Dose-Response Trend Tests for Tumorigenesis Adjusted for Differences in Survival and Body Weight across Doses

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A relationship between rodent body weight and tumor incidence for some tissue/organ sites has been demonstrated in many studies. It is not uncommon for a chemical tested for carcinogenicity to also affect body weight due to toxicity and/or food consumption. In such cases, comparisons of tumor incidence may be biased by body weight differences across dose groups. A simple procedure was investigated for reducing this bias. This procedure divides the animals into a few groups on the basis of body weight. Body weight at 12 months was used, before the appearance of a tumor was likely to affect body weight. Statistics for dose-response trend tests are calculated within body weight strata and pooled to obtain an overall dose-response trend test. This procedure is analogous to stratifying animals on the basis of age at the time of removal from a study to account for differences in ages of animals across dose groups that can affect comparisons of tumor incidence. In this paper, differences in survival times of animals were adjusted by the Poly-3 technique used by the National Toxicology Program. This technique does not require the assignment of cause of death. Several examples from rodent chronic bioassays were investigated, where the high dose group had reduced body weights and associated reductions in tumor incidence. When we analyzed the data by body weight strata, some positive dose-response trends for tumor incidence were demonstrated. In one case, the body weight adjusted analysis indicated that a negative dose-response trend in tumor incidence was a real effect in addition to a body weight reduction. These examples indicate that it is important to consider the effects of body weight changes as low as 10%, and perhaps less, as possibly being caused by chemicals in 2-year bioassays for carcinogenesis. The simple procedure of analyzing tumor incidence within body weight strata can reduce the bias introduced by body weight differences across dose groups.

Key Words: dose response; tumorigenesis; trend test; body weight; Poly-3.

Numerous studies have shown a positive correlation between rodent body weight and tumor incidence for some tissue sites (e.g., Kari and Abdo, 1995; Tannenbaum, 1940; Turturro et al., 1993). Hart and Turturro (1997) provide an overview of dietary intake, body weight, and tumor incidence. Gaylor and Kodell (1999) summarize potential mechanisms that relate tumor incidence to body weight. When toxicity or reduced food consumption results in lowered body weights as dosage increases, this could artificially result in reduced tumor incidence with dose. On the other hand, chemicals with therapeutic and/or nutritional components may result in higher body weights with increased dose, which could artificially inflate tumor rates.

Seilkop (1995) uses historical control animal data to provide a relationship between tumor incidence and body weight, upon which tumor rate adjustments are based. This provides a procedure when historical data are available, and the tumor incidence relationship to body weight for the current chemical under test follows historical trends. Gaylor and Kodell (1999) divide the experimental data into body weight groups from the current bioassay and calculate dose-response statistics within groups. An overall test for a dose-response trend is computed by pooling the statistics across weight groups in the same manner as age-adjusted analyses are currently calculated. Gaylor and Kodell (1999) used the procedure of Peto et al. (1980) within body weight groups to adjust for differences in survival across dose groups. In this paper, the Poly-3 method (Bailer and Portier, 1988) is used to adjust for differences in survival among animals within body weight groups. This approach adjusts for the number of animals at risk, based on survival. The Poly-3 dose-response trend test for tumorigenicity, which allows for variability in the estimate of the number of animals at risk (Bieler and Williams, 1993), is used in this paper. This procedure is computationally simpler than the procedure of Peto et al. (1980), but more importantly, the approach used in this paper does not require assigning whether or not the tumor of interest was the cause of death for each animal bearing that tumor type. Thus, this paper employs the simple technique of estimating dose-response trends within body weight strata, as proposed by Gaylor and Kodell (1999), to adjust tumor incidence associated with different body weights across doses, but employs the simpler Poly-3 method for adjusting for tumor incidence associated with different survival across doses without requiring the cause of death of tumor bearing animals. The weight adjustment approach is illustrated for 3 chemicals in which lower body weights in the high dose groups may be associated with lower tumor incidence.

