1. Introduction

A number of computer packages are commercially available for the acquisition and processing of toxicology and pathology data. Most have rudimentary statistical capabilities, but lack real sophistication in terms of state-of-the-art statistical methods. General purpose statistical packages such as SAS® software provide sophisticated statistical capabilities, but are not specifically designed for a specialty such as toxicology. In order to provide these enhanced capabilities to this selective set of users, Statistics Unlimited, Inc. has developed the TPSTAT™ system.

The purpose of this paper is to describe the design considerations involved in the development of such a system, the particular features of TPSTAT, some of the statistical and computational issues involved in the development process, the current implementation of TPSTAT under Version 6.06 of SAS, and plans we have for its future. In presenting this paper, we hope to provide the reader with an understanding of the problems we faced in the development of TPSTAT (some of which we still face), and an appreciation for the choices we made along the way, particularly the use of SAS as the analysis tool.

Every technical project of reasonable magnitude presents a set of alternative choices to the project team. For development of statistical software, this includes a choice in the statistical methods to be implemented, hardware and software for implementation, module structure, documentation, validation, and so on. There is even a choice as to which versions will be released to the public. As with most packages, we envision our system as continually evolving, and we expect that it will undergo a number of major enhancements throughout its lifetime.

2. Design Considerations

In designing a specialized statistical package such as TPSTAT, there are a number of considerations which impact on the design of the system. Several of the factors we considered during our system development were:

1. The most likely user is a toxicologist or pathologist with limited training in statistics. It is therefore critical that the system be easy to use for a non-statistician.

2. Given the many recent developments in the statistical field related to analysis of toxicology/pathology data, it is important to implement state-of-the-art procedures, if at all possible.

3. Some of the procedures implemented should be "standard" approaches. Because others may appear "exotic" to the toxicologists or pathologists using the system, it is important that some choices for analyses be familiar to them.

4. The system should perform statistical analyses on a wide variety of data types in toxicology.

5. The system should easily interface with current toxicology and pathology data base systems.

6. The system should provide users with understandable reports in convenient formats.

7. It is vital to allow for future expansion, so that new methods can be added and new types of data processed within the system.

8. The system should not be a burden to maintain, either by the developer or by a corporate systems group.

9. The system should be validated to the fullest extent possible. Such validations should satisfy Good Laboratory Practice (GLP) regulations.

10. The documentation should be clear and concise. It should supply information on how to use the system and on the statistical methodology implemented.

3. Features of TPSTAT

The current version of TPSTAT consists of two subsystems: TOXSTAT™, for the analysis and reporting of data from subchronic and chronic toxicology studies, and PATHSTAT™, for the analysis and reporting of survival and tumor data. TOXSTAT is implemented using SAS as a high level language and runs on Digital Equipment Corporation (DEC™) computers running the VAX/VMS™ operating system. Each subsystem is menu-driven, with appropriate menus coded in DEC command language. The end-product of the user dialogue with the system is a batch job which is submitted to the batch processor. Upon completion of the batch job, statistical reports are routed to an appropriate output printing device. These reports are also available as disk files.

3.1. The TOXSTAT System

TOXSTAT provides toxicologists and statisticians with ready to use statistical analysis and reporting modules for toxicology data. The system incorporates many state-of-the-art statistical techniques and accommodates a wide variety of experimental designs.

Perhaps the most widely used experimental design in toxicology is one which includes a single control group and several test groups at increasing levels of a test substance. TOXSTAT implements trend tests applicable to such designs. Both parametric (normal theory) and nonparametric (Jonckheere’s test) analyses are...
available. The parametric tests are the Welch t-test and the F test as specified by both (1983). The system also has the flexibility to include extra comparison groups (such as untreated control or positive control) in addition to the trend groups. These comparisons are performed using the t-test (normal theory) or the Wilcoxon test (nonparametric) with pairwise tests against the first control group. Finally, in situations without any natural ordering of treatment groups, TOXSTAT implements either a Dunnett’s test (normal theory) or a Dunn’s test (nonparametric) for pairwise comparisons of all groups against the control group. The range of statistical analysis choices is indicated by the Statistical Analysis Main Menu depicted in Figure 1.

