

Estimation and Testing of Tumor Incidence Rates in Experiments Lacking Cause-of-Death Data

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Abstract

Approximate nonparametric maximum likelihood estimation of the tumor incidence rate and comparison of tumor incidence rates between treatment groups are examined in the context of animal carcinogenicity experiments that have interval sacrifice data but lack cause-of-death information. The estimation procedure introduced by Malani and Van Ryzin (1988), which can result in a negative estimate of the tumor incidence rate, is modified by employing a numerical method to maximize the likelihood function iteratively, under the constraint that the tumor incidence rate is nonnegative. With the new procedure, estimates can be obtained even if sacrifices occur anywhere within an interval. The resulting estimates have reduced standard error and give more power to the test of two heterogeneous groups. Furthermore, a linear contrast of more than two groups can be tested using our procedure. The proposed estimation and testing methods are illustrated with an experimental data set.

KEY WORDS: Maximum Likelihood Estimate; Asymptotic Variance; Carcinogenicity; Tumor Incidence Rate.

1 Introduction

In carcinogenesis experiments, tumors are often undetectable until death, and cause of death is often not available. For such an experiment, valid comparisons of tumor rates between treatment groups are difficult to make. To address this problem, various analyses have been developed for experiments in which serial interim sacrifices are performed, since such experiments make the tumor incidence rate identifiable (McKnight and Crowley, 1984). Portier and Dinse (1987) introduced

a semiparametric test for comparing the tumor incidence rates of two treatments. McKnight and Crowley (1984), Dewanji and Kalbfleisch (1986) and Malani and Van Ryzin (1988) proposed nonparametric methods to estimate and test the tumor incidence function. Williams and Portier (1992a) proposed analytic expressions for maximum likelihood estimates of the tumor incidence rate, and later imposed a boundary condition for a nonnegative tumor incidence rate for up to three sacrifices (Williams and Portier, 1992b). On the other hand, methods requiring information on cause of death were suggested by Peto *et al.* (1980), Kodell, Shaw and Johnson (1982), Dinse and Lagakos (1982) and Turnbull and Mitchell (1984).

The maximum likelihood estimation procedure introduced by Malani and Van Ryzin (1988) is a good procedure for analyzing survival/sacrifice data when information on the cause of death is not available, provided that the estimated tumor incidence rate is nonnegative. However, their test suffers from a loss of power when the estimated tumor incidence rate is negative, as often happens. Such an undesirable property may occur when small numbers of natural deaths or sacrifices are observed in some intervals. Also, estimates can be obtained only if sacrifices occur at the end of the intervals. The purpose of our work is to develop a numerical method to maximize the likelihood function iteratively, under the constraint that the tumor incidence rate is nonnegative, so that the tumor incidence rate becomes stable. Thus, the resulting estimate reduces standard error and gives the test of two heterogeneous groups more power. The proposed estimating method can handle data having any number of interim sacrifices, and is adapted to deal with situations in which sacrifices occur anywhere in an interval. Furthermore, a linear contrast of more than two groups can be tested using our procedure. A computing procedure for the proposed method is developed for analyzing data from animal carcinogenicity studies. The algorithm is coded in a computer program obtainable from the authors. Preliminary results from this approach were presented by Ahn and Kodell (1993).

2 Notation

Consider an experiment with d treatment groups, a control and $d - 1$ dose groups. Suppose that n_i animals in the i th treatment group are followed over time for the development of irreversible tumors. We assume that all animals come from the same population and are born without tumor on day zero of the experiment. Let $0 = t_0 < t_1 < \dots < t_m$ be distinct times. Divide the time span into m intervals such that the j th interval is $(t_{j-1}, t_j]$, $j = 1, \dots, m$. Sequential sacrifice is

Table 1: Counts of key events

Interval j	With tumors	Without tumors	Total
Natural deaths	$N^1(j)$	$N^2(j)$	$N(j)$
Sacrifices	$N^3(j)$	$N^4(j)$	$S(j)$
Total	$N^1(j) + N^3(j)$	$N^2(j) + N^4(j)$	$N(j) + S(j)$

done at the end of each interval and all deaths and tumors are assumed to occur at the end of the interval, with tumors preceding deaths and deaths preceding sacrifices. This assumption permits us to assume that an animal actually dying in the middle of an interval without tumor would not have developed a tumor until the end of the interval if alive, and therefore we can count that animal as dead without tumor at the end of the interval.