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**Trend Test Adjusted for Body Weight**

It has been recognized for several decades that tumor rates increase dramatically with age. Differences in survival across dose groups in a chronic bioassay can affect tumor rates and bias dose-response trend tests. Bailer and Portier (1988) introduced a procedure that adjusts for differences in survival without requiring the cause of death of tumor-bearing animals. Their Poly-3 procedure incorporates a weighting scheme that allows fractional values for animals not at full risk for tumor development. This weighting scheme essentially modifies the denominators of the crude estimates of lifetime tumor incidence to approximate the total number of animals at risk in each group. The weight given an animal is \( w = (t/T)^i \), where \( T \) is the time of final sacrifice for the bioassay and \( t \) is the time of removal for an animal. If an animal lives to the end of the bioassay, it receives a full weight of one. An animal that gets a tumor of interest also counts as a full animal, regardless of the time of observance of the tumor. No assumption is required concerning the lethality of the tumor. The adjusted number of animals at risk in a group is \( n' = \sum w \), and the age-adjusted tumor incidence is \( (y/n') \) where \( y \) is the number of animals in the group that develop the tumor of interest.

The procedure to account for differences in tumor incidence due to differences in body weights across doses is accomplished by dividing the animals into body weight strata with approximately an equal number of animals in each stratum. Dose-response trends are estimated within each stratum and then pooled across body weight strata to obtain an overall dose-response trend test.

Let

\[
\begin{align*}
  i & = 1, 2, \ldots, s \text{ denote the body weight stratum;} \\
  j & = 1, 2, \ldots, g \text{ denote the dose group;} \\
  n_{ij} & = \text{the number of animals initially at risk in the } j^{th} \text{ dose of the } i^{th} \text{ body weight stratum;} \\
  n_i & = \sum_j n_{ij} \\
  d_j & = \text{the dose level of the } j^{th} \text{ dose which is the same value for each body weight stratum;} \\
  y_{ij} & = \text{the number of animals with tumors in the } j^{th} \text{ dose of the } i^{th} \text{ stratum;} \\
  y_i & = \sum_j y_{ij} \\
  w_{ijk} & = (t_{ijk}/T)^i \text{ sample size weight assigned the } k^{th} \text{ animal in the } j^{th} \text{ dose of the } i^{th} \text{ body weight stratum;} \\
  n'_{ij} & = \sum_k w_{ijk} \text{ effective number of animals at risk in the } j^{th} \text{ dose of the } i^{th} \text{ stratum;} \\
  n'_i & = \sum_j n'_{ij} \\
  p'_{ij} & = y_{ij}/n'_{ij} \text{ tumor incidence adjusted for survival in the } j^{th} \text{ dose of the } i^{th} \text{ stratum;} \\
  a_i & = \left( \frac{n'_i}{n_i} \right)^2 n_i, \\
  p'_i = \frac{\sum_j y_{ij}/n'_{ij}}{\sum_j n'_{ij}} = \frac{y_i}{n'_i}.
\end{align*}
\]

For the control group \( d_{i0} = 0 \), and to simplify calculations without loss of generality for test statistics, \( d_{i2} \) can be set equal to one for the group of lowest dosed animals and the other \( d_{ij} \) can be scaled accordingly, relative to that group.

Following Bieler and Williams (1993), a weighted least squares estimate of the slope of the dose-response trend for the \( i^{th} \) body weight stratum is

\[
b_i = \frac{\sum_j a_{ij} p'_j d_{ij} - (\sum_j a_{ij} d_{ij}) (\sum_j a_{ij} p'_j) / \sum_j a_{ij}}{\sum_j a_{ij} d_{ij}^2 - (\sum_j a_{ij} d_{ij})^2 / \sum_j a_{ij}} \\
= \frac{\text{(numerator of } i)}{\text{(denominator of } i)} \\
= \text{num/den}_i. \tag{1}
\]

Following Bieler and Williams (1993), the approximate variance of \( b_i \), which considers \( n'_{ij} \) a random variable (see Appendix A), is

\[
\mathrm{V}(b_i) = p'_i \left( \frac{n'_i}{n_i} - p'_i \right) / \text{den}_i = C_i / \text{den}_i \tag{2}
\]

where \( C_i = p'_i \left( \frac{n'_i}{n_i} - p'_i \right) \).