All trend tests are two-sided and conducted at the .05 level. All follow-up tests (after sequentially eliminating the highest dose group) are one-sided and in the direction of the overall trend. Each analysis in applied to a single data type collected in the study. TOXSTAT analyzes a variety of data types: body weights; food consumption; organ weights, organ weight/body weight ratios; serum chemistry data; hematology data; and urinalysis data. The Data Selection Menu is depicted in Figure 2.

TOXSTAT processes data which include missing values and "messy" data, although some restrictions apply (see the user manual for details). It is designed to use data files which are extracted from toxicology data base systems. These extract files must be standard ASCII files and compatible with TOXSTAT specifications.

For each statistical analysis conducted, two types of reports are prepared: a summary report and a raw data report. The summary report contains summary statistics (means, standard errors, and sample sizes) for each treatment group and time point, as well as results of the statistical analysis conducted (p-values). Statistical tests for baseline and time point boundaries are printed. For fatal tumors, intermediate results are printed for each group and time point at which a death with tumor occurs. Both the number of animals at risk and the number of animals dying with tumor per group are printed. For Peto analyses, the interval boundaries are printed along with the number of animals dying in the interval and the number of animals with tumor. The logistic scores intermediate printout provides only group totals because intervals are not defined with this approach.

3.3. The Structure of TOXSTAT and PATHSTAT

Both TOXSTAT and PATHSTAT are composed of four kinds of modules: DEC Command Language (DCL) modules, SAS "control" modules, SAS analysis modules, and SAS reporting modules. The DCL modules provide the link from the users and their menu choices to the batch programs performing the analysis and reporting. They contain assignment statements which provide the correspondence between logical and physical data files.

SAS control modules serve two purposes. The first modules perform "housekeeping" functions, take data from input files, and prepare it for processing by the analysis modules. Secondly, after the analysis modules have been processed, control modules take the output and prepare it for the reporting modules.

All analysis modules use SAS/IML.
software to perform statistical calculations. TOOLSTAT calculates group means, standard errors, sample sizes, and p-values from statistical tests. Some of the tests require difficult calculations for p-values, but the power and flexibility of SAS/IML produces results quickly and accurately. In PATHSTAT, the analysis modules calculate test statistics, their p-values, and summary statistics displayed in "intermediate" reports.

Reporting modules generate the TOOLSTAT and PATHSTAT reports. These modules make extensive use of the report-writing and formatting capabilities of SAS software.

3.4. The Implementation of TOOLSTAT under Version 5.06 of SAS

With the recent release of Version 5.06 of SAS for VAX/VMS, we were able to take advantage of the SAS macro facility to rewrite portions of the TOOLSTAT system. The changes were much more extensive for TOOLSTAT than for PATHSTAT, where only minimal changes have been made to provide compatibility with Version 6.

Version 1.03 of TOOLSTAT has recently been released. This version uses the macro language extensively. In doing so, we have made TOOLSTAT more powerful and easier to maintain. Some of the enhancements include:

1) With the new version of TOOLSTAT, there is considerable flexibility in parameter definition. Parameter codes are table driven, rather than being hard-coded in the SAS source code. The user can specify formats for labels, units, raw data, means, and standard errors. Changes can easily be made by editing appropriate code files in the VAX. New parameters can be added in the same way.

To implement this feature, we re-programmed many of the control and analysis modules using symbolic parameters. For example, in version 1.01 of TOOLSTAT, we had many PUT statements in our report-writers of the form:

```
IF CODE='HCT' THEN PUT #L @(COL-1)
MEAN 5.2 #(L+1) @COL SE 4.2;
```

Therefore, changing the "code" for hematocrit, the format for its mean, or the format for its standard error would necessitate changing this line of code (and similar lines elsewhere). In our recent version, we use symbolic parameters for codes, mean formats, and standard error formats. An initial macro (WRITEMS) is set up to generate a series of PUT statements, and this macro is then called by the report-writing module:

```
%MACRO WRITEMS;
%DO J=1 TO 60TPARM;
  IF CODE='APARMSJ' THEN PUT #L @((COL-1)
  MEAN 5.2 #(L+1) @COL SE 4.2;
%END;
%END WRITEMS;
```

Similarly, customized parameter titles and units are processed from code files and incorporated into report-writing modules via macros (LABELS and UNITS):

```
%MACRO LABELS;
PROC FORMAT; VALUE $LABEL
%DO J=1 TO 60TPARM;
  "$APARMSJ" = "$APARMSJ"
%END;
%END LABELS;

%MACRO UNITS;
PROC FORMAT; VALUE $UNIT
%DO J=1 TO 60TPARM;
  "$APARMSJ" = "$APARMSJ"
%END;
%END UNITS;
```

(1) The previous version of TOOLSTAT was somewhat cumbersome in that multiple batch jobs were necessary to perform a single analysis. The first job in the sequence wrote SAS code to files for use by later jobs. In version 1.03 of TOOLSTAT, an analysis runs as a single batch job, and this allows for simultaneous runs by multiple users.

