$13,500	$13,600	$27,000	$44,700	$44,000	$87,300
D	.	.	.	.	$19,436	$21,981	$22,142	$28,928	$54,879	$61,021	$96,365
E	$6,100	$8,700	$6,700	$9,850	$12,200	$11,300	$11,300	$22,760	$62,250	$59,700	$67,900
F	$2,000	$2,000	.	.	$12,000	$12,000	$12,000	$20,000	$36,000	$36,000	$50,000
G	.	.	.	.	$10,500	$11,900	$11,900	$17,600	$39,000	$33,800	$70,500
H	$8,800	$9,200	$17,600	$18,000	$12,200	$15,500	$13,800	$19,000	$34,000	$32,100	$28,800
I	$6,000	$6,500	$5,000	$6,500	.	.	.	.	.	.	.
J	$4,000	$4,000	$4,000	$4,000	.	.	.	.	.	.	.
K	$1,400	$1,625	$7,000	$5,500	$14,000	$16,500	$16,500	$20,500	$44,200	$44,000	$45,000
L	$15,000	$16,000	$30,000	$75,000	$60,000	$55,000	$50,000	$70,000	$150,000	$150,000	$75,000
MIN	$1,400	$1,625	$4,000	$4,000	$10,500	$11,300	$11,300	$17,600	$34,000	$32,100	$28,800
MAX	$15,000	$16,000	$30,000	$75,000	$60,000	$55,000	$50,000	$70,000	$150,000	$150,000	$96,365
MED	$6,050	$7,500	$6,850	$8,175	$14,500	$16,000	$15,150	$23,880	$44,700	$44,000	$67,900
MEAN	$6,350	$7,066	$11,717	$19,808	$20,034	$20,068	$19,424	$27,579	$56,725	$56,680	$64,874

* Cost Estimates are listed in U.S. Dollars
**Laboratories are designated by letters to maintain confidentiality of the estimates.

Table 4: Costs* of Assays Included in EPA's EDSTAC Report[3] Recommended Tier 1 Screening (T1S) Battery & EDSTAC Recommended Alternatives

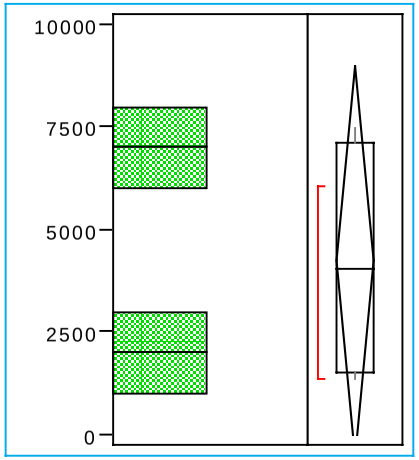
Assays	No. Animals	Mean Costs	Median Costs	Recommended T1S Battery	Alternative 1 T1S Battery	Alternative 2 T1S Battery
In Vitro						
Estrogen receptor binding		$6,350	$6,050	**x**	**x**	**x**
Androgen receptor binding		$7,066	$7,500	**x**	**x**	**x**
Steroidogenesis	10	$11,717	$6,850	**x**		
Placental aromatase		$19,808	$8,175		**x ****	
In Vivo						
Uterotrophic (OVX / Oral)***	36	$20,034	$14,500	**x**	**x**	**x**
Hershberger	54	$27,579	$23,880	**x**		
Pubertal female	60	$56,725	$44,700	**x**		
Intact male	75	$56,680	$44,000		**x**	
Pubertal male	45	$64,874	$67,900			**x**
Total Number of Animals				**160**	**111**	**81**
Mean Estimates Total				**$129,471**	**$109,938**	**$98,324**
Median Estimates Total				**$103,480**	**$80,225**	**$95,950**

*Cost Estimates are listed in U.S. Dollars; Analytical costs not included in estimates.
**There is some question as to whether the placental aromatase assay is redundant with endpoints measured in the intact male assay, and therefore, unnecessary in this alternative battery.
***Costs may vary slightly depending on the protocol selected for conducting the uterotrophic assay (see Table 3 and Figure 1).