An overall estimate of the dose-response slope \( b \) is obtained by weighting the \( b_i \) by the reciprocal of their variances

\[
b = \sum_i b_i / \mathrm{V}(b_i) / \sum_i 1 / \mathrm{V}(b_i) \\
= \left( \sum_i \text{num}/C_i \right) / \left( \sum_i \text{den}/C_i \right). \tag{3}
\]

A test of the null hypothesis that dose has no effect on tumor incidence, i.e., true slope \( = 0 \), is derived in Appendix B.

\[
Z_c = \frac{\sum_i \frac{\text{num}_i}{C_i} - \Delta \sum_i \frac{1}{C_i}}{\left( \sum_i \frac{\text{den}_i}{C_i} \right)^{1/2}} \tag{4}
\]

where \( Z \) is approximately a standard normal deviate and \( \Delta \) is the maximum difference between adjacent doses. Note that the continuity correction, negative term in the numerator, approximates the usual value of \( \Delta/2 \) if the \( C_i \) are approximately equal.

If the continuity correction is not included, the approximate test given in Equation 4 may underestimate the true \( p \) value when the total number of tumor occurrences across dose groups is small. An alternative to the test in Equation 4 for small tumor frequencies (e.g., 10 or fewer tumor-bearing animals) is to use an exact version of the test without the continuity correction (Kodell et al., 2000).

In older animals, the body weight might be influenced by the
presence of disease, particularly a growing tumor, rather than the occurrence of a tumor influenced by the body weight. Seilkop (1995) investigated body weights at different ages and used the body weight of the animals after one year in a study for adjustments. Turturro et al. (1993) show that body weights taken earlier than 12 months may have a higher correlation with tumor incidence at some tissue sites. For purposes of illustration, 12 month body weights are used here. For a given chemical, the animals are divided into 2, 3, 4, etc. weight groups, with approximately equal numbers of animals per weight group. As the numbers of weight groups are increased, the body weights within a group become more homogeneous, but the number of animals per dose-weight group become smaller. The number of weight groups is limited by the requirement to have animals in at least 2 dose groups in order to estimate the slope (dose-response trend) within each body weight stratum.

Examples

*p-Nitrobenzoic acid.* In the first example, data from the National Toxicology Program (1994) 2-year bioassay for p-nitrobenzoic acid are used to illustrate the weight stratification process for dose-response tests for carcinogenicity. In this study, the average body weights for female rats at 12 months were 271, 269, 260, and 243g in the 0, 1250, 2500, and 5000 ppm dietary groups, respectively. The high dose group weighed just 10% less than the control group. Seilkop (1995) and Turturro et al. (1993) indicate that mammary tumor incidence in female Fischer 344 rats is correlated with 12 month body weight. Hence, a weight-adjusted, dose-response analysis was conducted for mammary fibroadenoma.

Initially, 50 animals were started in each dose group. The calculations of the numbers of animals at risk, adjusted for mortality before the presence of a tumor or the terminal sacrifice by the Poly-3 technique proposed by Bailer and Portier (1988), were 45.9, 43.6, 42.6, and 44.0 for the 0, 1250, 2500, and 5000 ppm doses, respectively. The lifetime incidences of mammary fibroadenoma, unadjusted for body weight differences, were 17/45.9, 15/43.6, 19/42.6, and 19/44.0, respectively, showing no dose-response trend. However, e.g., when the animals were stratified into 3 body groups, the number of animals with tumors divided by the Poly-3 number of animals at risk gave the survival and weight-adjusted mammary fibroadenoma incidence rates in Table 1.

