ABSTRACT
In animal toxicity studies, treatments are composed of a series of increasing doses including a vehicle control. The Tukey, Ciminera and Heyse (1985) trend test, referred to as Tukey’s trend test, is often performed to test for linear trend in the response curve corresponding to three dose scales and possibly different dose ranges by deleting the higher doses, (2) identify the NOSTASOT (NO STATistical Significance Of Trend) dose: the maximum dose concentration which is not significantly different from the vehicle group. This is an iterative and tedious process. A SAS macro was developed to automate Tukey’s trend test. The key to automate this process is to convert the sequential use of Tukey’s trend test in identifying NOSTASOT dose into a set of contrast tests where each contrast corresponds to a different dose scale and dose range combination. In this macro, we utilized and implemented an algorithm for contrast coefficient calculation that works for both balanced and unbalanced studies with equal or unequal dose intervals and equal or unequal sample sizes for one-way designs. The output contrast coefficients can be entered in a set of contrast statements of SAS PROC GLM or PROC MIXED to perform trend test. The macro requires minimum manual intervention to input information about doses and sample sizes.

INTRODUCTION
Tukey’s trend test is used frequently in toxicity studies for two purposes (1) to detect a linear trend in the response over the dose range. A nonzero (increasing or decreasing) trend is an evidence of treatment affecting the response of interest, thus an indication of toxicity; (2) to identify the NOSTASOT dose. The procedure for (2) involves a sequence of trend tests, each applied to different range of doses. This iterative procedure can be described as follows. Suppose \( d_1, \ldots, d_n \) are the doses to be examined in the study with \( d_1 = 0 \) being control. First, we apply trend test through the entire dose range \( d_1 \to d_n \). If the trend is not statistically significant (P>0.05), then the NOSTASOT dose is defined to be the highest dose level in the range. If the trend is statistically significant, we delete the highest dose \( d_n \) and repeat trend test through the range \( d_1 \to d_{n-1} \). Repeat trend analysis in this fashion until P>0.05 is observed. The NOSTASOT dose is defined to be the largest dose that yields P>0.05 and no additional P-values are computed. If the lowest active dose group yields P≤0.05, the NOSTASOT dose is specified only as below the lowest dose in the experiment.

There are three sets of “carriers” \( \{X_i \}_{i=1}^n \) frequently used as candidate dose scalings. They span a fairly broad range of possible dose-response relationships: for \( i = 1, \ldots, n \)

(i) arithmetic \( x_i = d_i \)

(ii) ordinal (equally-spaced) \( x_i = i \)

(iii) arithmetic-logarithmic \( x_i = \log d_i \), for \( i > 1 \), and \( x_1 = \log d_2 - \log d_1 \) and \( d_i = \log d_{i+1} - \log d_i \)

The statistical assessment of Tukey’s trend is performed on each of the three candidate scales separately. The minimum p-value is reported. This procedure may result in an inflated type I error rate. This can be justified in toxicity studies since the primary concern is the safety of the treatment. For efficacy studies, Cappizzi et al (1992) proposed an adjusted trend test by adjusting the p-value using the joint distribution, a trivariate t-distribution, of the three test statistics for the three dose scalings. Similar to Tukey’s trend test, this adjusted trend test is applicable for one-way designs or balanced two-way designs. Further extension to unbalanced two-way designs can be found in Quan and Cappizzi (1999).

As described above, the step-down analyses of Tukey’s trend test is a tedious process. Thus, it is highly desirable to simplify this process and to automate the analysis. This is the focus of this paper.

Statistically, Tukey’s trend test is a sequence of regression analyses to assess the significance of the slopes of regression lines corresponding to different combinations or dose scales and dose ranges when deleting the higher dose(s). It should be pointed out that when we delete a dose, we delete it from the calculation of contrasts, but not from the model fit (ie the pooled within group variance are always estimated from data in all dose groups).

Testing the slope of a regression line is equivalent to testing a contrast among the treatment means where the contrast coefficients are properly constructed (Mehta et al 1984). This analysis is equivalent to the orthogonal polynomial analysis of dose response curve when we want to assess the significance of the linear components of the curve. There is an algorithm available (Roger E. Kirk) to convert the orthogonal polynomial analysis to contrast analysis. In this paper, we utilize the approach of converting Tukey’s trend test to contrast analysis to simplify the recursive trend test process, and then apply the algorithm (Roger E. Kirk) for orthogonal linear component analysis to contrast the contrast coefficients. The algorithm, SAS macro source code, and examples are provided.