Using notation of Malani and Van Ryzin (1986, 1988), let X_T denote time to tumor and X_D be time to death, which are both discrete random variables, taking on values $j = 1, \dots, m$. For group i and interval j , let $N_i^1(j)$ and $N_i^2(j)$ denote the number of natural deaths with and without tumors, respectively, and let $N_i^3(j)$ and $N_i^4(j)$ denote the number of sacrifices with and without tumors, respectively. Also, let $S_i(j) = N_i^3(j) + N_i^4(j)$ and $N_i(j) = N_i^1(j) + N_i^2(j)$ denote the number of sacrifices and natural deaths, respectively, and let $A_i(j)$ denote the number of animals alive at the start of interval j . Table 1 summarizes the key events corresponding to $N^1(j), \dots, N^4(j)$. Let $p^A(j) = P(X_T \leq j | X_D > j)$ be the tumor prevalence rate among live animals, $p^D(j) = P(X_T \leq j | X_D = j)$ be the tumor prevalence rate among dying animals, $\lambda^D(j) = P(X_D = j | X_D \geq j)$ be the discrete hazard rate for X_D , and $\lambda^S(j) = P(X_S = j | X_S \geq j)$ be the hazard function for the time to sacrifice. The tumor incidence rate in interval j is given by

$$\begin{aligned} \lambda^T(j) &= P(X_T = j | X_D \geq j, X_T \geq j) \\ &= 1 - \{[1 - p^A(j)]\{1 - \lambda^D(j)\} + [1 - p^D(j)]\lambda^D(j)] / [1 - p^A(j-1)]\}, \end{aligned} \quad (1)$$

A derivation of $\lambda^T(j)$ is provided in Appendix A.

3 Existing estimation and testing procedure

According to Malani and Van Ryzin (1986), the log-likelihood function is defined as

$$l = \sum_{j=1}^m [N^1(j) \log p^D(j) + N^2(j) \log\{1 - p^D(j)\} + N^3(j) \log p^A(j) + N^4(j) \log\{1 - p^A(j)\}]$$

$$\begin{aligned}
& +N(j) \log \lambda^D(j) + \{A(j) - N(j)\} \log \{1 - \lambda^D(j)\} + \sum_{j=2}^m [S(j-1) \log \lambda^S(j-1) \\
& + A(j) \log \{1 - \lambda^S(j-1)\}] + c,
\end{aligned} \tag{2}$$

where c is a constant. See Malani and Van Ryzin (1986) for detailed derivation of l . The maximum likelihood estimates of the parameters are $\hat{p}^A(j) = N^3(j)/S(j)$, $\hat{p}^D(j) = N^1(j)/N(j)$, $\hat{\lambda}^D(j) = N(j)/A(j)$, $j = 1, \dots, m$, and $\hat{\lambda}^S(j) = S(j)/\{A(j) - N(j)\}$, $j = 1, \dots, m-1$. By substituting these in (1), the maximum likelihood estimates $\hat{\lambda}^T(j)$ of the tumor incidence rate are obtained.

It is easy to see that the estimate of the tumor incidence rate λ_{ij} proposed by Williams and Portier (1992a) and $\hat{\lambda}_i^T(j)$ are identical, and that the estimate of the overall death rate ϕ_{ij} in Williams and Portier (1992a) is identical to $\hat{\lambda}_i^D(j)$.