The 2 lower body weight groups indicate a dose-response trend. The overall test result across the 3 body weight groups is given in Table 2.

The animals were divided into 2, 3, 4, 6, and 12 body weight groups, with nearly equal numbers per group, and slopes (dose-response trends) and variances of the slopes were calculated within each body weight stratum. Weighted averages of the slopes were calculated from Equation 3 and tested for significant differences from zero using Equation 4. The results are summarized in Table 2. With 2 weight groups, the body weight ranges were from 217–259 g and 260–319 g at 12 months. With such diverse body weights within these 2 groups, no improvement was obtained over using no stratification of body weights. With 12 body weight groups, many of the groups had a weight range of only 5 grams. No demonstrable difference in tumor incidence would be expected among animals within these groups. Hence, more body weight groups were not examined. Also, as the number of body weight groups is increased, the number of animals per body weight-dose group decreases and frequently is zero. Thus, it is not uncommon, with a large number of body weight groups, to have animals for only 2 doses within a body weight group. In the comparison of the controls with the high dose group (Table 3), it was not possible to obtain 12 body weight groups with animals in both the controls and high dose groups. Three adjacent weight groups had to be pooled, resulting in 9 body weight groups. From the estimated slope (b), using Equation 3, the estimated difference between the control and 5000-ppm high-dose groups was 5000 × b (Table 3).

In this study, failure to recognize the lowered incidence of mammary fibroadenomas in the high dose group associated with lower body weights results in no detection of a dose-response trend. Accounting for differences in body weight across doses, by examining dose-response trends within body weight strata, resulted in a dose-response slope about 3 times

### TABLE 1

| p-Nitrobenzoic Acid Administered to Female Fischer 344 Rats for Two Years in Feed |
|-------------------|-----------------|-----------------|-----------------|-----------------|
| bw g              | 0 ppm           | 1250 ppm        | 2500 ppm        | 5000 ppm        |
| 0-249 g           | 1/4.8 (21%)     | 1/4.0 (25%)     | 2/7.0 (29%)     | 13/35.1 (37%)   |
| 250–269 g         | 3/11.9 (25%)    | 7/20.1 (35%)    | 14/25.4 (55%)   | 4/5.5 (73%)     |
| ≥ 270 g           | 13/29.3 (44%)   | 7/19.5 (36%)    | 3/10.2 (29%)    | 2/3.4 (59%)     |

*Note.* Values represent number of animals with mammary fibroadenoma divided by the Poly-3 number of animals at risk, stratified by body weight at 12 months; bw, body weight.

### TABLE 2

<table>
<thead>
<tr>
<th>Number of bw groups</th>
<th>Slope (b), tumor incidence/ppm</th>
<th>Slope test statistic (ZC)</th>
<th>Right-sided p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$1.6 \times 10^{-5}$</td>
<td>0.729</td>
<td>0.23</td>
</tr>
<tr>
<td>2</td>
<td>$1.3 \times 10^{-5}$</td>
<td>0.403</td>
<td>0.34</td>
</tr>
<tr>
<td>3</td>
<td>$4.2 \times 10^{-5}$</td>
<td>1.464</td>
<td>0.07</td>
</tr>
<tr>
<td>4</td>
<td>$3.2 \times 10^{-5}$</td>
<td>1.134</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>$4.2 \times 10^{-5}$</td>
<td>1.566</td>
<td>0.06</td>
</tr>
<tr>
<td>12</td>
<td>$4.6 \times 10^{-5}$</td>
<td>2.016</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Note.* bw, body weight.
larger than the unadjusted slope. The adjusted slope achieved a one-sided $p$ value of 0.02 when 12 relatively homogeneous body weight groups were used (Table 2).