ALGORITHM
Assuming there are \( i = 1, \ldots, n \) different dose levels, we use the following notations:

\[ m_i \quad \text{sample size at the } i \text{th dose level.} \]
\[ X_i \quad \text{i th dose level, in one of the three scales.} \]
\[ m_i c_i \quad \text{contrast coefficient corresponding to } X_i \]
\[ k \quad \text{number of doses being included in the step-down Tukey’s trend test, } k = n, n-1, \ldots, 3. \]

Then,
\[ c_i = \alpha_i + X_i, \quad i = 1, \ldots, k; \quad k = n, n-1, \ldots, 3. \]

The orthogonal condition is that the coefficients should satisfy the equation:
\[ \sum m_i c_i = 0, \quad i = 1, \ldots, k; \quad k = n, n-1, \ldots, 3. \]

To find \( c_i \), first, solve for \( \alpha_i \) from:
\[ \sum m_i c_i = \sum m_i (\alpha_i + X_i) = 0 \]

Then, for \( i = 1, \ldots, k; \quad k = n, n-1, \ldots, 3 \), substitute \( \alpha_i \) into (1) to solve for \( c_i \). This results in the solution for coefficients:
\[ m_i c_i = m_i \left( X_i - \frac{\sum m_i X_i}{\sum m_i} \right) = m_i (X_i - \bar{X}). \]
AN EXAMPLE
There are four dose levels as listed in column (1) in Table 1 with corresponding sample sizes listed in column (2). Note that this is a general unequal intervals and unequal sample sizes case. The solution of coefficients in arithmetic scale for \( k = n = 4 \) is listed in column (5). The solution of coefficients in other two scales can be obtained similarly by replacing column (1) with dose levels in the corresponding scales. Coefficients with the highest dose deleted in the sequential test can be constructed similarly.

**TABLE 1: COMPUTATION OF COEFFICIENTS**

<table>
<thead>
<tr>
<th>( X_i )</th>
<th>m</th>
<th>( c_1 = a_1 X_i )</th>
<th>m ( c_2 = a_2 + m_3 X_i )</th>
<th>m ( c_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 5</td>
<td>5</td>
<td>( c_1 = a_1 + 0 )</td>
<td>( 5c_1 = 5a_1 + 5(0) )</td>
<td>-5(29/18 )</td>
</tr>
<tr>
<td>1 5</td>
<td>5</td>
<td>( c_2 = a_1 + 1 )</td>
<td>( 5c_2 = 5a_1 + 5(1) )</td>
<td>-5(11/18 )</td>
</tr>
<tr>
<td>2 4</td>
<td>4</td>
<td>( c_3 = a_1 + 2 )</td>
<td>( 4c_3 = 4a_1 + 4(2) )</td>
<td>4(7/18 )</td>
</tr>
<tr>
<td>4 4</td>
<td>4</td>
<td>( c_4 = a_1 + 4 )</td>
<td>( 4c_4 = 4a_1 + 4(4) )</td>
<td>4(43/18 )</td>
</tr>
</tbody>
</table>

\[ 0 = 18a_1 + 29 \]

THE MACRO

INPUT
A SAS MACRO has been developed to perform the contrast coefficient calculation for three dose scales and for the dose ranges of \( k = n, n-1, \ldots \), down to 3 dose levels. The user intervention include the inputting of four global macro variables: # of dose levels \( n \), and three lowest dose levels in original (arithmetic) scale. User also needs to input a SAS data containing the sample size and the corresponding dose levels in each treatment. The SAS code for the above example is as follows:

```sas
/*%%%%%%%%%%%%%%%%%%%%%%%%%%%%*/
/ ** change n, d1-d3 and cards each **/  
/ ** time for a new problem **/  
/%%%%%%%%%%%%%%%%%%%%%%%%%%%%*/
%global n d1 d2 d3;
%let n=4; /* # of different doses */
%let d1=0; /* lowest dose level in original scale */
%let d2=1; /* 2nd lowest dose level */
%let d3=2; /* 3rd lowest dose level */
/* enter the doses and corresponding sample sizes */
data dose;
cards;
0 5
1 5
2 4
4 4
run;
```

OUTPUT
An example of output is given to demonstrate the macro generated output. The data shown in Table 1 is used. There are 4 dose levels: 0, 1, 2, 4 in original scale (arithmetic) with corresponding sample sizes: 5, 5, 4 and 4. The macro output is presented in Table 2 below:

**Table 2. Coefficients of Contrasts**

<table>
<thead>
<tr>
<th>dose</th>
<th>m</th>
<th>k</th>
<th>d</th>
<th>acoef</th>
<th>lcoef</th>
<th>ocoef</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 5 4 0</td>
<td>-6.056</td>
<td>-4.814</td>
<td>-6.944</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 5 4 0</td>
<td>-3.056</td>
<td>-1.348</td>
<td>-1.944</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 4 4 0</td>
<td>1.556</td>
<td>1.694</td>
<td>2.444</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 4 4 0</td>
<td>9.556</td>
<td>4.467</td>
<td>6.444</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 5 3 1</td>
<td>-4.643</td>
<td>-3.218</td>
<td>-4.643</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 5 3 1</td>
<td>0.357</td>
<td>0.248</td>
<td>0.357</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 4 3 1</td>
<td>4.286</td>
<td>2.397</td>
<td>4.286</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The seven variables in the output are:
- **dose**: Dose levels.
- **m**: Sample size in the corresponding dose level.
- **k**: The number of doses included; \( k = n-1 \); all 4 doses are included; \( k = n-1; k = 3 \); the highest dose is deleted.
- **d**: The number of higher dose(s) deleted.
- **acoef**: Coefficients for dose in arithmetic scale.
- **lcoef**: For arithmetic-logarithmic scale.
- **ocof**: For ordinal scale.