[3] http://www.epa.gov/scipoly/oscpendo/history/finalrpt.htm

Figure 1. Statistical Parameters and Distributions Calculated from the Cost Estimate Data

ER Binding



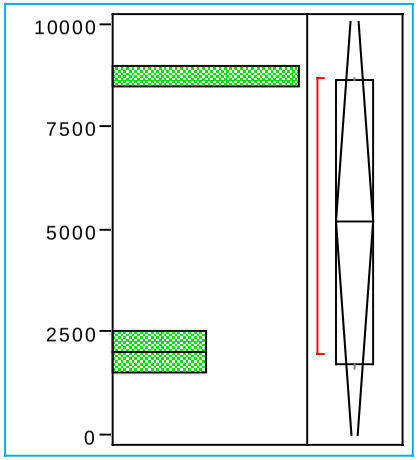
Quantiles

maximum	100.0%	7500.0
	99.5%	7500.0
	97.5%	7500.0
	90.0%	7500.0
quartile	75.0%	7150.0
median	50.0%	4050.0
quartile	25.0%	1550.0
	10.0%	1400.0
	2.5%	1400.0
	0.5%	1400.0
minimum	0.0%	1400.0

Moments

Mean	4250.000
Std Dev	3009.430
Std Error Mean	1504.715
Upper 95% Mean	9038.755
Lower 95% Mean	-538.755
N	4.000
Sum Weights	4.000

AR Binding



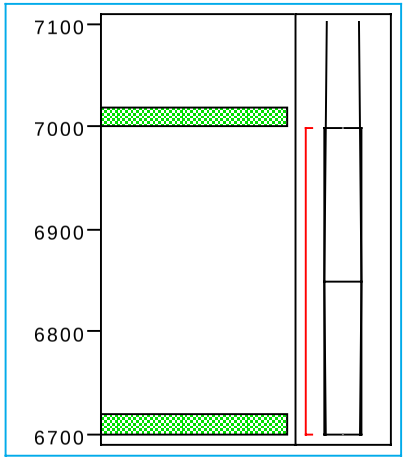
Quantiles

maximum	100.0%	8700.0
	99.5%	8700.0
	97.5%	8700.0
	90.0%	8700.0
quartile	75.0%	8650.0
median	50.0%	5250.0
quartile	25.0%	1718.8
	10.0%	1625.0
	2.5%	1625.0
	0.5%	1625.0
minimum	0.0%	1625.0

Moments

Mean	5206.25
Std Dev	3922.60
Std Error Mean	1961.30
Upper 95% Mean	11448.09
Lower 95% Mean	-1035.59
N	4.00
Sum Weights	4.00

Steroidogenesis



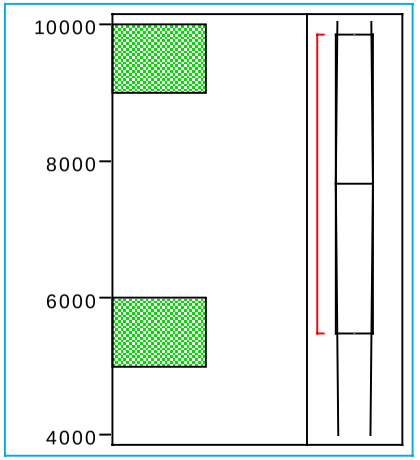
Quantiles

maximum	100.0%	7000.0
	99.5%	7000.0
	97.5%	7000.0
	90.0%	7000.0
quartile	75.0%	7000.0
median	50.0%	6850.0
quartile	25.0%	6700.0
	10.0%	6700.0
	2.5%	6700.0
	0.5%	6700.0
minimum	0.0%	6700.0

Moments

Mean	6850.000
Std Dev	212.132
Std Error Mean	150.000
Upper 95% Mean	8755.900
Lower 95% Mean	4944.100
N	2.000
Sum Weights	2.000

Aromatase



Quantiles

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	97.5%	9850.0
	90.0%	9850.0
quartile	75.0%	9850.0
median	50.0%	7675.0
quartile	25.0%	5500.0
	10.0%	5500.0
	2.5%	5500.0
	0.5%	5500.0
minimum	0.0%	5500.0

Moments

Mean	7675.00
Std Dev	3075.91
Std Error Mean	2175.00
Upper 95% Mean	35310.55
Lower 95% Mean	-19960.55
N	2.00
Sum Weights	2.00

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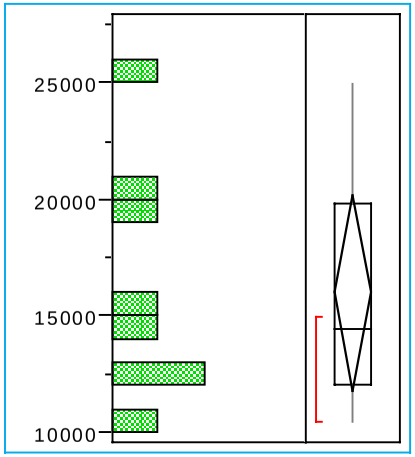
Figure 1. (continued)