If a comparison of this result is made to the body weight-adjusted analysis using a trend test based on Peto et al. (1980), note that Gaylor and Kodell (1999) reported 2-sided $p$ values.

O-Nitroanisole. Female B6C3F1 mice were administered O-nitroanisole in the diet at 0, 666, 2000, and 6000-ppm concentrations in a National Toxicology Program (1993) study. At 12 months, the average body weights were 44g, 43g, 38g, and 25g, respectively. Initially, 50 animals were started on the study in each dose group. The calculations of the number of animals at risk, adjusted for mortality before the presence of tumor or the terminal sacrifice, were 45.7, 44.8, 46.5, and 47.8 for the respective dose groups, by the Poly-3 technique proposed by Bailer and Portier (1988). The lifetime incidence of hepatocellular adenoma or carcinoma, unadjusted for body weight difference, were 17/45.7, 21/44.8, 37/46.5, and 20/47.8 at 0, 666, 2000, and 6000 ppm, respectively, showing no dose-response trend. However, when the animals were divided into 3 body weight groups, e.g., the Poly-3 survival adjusted liver tumor incidence rates are given in Table 4.

A dose response is apparent in the 2 larger body weight groups. Combined over the three body weight groups, the dose response has a one-sided $p$ value of less than 0.01 (Table 5).

The results of using up to 4 body weight groups are summarized in Table 5. Ignoring the decreased liver tumor incidence associated with lower body weights in the higher dose groups failed to show the tumorigenicity of o-nitroanisole.

There was no overlap in body weights between the controls and the high dose group; thus, a comparison of these 2 groups is meaningless. This may be an indication that 6000 ppm exceeded the maximum tolerated dose.

Doxylamine succinate. Jackson and Blackwell (1993) present the results of a 2-year carcinogenicity study conducted at the National Center for Toxicological Research, in which Fischer 344 rats were administered 0, 500, 1000, and 2000 ppm of doxylamine succinate in the diet. The average body weights for female rats, after 12 months on the study, were 295, 282, 263, and 229g in the 0, 500, 1000, and 2000 ppm groups, respectively. If ignoring the decreases in body weight with higher doses, results show a highly significant negative dose-response trend for mammary tumors. The Poly-3 survival-adjusted lifetime incidence rates for mammary fibroadenomas were 21/47.0, 18/46.5, 7/43.9, and 3/45.7, respectively. When the animals were divided into 4 body weight groups, the negative dose-response trend was greatly diminished (Table 6).

The results of using up to 4 body weight groups are summarized in Table 7. Adjusting for the decreased mammary fibroadenoma incidence associated with lower body weights in...
the higher dose groups diminishes the negative dose-response trend.

There was such small overlap in similar body weights between the control group (3 animals) and the high dose group (9 animals) that a comparison of their incidence rates would be meaningless.

Even when the dose-response trend tests were adjusted for lower body weights as dose increased, the negative trend appeared to remain. This suggests that doxylamine succinate may cause a decrease in mammary tumors by a mechanism in addition to or in conjunction with a reduction in body weight.

**DISCUSSION**

Several studies have shown a positive correlation for some tissue sites between rodent body weight and tumor incidence. This indicates that weight-adjusted comparisons of tumor rates are needed when animal body weights differ across dose groups. A standard covariance analysis of tumor incidence and body weight may not be appropriate because the treatment (chemical) also causes the difference in body weights. Seilkop (1995) uses historical tumor rates from control animals to provide a relationship between tumor incidence and body weight upon which tumor rate adjustments are made. This requires that the current study follow historical norms, if they are available.

The procedure proposed in this paper divides the animals into body weight groups and calculates dose-response trend statistics within these groups. An overall test for a dose-response trend is calculated by pooling the test statistics across body weight groups, in the same manner as age-adjusted analyses are often calculated. Hence, no external data to the bioassay or additional assumptions are required. The dose-response trend tests used in this paper follow the procedures of Bieler and Williams (1993), using Poly-3 adjustments for survival. However, body weight stratification can be used for any statistical dose-response test. The proposed test for trend pools results across body weight groups weighted inversely by their estimated variances.