Remark: Note that ‘acoef’, the coefficients in arithmetic scale, match the values in column (5) in Table 1.

CONTRAST ANALYSIS
We illustrate the contrast analysis using the macro generated coefficients by an example. An experiment was undertaken to evaluate the possible toxicity effect of a compound on the growth of cucumber. The five treatment groups involved were control (0 ppm), compound at nominal concentrations of 1.0, 10, 100, and 1000 ppm. It’s a balanced design with equal sample size \( m \). Shoot weights were measured at the end of the study. Tukey’s trend test was performed to identify the NOSTASOT dose. The following table lists the contrast coefficient results.

<table>
<thead>
<tr>
<th>dose</th>
<th>k</th>
<th>d</th>
<th>acoef</th>
<th>lcoef</th>
<th>ocoef</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 5 0</td>
<td>-222.2</td>
<td>-2.96778</td>
<td>-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 5 0</td>
<td>-221.2</td>
<td>-2.71193</td>
<td>-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 5 0</td>
<td>-212.2</td>
<td>-0.40935</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 5 0</td>
<td>-122.2</td>
<td>1.89324</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 5 0</td>
<td>777.8</td>
<td>4.19582</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 4 1</td>
<td>-27.75</td>
<td>-1.91882</td>
<td>-1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 4 1</td>
<td>-26.75</td>
<td>-1.66298</td>
<td>-0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 4 1</td>
<td>-17.75</td>
<td>0.63961</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 4 1</td>
<td>72.25</td>
<td>2.94219</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 3 2</td>
<td>-3.667</td>
<td>-0.93809</td>
<td>-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 3 2</td>
<td>-2.667</td>
<td>-0.68225</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 3 2</td>
<td>6.333</td>
<td>1.62034</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SAS code for contrast analysis is listed below. The contrast label ‘aco0’ refers to the arithmetic scale coefficient with no dose deleted; ‘oc0c’ refers to the ordinal scale with two highest doses deleted. Notice that arithmetic scale coefficients were rescaled.

```sas
proc glm data=a;
class trt;
model shoot=trt;
contrast 'aco0' trt -1.000 0.000 1.000 -2.000 1.000 0.000 0.000;
contrast 'aco1' trt -1.222 7.778 -2.968 2.712 -0.409 1.893 4.196;
contrast 'aco2' trt -1.500 0.000 -2.667 1.662 0.500 1.500 0.000;
contrast 'aco3' trt -2.775 2.675 1.919 1.663 0.640 2.942 0.000;
contrast 'aco4' trt -3.667 2.667 1.500 0.000 0.500 1.500 0.000;
contrast 'aco5' trt -0.938 0.682 1.620 0.000 0.000 0.000 0.000;
run;
```

From the above SAS code, it is noticeable that:
(i) a pooled estimation of standard error from all data is used when we delete higher dose(s).
(ii) the multiple contrast statements are for testing the hypotheses
$$\sum_{k=1}^{m} c_k y_k = 0, \quad k = n-1, \ldots, 3.$$ 

SOURCE CODE

Below is the source code of the SAS macro.

```sas
/* change n, d1-d3 and cards each time for a new problem */
%macro coef(i);
   %global n d1 d2 d3;
   %let n=4; /* # of different doses */
   %let d1=0; /* lowest dose in original scale */
   %let d2=1; /* 2nd lowest dose level */
   %let d3=2; /* 3rd lowest dose level */
   /* enter the doses and corresponding sample sizes */
   data dose;
      input dose m;
      cards;
      0 5
      1 5
      2 4
      4 4
      run;
   %macro loop;
   %do j=1 %to (&n-3);
      %let num=(&n-&j);
      %coef(&num)
      proc append base=allcoef data=coef;
   %end;
   %mend loop;
   %loop
   data allcoef; set allcoef;
   acoef=round(acoef, 0.001);
   lcoef=round(lcoef, 0.001);
   ocoef=round(ocoef, 0.001);
   proc print noobs data=allcoef;
   var dose m k d acoef lcoef ocoef;
   title 'Tukey Trend Test Contrasts Coefficients ';
   run;
%end;
```

REFERENCES


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