Again, this efficiency is accomplished by extensive use of macro and symbolic parameters. For example, in the analysis of most TOOLSTAT data, a separate call is made to the analysis module for each sex (male or female) and time point combination. This is programmed by incorporating the analysis routine (with INCLUDE in a large %DO loop:

```
%IF SEX=M OR SEX=B THEN %DO;
DATA XDATA; SET DATA.M;
IF DAY<=60DAY; DROP DAY;
%INCLUDE &TREND.M.SAS;
DATA OUT.TREND.M; SET TREND.M.
%END;
```

Here, SEX takes on the value M for male data only, F for female data only, and B for both. TREND is a prefix for the appropriate analysis module with output data set TREND.M (for males), 60Y1, 60Y2, . . . are the day value for analysis, and 60DAY is another representation of the day value.

(3) By combining similar modules and by using macros and symbolic parameters, the total number of modules (SAS programs and DEC command language files) has been reduced from almost 400 to under 200. Thus, the new version will be easier to maintain.

(4) Through the use of symbolic parameters, many previous limitations have been
3.5. Current Status

Both TOXSTAT and PATHSTAT were designed to interface with toxicology/pathology data base systems. The current version of TOXSTAT will accept XYBION PATH/TOX toxicity data extract files, and is currently being used by a number of pharmaceutical and chemical companies. The current version of PATHSTAT is set up to process survival and tumor data in ETA file format (Fairweather 1989). Interfaces with pathology data base systems are similarly feasible with PATHSTAT and will be completed in the near future. Several companies are also currently using PATHSTAT.

4. Documentation and Validation

There are two types of documentation for TOXSTAT: user documentation and internal documentation. The user documentation takes the form of the TOXSTAT user manual. This manual is intended to provide the user with the fundamentals on how to use TOXSTAT, as well as an understanding of the statistical methodology implemented. The table of contents for the TOXSTAT user manual is displayed in Figure 4.

System documentation is for the Statistica Unlimited, Inc. staff to use in maintaining the TOXSTAT system. This documentation includes the code for the DEC command language modules and SAS programs which comprise the system, and data flow diagrams for representative portions of TOXSTAT.

Considerable effort has been undertaken to validate TOXSTAT in accordance with GLP regulations. To validate TOXSTAT, computer simulated data were generated for all six data types. Cases considered studies with (1) data from male animals only, (2) data from female animals only, (3) data from both males and females analyzed separately, or (4) data from both sexes analyzed together. For each data type (6 such) and each analysis type (7 such), statistical analyses were performed for each of these four cases. Two or three parameters were randomly selected from at least one treatment time point. Each run was independently performed to compare with the output provided by TOXSTAT. In each instance, results matched exactly except for negligible rounding differences.

PATHSTAT validations were conducted using published data sets for which output of PATHSTAT could be compared against results in the literature. In each instance, results matched exactly except for negligible rounding differences.

5. Statistical Issues

In developing any statistical package, the developer is faced with a number of decisions about statistical methods and their implementation. In the development of TOXSTAT, choices were made regarding the statistical methods to be implemented for toxicity data (TOXSTAT) and the methods implemented for survival and tumor data (PATHSTAT). These choices were motivated by the considerations listed in section 2 and by our knowledge of applicable methods. Other statisticians might have made alternative choices.

A fundamental aspect of current TOXSTAT reports for both raw and summary data is that they always present means and standard errors as summary statistics. Regardless of whether normal theory or nonparametric tests have been performed. An alternative choice here would have been to use other measures of central tendency (e.g., median, trimmed mean) and variation (e.g., interquartile range), especially with nonparametric methods. We chose to present means and standard errors because of their widespread use in most toxicology data base packages and familiarity for the end user. Even with normal theory procedures, one might consider an alternative measure of variation per group based on pooled variance for those procedures using pooling.

All nonparametric procedures base p-value calculations on asymptotic normality. Here, the choice is motivated more by computational convenience rather than by always having large sample sizes.