When a chemical causes an increase in body weight and subsequent increase in tumor incidence, the analysis stratified body weight will tend to decrease a spurious positive dose-response trend, and hence decrease the statistical significance, if any, of a positive dose-response trend. When a chemical causes a decrease in body weight and a subsequent decrease in tumor incidence, the analysis within body weight groups will tend to disclose an increase in the dose-response trend. For example, without an adjustment for differing body weights across dose groups, p-nitrobenzoic acid did not exhibit a dose-response trend for mammary fibroadenoma. However, when animals were sorted into body weight groups, there is statistical evidence of an effect of p-nitrobenzoic acid on mammary fibroadenoma (Table 2). Seilkop (1995) also found increases in mammary tumors, when compared to historical controls, with the same 12-month body weights.

For female B6C3F1 mice administered o-nitroanisole, the decrease in body weight at the high dose and accompanying low incidence of hepatocellular tumors resulted in no dose-response trend. However, when the data were analyzed by weight groups, a highly significant dose-response trend was noted. Seilkop (1995) also found increases in the incidence of liver tumors when compared to historical controls, with the same average 12-month body weights.

For doxylamine succinate administered to female Fischer 344 rats, the negative dose-response trend for mammary tumors remains even after the weight adjusted analysis. This indicates that doxylamine succinate may have a beneficial effect for mammary tumors in addition to or in conjunction with the effect from body weight reduction.

Ames and Gold (1990) suggest that lower body weight may indicate cytotoxicity which could cause compensatory cell proliferation providing increased opportunities for mutations, and as a result artificially inflate tumor incidence at high doses. Analysis of tumor incidence results by body weight strata should also adjust for such a negative association between tumor incidence and body weight.

This paper does not address the issue of when it is necessary to account for differences in body weight across dose groups. In the example with p-nitrobenzoic acid, substantial effects on dose-response trend tests were obtained with a 10% difference in body weights. Seilkop (1995) and Turturro et al. (1993) show effects on tumor incidence for body weight differences less than 10%. Since stratifying by body weight is a simple procedure that imposes no additional assumptions, it can be applied universally. No information is lost in those cases where body weight has no influence.

Much of the data indicating a relationship between body weight and tumor incidence come from caloric restriction studies. Suggested mechanisms for influencing tumor incidence appear to be related to caloric intake rather than body weight (Hart and Turturro, 1997). Body weight serves as a simple, direct surrogate for dietary intake. It might be of interest for future research to investigate if stratifying on dietary intake and body weight provide similar results.
Any procedure to adjust for body weight changes must make the assumption that a body weight change has the same impact on tumor incidence regardless of the cause. For example, the implicit assumption is that a similar decrease in body weight due to reduced caloric intake or chemical toxicity has a similar effect on tumor incidence.

It appears that the animals should be divided into as many weight groups as possible and still maintain some animals in most dose groups in each weight group. When there is little overlap in body weights between the control animals and the high dose animals, as was the case with o-nitroanisole and doxylamine, only a few body weight groups may be feasible.

It could be argued that corrections of tumor incidence for body weight changes should not be made. If this is part of the mechanism through which a chemical influences tumor rate, perhaps it should contribute accordingly to the risk. On the other hand, it can be argued that at low doses body weights may not differ from unexposed control levels and, therefore, tumor rates should be adjusted for their effects at higher doses. The adjusted trend test proposed here to accommodate differences in body weight across dose groups is similar to adjusted trend tests commonly in use to accommodate differences in noncancer mortality across dose groups.

The above examples indicate that it is absolutely important to consider the differences in tumor incidence resulting from body weight differences caused by chemicals in 2-year bioassays for carcinogenesis. Such effects on tumor incidence can be significant even in studies where average body weight differences are 10% or perhaps less. The simple procedure of dividing the animals into a few groups stratified by body weight (12-month body weight was used in these analyses) and pooling dose-response trend statistics from body weight groups provides an easy method to adjust for body weight differences across dose groups.