By a Taylor's series expansion, Malani and Van Ryzin (1988) obtained the asymptotic variance of $\hat{\lambda}^T(j)$

$$\begin{aligned}
\text{Var}[\hat{\lambda}^T(j)] &= [\{1 - \lambda^T(j)\}^2 p^A(j-1) \{1 - p^A(j-1)\} \{1 - \lambda^S(j-1)\} / \lambda^S(j-1) \\
&\quad + a(j) + \{1 - \lambda^D(j)\} b(j) + c(j)] / k(j),
\end{aligned} \tag{3}$$

for $j = 1, \dots, m$, where $\alpha(j) = \prod_{k=0}^{j-1} \{1 - \lambda^D(k)\} \{1 - \lambda^S(k)\}$,

$$a(j) = \lambda^D(j) p^D(j) \{1 - p^D(j)\}, \tag{4}$$

$$b(j) = p^A(j) \{1 - p^A(j)\} / \lambda^S(j), \tag{5}$$

$$c(j) = \lambda^D(j) \{1 - \lambda^D(j)\} \{p^A(j) - p^D(j)\}^2, \tag{6}$$

$$k(j) = n \alpha(j) \{1 - p^A(j-1)\}^2, \tag{7}$$

and the non-zero covariance

$$\text{Cov}[\hat{\lambda}^T(j'), \hat{\lambda}^T(j)] = [\partial \{1 - \lambda^T(j')\} / \partial p^A(j')] [\partial \{1 - \lambda^T(j)\} / \partial p^A(j')] \text{Var}[\hat{p}^A(j')]$$

for $j' = j-1$, $j = 2, \dots, m$. Thus, the asymptotic covariance of $\hat{\lambda}^T(j-1)$ with $\hat{\lambda}^T(j)$ is

$$\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] = -p^A(j-1) \{1 - \lambda^T(j)\} \{1 - p^A(j-2)\} / \{k(j-1) \lambda^S(j-1)\} \tag{8}$$

and therefore, the variance of $\hat{\Lambda}^T(j) = \sum_{k=1}^j \hat{\lambda}^T(k)$ is

$$\sigma^2(j) = \text{Var}[\hat{\Lambda}^T(j)] = \sum_{k=1}^j \text{Var}[\hat{\lambda}^T(k)] + 2 \sum_{k=2}^j \text{Cov}[\hat{\lambda}^T(k-1), \hat{\lambda}^T(k)], \quad j = 2, \dots, m.$$

From Malani and Van Ryzin (1988),

$$Z_T = \{\hat{\Lambda}_r^T(m) - \hat{\Lambda}_s^T(m)\} / \sqrt{\hat{\sigma}_r^2(m) + \hat{\sigma}_s^2(m)} \quad (9)$$

converges in distribution to $N(0, 1)$ under $\lambda_r^T(j) = \lambda_s^T(j)$, $j = 1, \dots, m$, where the subscripts r and s are for the r th and s th groups, respectively. However, the estimate of the tumor incidence rate $\lambda^T(j)$ is negative in many cases, resulting in a decreasing estimate of the cumulative tumor incidence rate. In addition, the parameter space becomes bigger if negative estimates of $\lambda^T(j)$ are allowed. Therefore, its standard error is overestimated, and the test of two heterogeneous groups has too little power. It should be noted that formulas (3) and (9) differ from those of Malani and Van Ryzin (1986, 1988), due to our correction of errors in their formulation. However, the corrected formulas appear in Malani and Lu (1993).

4 Improved estimating procedure

To avoid having a negative estimate of the tumor incidence rate, the likelihood function needs to be maximized under the constraint that the tumor incidence rate is nonnegative. However, it is difficult to obtain the maximum likelihood estimates algebraically under that constraint. Alternatively, we introduce a sufficient condition for $\lambda^T(j) \geq 0$ and we get approximate maximum likelihood estimates under that condition. First, we can easily see from (1) that at least one of the following conditions needs to be satisfied to make the tumor incidence rate nonnegative:

- The tumor prevalence rate among live animals in the j th interval ($p^A(j)$) needs to be at least as large as in the $(j-1)$ st interval ($p^A(j-1)$).
- The tumor prevalence rate among dying animals in the j th interval ($p^D(j)$) needs to be at least as large as the tumor prevalence rate among live animals in the $(j-1)$ st interval ($p^A(j-1)$).