6. Computational Issues

Several choices regarding computation were also made during the development of TOXSTAT. In TOXSTAT, both the E' and Welch trend modules have p-value calculations which are considerably more difficult when the total number of treatment groups (including vehicle control) is greater than five. In such cases, we opted to implement alternative statistical procedures with simpler p-value calculations. Dunnett's multiple comparison test generally assumes equal group sizes in all treated groups (not necessarily the same as for the control group). In addition, the simplest case is when all groups, including vehicle control, have the same sample size. Our present implementation makes this simplifying assumption. In terms of correlations, this approach assumes that the correlation between each pairwise difference (treatment group mean minus control group mean) is constant and equal to .5. We believe that this approximation
will be satisfactory in most practical instances.

7. The Future

Although we are quite satisfied with our current version of TOXSTAT, we realize that this is only a small step in a potentially long process. The utility of any package is determined by its acceptance by users and its ability to successfully meet their individual objectives. We therefore expect to revise and enhance this version in order to best meet our users' needs.

We can segment our future activities into three categories: the immediate future, the near-term future, and long-term future activities. In the immediate future we plan to assist current users of both TOXSTAT and PATHSTAT and resolve any problems with the current versions. We hope to attract new users for TPSTAT and obtain additional feedback on its utility.

In the near future, we expect to expand its capabilities in the most desired directions. For example, we hope to offer a range of data transformations prior to normal theory tests. Some users have expressed an interest in graphics within TPSTAT (e.g., plots of Kaplan-Meier survival curves, tumor onset curves, and plots of mean values for body weight and food consumption over time). We have also spoken with several statisticians, both in industry and at universities, who have agreed to serve on an informal "advisory" committee to give us feedback and make suggestions about future directions for TPSTAT.

In the long-term, we foresee many possible extensions of the TPSTAT package, not only for toxicology and pathology data, but in other areas as well. We envision similar subsystems for reproductive studies (REPROSTAT), genotoxicity (GENOSTAT), immunotoxicity (IMUNOSTAT), and quantitative risk assessment (RISKSTAT).

8. Footnotes

1Data base systems providing equivalent information, but with modified record formats or file names, can be accommodated by TOXSTAT with minimal reprogramming.

2XYBION Medical Systems Inc., Cedar Knolls, NJ.

3The design of TPSTAT is such that many of the analysis type/data type combinations have identical or similar module structures.

9. References


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Statistical Analysis Main Menu

Enter 1 Welch Trend Test
2 Jonckheere Trend Test
3 E Bar Square Trend Test
4 Dunnett's Test
5 Dunn's Test
6 Welch Trend Test (Dose Groups)
T-tests (All Other Groups)
7 Jonckheere Trend Test (Dose Groups)
Wilcoxon Test (All Other Groups)
8 Purge old SAS logs and listings
9 or <cr> to exit

Select Statistical Analysis Option >

Figure 1. TOXSTAT Statistical Analysis Main Menu
Data Selection Menu

Enter 1 Serum Chemistry Data
2 Hematology Data
3 Urinalysis Data
4 Organ Weights
5 Body Weight
6 Food Consumption
7 or <CR> to return to previous menu
Select Data Option >

Figure 2. TOXSTAT Data Selection Menu

STATISTICAL ANALYSIS OF PATHOLOGY DATA

MAIN MENU

Enter
1 Provide/change header and dose group info.
2 Analyze survival data
3 Analyze tumor data without time adjustment
4 Time-adjusted analysis - Peto method
5 Time-adjusted analysis - Logistic score
6 or <CR> to Exit

Select statistical analysis option >

Figure 3. PATHSTAT Statistical Analysis Menu

(1) The TPSTAT System (introductory material and table of contents for user manual)
(2) Introduction to TOXSTAT
(3) Using TOXSTAT (a guide to the TOXSTAT menu)
(4) TOXSTAT Analysis Modules (description of TOXSTAT statistical methods)
(5) Sample TOXSTAT Reports (annotated)
(6) Introduction to PATHSTAT
(7) Using PATHSTAT (a guide to PATHSTAT menu)
(8) PATHSTAT Analysis Modules (description of PATHSTAT statistical methods)
(9) Sample PATHSTAT Reports (annotated)
(10) Appendices

Figure 4. TPSTAT User Manual Table of Contents