**APPENDIX A**

**Variance of \( b_i \)**

From Equation 9 of Bieler and Williams (1993) with \( n_i' \) treated as a random variable, the variance of \( p_i' \) is approximately

\[
V(p_i') = n_iC/(n_i')^2. \tag{A1}
\]

From the Taylor’s series expansion of \( p_i' \), the variance of \( p_i' \) is approximately

\[
V(p_i') = V\left( \frac{y_i}{n_i'} \right) = \frac{V(y_i)}{(n_i')^2} + \left[ \frac{y_i}{(n_i')^2} \right]^2 V(n_i') - \frac{2y_i}{(n_i')^2} \text{Cov}(y_i, n_i') \tag{A2}
\]

where

\[
y_i = n_i'p_i'
\]

\[
V(y_i) = n_i'p_i'(1 - p_i')
\]

and based on a Poisson distribution approximation,

\[
V(n_i') = V[n_i - (n_i - n_i')] = (n_i - n_i')
\]

\[
\text{Cov}(y_i, n_i') = \text{Cov}(n_i'p_i', n_i') = p_i'(n_i - n_i').
\]

Substituting the above values into (A2) gives

\[
V(p_i') = \frac{p_i'}{n_i} \left( \frac{n_i}{n_i'} - p_i' \right). \tag{A3}
\]

where \( a_i = (n_i')^2/n_i \).

Equating this result to Equation A1 gives

\[
C_i = p_i\left( \frac{n_i'}{n_i} - p_i' \right). \tag{A4}
\]

Following the approach of Bieler and Williams (1993), the test of the hypothesis of no dose effect, i.e., true slope = 0, contains the term \( C_i \) in Equation 4. Under the null hypothesis of no dose effect, \( p_i' = p_i' \) for all \( j \). Hence, Equation A4 becomes

\[
C_i = p_i\left( \frac{n_i'}{n_i} - p_i' \right). \tag{A5}
\]

Replacing \( (n_i'/n_i) \) by the average of the \( (n_i'/n_i) \) weighted by \( n_i \) gives

\[
C_i = p_i\left( \frac{n_i'}{n_i} - p_i' \right) \tag{A6}
\]

as used in Equations 2–4 for \( V(b_i) \), \( b_i \), and the test statistic, \( Z_C \), respectively.

**APPENDIX B**

**Dose-Response Trend Test**

The overall estimate \( b \) of the weighted slopes within body weight strata is given in Equation 3. The variance of \( b \) is,

\[
V(b) = 1/\sum_i 1/V(b_i). \tag{B1}
\]

From Equation 2, \( V(b_i) = C_i/den_i \), giving,

\[
V(b) = 1/\sum_i den_i/C_i. \tag{B2}
\]

The ratio of \( b \) to \( \sqrt{V(b)} \) is distributed approximately as a standardized normal deviate,

\[
Z = \frac{b}{\sqrt{V(b)}} = \frac{\left( \sum_i \text{num}/C_i \right)/\left( \sum_i \text{den}/C_i \right)}{1/\left( \sum_i \text{den}/C_i \right)^{1/2}} = \frac{\left( \sum_i \text{num}/C_i \right)}{\left( \sum_i \text{den}/C_i \right)^{1/2}}. \tag{B3}
\]

The Z-score is adjusted further by the continuity correction,

\[
Z_C = \frac{\sum_i \text{num}/C_i - \Delta/2}{\sum_i \text{den}/C_i^{1/2}} \tag{B4}
\]

where \( \Delta \) is the maximum difference in dose between 2 adjacent doses. Note that the continuity correction term approximates the usual value of \( \Delta/2 \) if the \( C_i \) are approximately equal.
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REFERENCES


