III. SURVEY DESIGN

The Cost Estimate Survey for Endocrine Disrupter Screening and Testing Batteries developed by APT included the following:

- standardized protocols developed by APT;
- Chapter 5 and Appendices J, K, L, and P of the EDSTAC April 3, 1998 Draft Report;
- a cover letter explaining the purpose, design, specific requests and reporting deadlines of the survey;
- a Cost Estimate Form for reporting estimates to APT.

Based upon Chapter 5 and Appendices J, K, L, and P of the April 3, 1998 Draft Report of the EDSTAC, APT developed detailed protocols for thirteen screening assays and seven tests according to a standardized format. The purpose of drafting standardized protocols was to help ensure that each laboratory provided estimates based upon a similar set of assumptions regarding the details of the assays. In general, the protocols drafted by APT included all of the EDSTAC suggested endpoints and study enhancements. Single cost estimates to include all endpoints were requested for each protocol. A range of analytical cost estimates for each protocol was requested as a separate estimate.

The Project Director, Christopher J. Borgert, Ph.D. contacted six industry toxicology laboratories and twelve contract laboratories, explained the purpose and format of the survey and extended the invitation to participate. Surveys were sent to the laboratories on May 18, 1998 by Federal Express overnight. Cost Estimate Forms were to be returned to APT by Friday, May 29, 1998.

Survey responses were obtained under the assurance of confidentiality. Confidentiality was assured both verbally and in the cover letter that accompanied each survey which stated that individual cost estimates would not be attributable to any particular responding laboratory. Anonymity was assured to encourage estimates based purely on professional judgement of the expected costs, to discourage competitive bidding as an influencing factor, and to encourage laboratories to respond without fear of placing themselves at a competitive disadvantage should their estimate be higher than that of a competitor.

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.

IV. PROTOCOL DESIGN

The following features were common to each protocol:

- conductance under Good Laboratory Practice guidelines required;
- a dose range finding pilot study to be conducted as an integral component of the study;
- *in vivo* assays conducted using three dose levels of the test substance and *in vitro* assays as per the EDSTAC report;
- endpoints listed as "optional" in the EDSTAC report were required in the protocols;
- data summary and final report required as part of the study;
- analytical chemistry to verify the purity and stability of the test agent to be conducted by the study sponsor;
- a separate cost estimate for conducting the analytical chemistry was requested assuming that the study sponsor provided only the analytical protocol (separate lines provided for this figure on the Cost Estimate Survey).

For Tier 2 Tests that are recommended enhancements of an existing EPA guideline study, a study enhancement protocol was developed and a photocopy of the appropriate EPA guideline was included. Some study designs were not clearly outlined in the EDSTAC report, including the Fish Gonadal Recrudescence Tier 1 Screen, the Frog Metamorphosis Tier 1 Screen, Enhancements to the Avian Reproduction Toxicity study, and the Amphibian Reproductive and Developmental Toxicity Test. These required a review of the scientific literature cited in Appendix J and consultation with experts in these methods in order to provide protocols for the survey. Dr. William Benson of the University of Mississippi provided preprints of his publications in press to assist the development of the Fish Gonadal Recrudescence protocol. Dr. Benson's assistance is greatly appreciated. To develop the protocol Enhancements to the Avian Reproductive Toxicity Test, literature concerning the cold stress test, the cliff test and the nest attentiveness test were reviewed. In addition, extensive conversations were held with Mark Jaber of Wildlife International, Ltd. and with Andrew Marias of Bio-Life Associates, Ltd. concerning avian husbandry, species characteristics and feasibility of various procedures. Their patient assistance is greatly appreciated. Publications provided by Dr. Douglas Fort of the Stover Group, Inc. were used directly as protocols for the Frog Metamorphosis Assay and the Amphibian Reproductive and Developmental Toxicity test. His kind assistance is greatly appreciated.

V. UNCERTAINTIES AND VARIANCE

Many of the respondents have followed the EDSTAC process and were quite familiar with EDSTAC's recommendations before being contacted to participate in the survey. Respondents commented that they were generally able to follow the protocols and found them useful for generating cost estimates. However, APT's internal review of the survey as well as comments from some respondents identified several sources of variability and uncertainty in the responses.

1. In some instances, participants may have had a different interpretation of the EDSTAC recommendations than were specified in APT's protocols. There was some variation among respondents with regard to the endpoints and analyses they included as part of their estimates. Since hormone analyses constitute a significant portion of the expense of many of these assays, lack of uniformity of interpretation undoubtedly created variance in the results.
2. The Steroidogenesis Assay in Minced Testis Culture (S. T1S) specifies an HPLC analysis of hormone levels, but in fact, radioimmunoassay is the method of choice. In most cases, respondents assumed the use of radioimmunoassay without being contacted. Lack of uniformity on this point could have contributed to some variability in the estimates for this assay.
3. Hormone analyses were not listed explicitly for the alternative *in vivo* mammalian screening assays (T1S 11, 12 and 13), though they were intended to be required. Most respondents assumed inclusion of the appropriate hormone levels without being contacted verbally, stating that the inclusion of these endpoints was implicit.
4. Many laboratories utilize standardized protocol formats and cost tables to generate estimates. The format of these standardized tables are likely different from laboratory to laboratory, and may include factors not specified in the APT protocols. Use of different formats to generate estimates may have produced some of the variability.
5. Some Tier 2 Tests involve guideline studies for which all endpoints are not routinely required. APT's protocols did not specify whether to include or omit endpoint measurements that would be triggered by certain results in these studies. Different assumptions regarding the inclusion of such endpoints could have caused some variation among the estimates for these assays.