Uterotrophic / OVX



Quantiles

maximum	100.0%	25000
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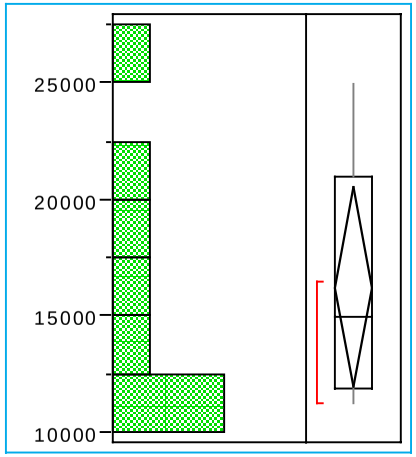
	97.5%	25000
	90.0%	25000
quartile	75.0%	19859
median	50.0%	14500
quartile	25.0%	12050
	10.0%	10500
	2.5%	10500
	0.5%	10500
minimum	0.0%	10500

Moments

Mean	16017.00
Std Dev	4992.76
Std Error Mean	1765.21
Upper 95% Mean	20191.09
Lower 95% Mean	11842.91
N	8.00
Sum Weights	8.00

Uterotrophic Imm. Oral



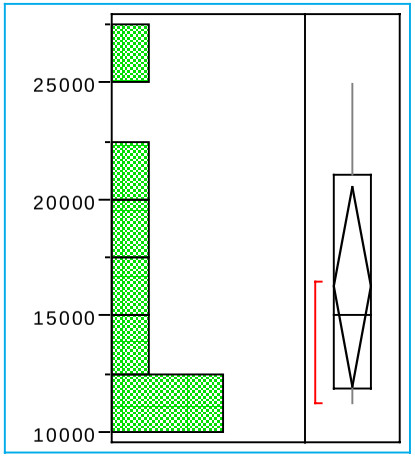
Quantiles

maximum	100.0%	25000
	99.5%	25000
	97.5%	25000
	90.0%	25000
quartile	75.0%	20986
median	50.0%	15000
quartile	25.0%	11925
	10.0%	11300
	2.5%	11300
	0.5%	11300
minimum	0.0%	11300

Moments

Mean	16272.62
Std Dev	5093.10
Std Error Mean	1800.68
Upper 95% Mean	20530.60
Lower 95% Mean	12014.65
N	8.00
Sum Weights	8.00

Uterotrophic Imm. SC



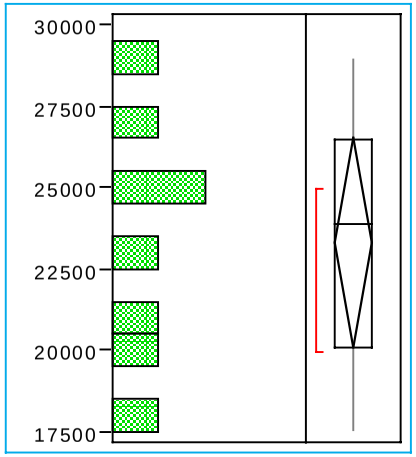
Quantiles

maximum	100.0%	25000
	99.5%	25000
	97.5%	25000
	90.0%	25000
quartile	75.0%	21106
median	50.0%	15050
quartile	25.0%	11925
	10.0%	11300
	2.5%	11300
	0.5%	11300
minimum	0.0%	11300

Moments

Mean	16305.25
Std Dev	5111.45
Std Error Mean	1807.17
Upper 95% Mean	20578.57
Lower 95% Mean	12031.93
N	8.00
Sum Weights	8.00

Hershberger



Quantiles

maximum	100.0%	28928
	99.5%	28928
	97.5%	28928
	90.0%	28928
quartile	75.0%	26500
median	50.0%	23880
quartile	25.0%	20125
	10.0%	17600
	2.5%	17600
	0.5%	17600
minimum	0.0%	17600

Moments

Mean	23348.50
Std Dev	3829.16
Std Error Mean	1353.81
Upper 95% Mean	26549.78
Lower 95% Mean	20147.22
N	8.00
Sum Weights	8.00

Figure 1. (continued)