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VI. SPECIFIC COMMENTS REGARDING ASSAYS, TESTS AND COSTS

A. Tier 1 Screening

Several respondents commented that 10 animals per each dose group, as specified in the *in vivo* Tier 1 Screening protocols, is insufficient to detect differences in hormone levels between treatment groups. The use of 15 animals per group was suggested as the minimum number of animals that should comprise a treatment group. APT chose 10 animals per group based upon study designs for some of EDSTAC's recommended *in vivo* Tier 1 screens. The use of 15 rather than 10 animals per treatment group would increase the cost of most of the *in vivo* Tier 1 Screening Assays by roughly 30 - 40%.

The APT protocols included both the endpoints listed as "required" in the EDSTAC report appendices as well as those listed as "optional". Several respondents commented that inclusion of the optional endpoints increased their cost estimates significantly, and also suggested that many of these optional endpoints may prove to be redundant and potentially uninformative regarding effects on the endocrine system. It is APT's opinion that these suggestions underscore the need for a rigorous validation and standardization effort capable of identifying the endpoints that are both reliable and relevant for endocrine disruption.

Some respondents based their estimates for Tier 1 *in vitro* Screening assays 1 and 2 on the use of commercially available purified receptor preparations, which are significantly less costly than *de novo* purification procedures.

B. Tier 2 Testing

For an avian reproduction test to assess endocrine endpoints, the EDSTAC recommends specific enhancements to the one-generation guideline study (OPPTS 850.230), including extension to a second generation. Though the guideline specifies Bobwhite Quail or Mallard Duck, EDSTAC recommends Japanese Quail for two-generation studies (Appendix P). Respondents to this survey agreed that for assessing endocrine-mediated effects, a two-generation study in Japanese Quail would be preferred over the current one-generation study guideline. However, respondents questioned the feasibility of assessing the endpoint enhancements recommended by EDSTAC as integral components of either a one-generation or two-generation reproduction study in birds. Neither species of Quail could be used to assess nesting. Separate studies would have to be conducted to address the other recommended enhancements due to logistical problems that would be encountered in assessing other required endpoints.

Though an imperfect solution to this dilemma, APT specified a one-generation study design in Mallard duck for the cost estimate survey. This decision was intended to encourage consistency in estimates by following the OPPTS guideline as closely as possible and to enable an evaluation of nesting in the same species used for the main study. The cost of performing a two-generation study in Japanese Quail was estimated to be within a two-fold range of the specified protocol (17. T2T).

In general, respondents stressed the need for determining statistically the number of hatches and numbers of offspring to be reared in two generation wildlife studies. Respondents generally commented that rearing the same number of offspring as were included in the parental generation would seem sufficient. They also noted that costs could vary substantially depending on the actual numbers of offspring required.

C. Analytical Cost Estimates

There was a wide range of interpretation regarding the request for an estimate of analytical costs. Some respondents interpreted the protocols to request only verification of the purity of the test substance, while others assumed verification of the dosing concentrations as well. Consequent to the various interpretations regarding analytical work required, respondents gave a wide range of cost estimates for analytical work, especially for the Tier 1 Screening Assays.

In addition, a wide range of costs is expected due to the wide range of complexity in analyzing chemicals with very different characteristics. A wide range of instrumentation can be required depending upon the physical-chemical properties of a test substance. Some analytical procedures are extremely complex, time consuming and costly, while others are relatively simple and inexpensive.

It should be noted that the estimates provided do not include the cost of developing a suitable analytical protocol when none exists. The expense of developing an analytical protocol can exceed the cost of its use by many times. The estimates do not necessarily include analytical costs for any but the most common routes of exposure or verification of dose stability in long-term dosing experiments. These factors can significantly increase analytical costs. On the other hand, few factors would decrease actual analytical costs below those estimated in this survey. Therefore, the higher ends of the analytical cost estimate ranges are likely to be more appropriate for estimating probable costs of the Screening and Testing batteries, and may actually underestimate actual analytical costs.

D. Quantity Cost Reductions

Several respondents noted that there is considerable economy of scale for performing hormone analyses. Thousands of analyses can be performed for little more than the cost of one hundred assays. Therefore, quantity cost reductions would depend upon the number of samples being run simultaneously by a single laboratory. Little economy of scale appears likely for Tier 2 Tests due to the duration and complexity of these studies.

Totals including analytical costs listed in Table 2 for Tier 1 Screening were calculated assuming the assays would be conducted in a single laboratory and that one analytical determination could suffice for all the assays. In practice, this may not be possible nor feasible. It should be kept in mind that analytical costs would be greater if it is necessary to run separate analyses for each assay.