Then, from (1), we see that the combination of the above two conditions gives a sufficient condition to have a nonnegative tumor incidence rate. To illustrate, let

$$E = \{\lambda^T(j) \geq 0, j = 1, \dots, m\},$$

$$F = \{p^A(j) \geq p^A(j-1) > p^D(j), j = 1, \dots, m\},$$

$$G = \{p^D(j) \geq p^A(j-1) > p^A(j), j = 1, \dots, m\},$$

$$H = \{p^D(j) < p^A(j-1), p^A(j) < p^A(j-1), j = 1, \dots, m\}$$

and

$$J = \{p^D(j) \geq p^A(j-1), p^A(j) \geq p^A(j-1), j = 1, \dots, m\}. \quad (10)$$

Then J is a sufficient condition for E , since E is a subset of J . Our examples show that we have achieved substantial improvement in the estimation of the tumor incidence rate and the comparison of treatments under the nonnegativity constraint. We provide the details of the estimation procedure in the next two sections.

4.1 Estimation of the tumor prevalence rate among live animals and the tumor prevalence rate among dying animals

Starting from the first interval, we estimate $\lambda^T(j)$, $j = 1, \dots, m$. If $\hat{\lambda}^T(j) \geq 0$, we can use the estimates described in Section 3. Otherwise, in the j th interval ($j \geq 2$), the modification is performed using isotonic regression estimates for the parameters of a multinomial distribution in the following cases:

1. (F) In the case that $\hat{p}^A(j) \geq \hat{p}^A(j-1) > \hat{p}^D(j)$, set $p^D(j) = p^A(j-1)$. The maximum likelihood estimate under the constraint is

$$\hat{p}^D(j) = \hat{p}^A(j-1) = \{N^1(j) + N^3(j-1)\} / \{N(j) + S(j-1)\},$$

and it is equivalent to

$$\hat{p}^D(j) = \hat{p}^A(j-1) = \{\hat{p}^D(j)N(j) + \hat{p}^A(j-1)S(j-1)\} / \{N(j) + S(j-1)\}. \quad (11)$$

Replace $\hat{p}^A(j-1)$ and $\hat{p}^D(j)$ with $\hat{\hat{p}}^A(j-1)$ and $\hat{\hat{p}}^D(j)$, respectively. Equation (11) is a weighted average of $\hat{p}^D(j)$ and $\hat{p}^A(j-1)$, since the right hand side of the equation is the same as $\{N^1(j) + N^3(j-1)\}/\{N(j) + S(j-1)\}$ in the first iteration. By using (11), we have

$$1 - \hat{\lambda}^T(j) = \{1 - \hat{p}^A(j)\}\{1 - \hat{\lambda}^D(j)\}/\{1 - \hat{p}^A(j-1)\} + \hat{\lambda}^D(j). \quad (12)$$

2. (G) In the case that $\hat{p}^D(j) \geq \hat{p}^A(j-1) > \hat{p}^A(j)$, use the maximum likelihood estimate (a weighted average) of $p^A(j) = p^A(j-1)$

$$\hat{\hat{p}}^A(j) = \hat{\hat{p}}^A(j-1) = \{\hat{p}^A(j)S(j) + \hat{p}^A(j-1)S(j-1)\}/\{S(j) + S(j-1)\} \quad (13)$$

of $\hat{p}^A(j)$ and $\hat{p}^A(j-1)$, and replace them with $\hat{\hat{p}}^A(j-1)$ and $\hat{\hat{p}}^A(j)$, respectively. Then

$$1 - \hat{\lambda}^T(j) = 1 - \hat{\lambda}^D(j) + \{1 - \hat{p}^D(j)\}\hat{\lambda}^D(j)/\{1 - \hat{p}^A(j)\}. \quad (14)$$

3. (H) In the case that $\hat{p}^A(j) < \hat{p}^A(j-1)$ and $\hat{p}^D(j) < \hat{p}^A(j-1)$, use the maximum likelihood estimate (a weighted average) of $p^A(j) = p^A(j-1) = p^D(j)$

$$\begin{aligned} \hat{\hat{p}}^A(j) &= \hat{\hat{p}}^A(j-1) = \hat{\hat{p}}^D(j) \\ &= \{\hat{p}^D(j)N(j) + \hat{p}^A(j)S(j) + \hat{p}^A(j-1)S(j-1)\}/\{N(j) + S(j) + S(j-1)\} \end{aligned} \quad (15)$$

of \hat{p}^A , $\hat{p}^A(j-1)$ and $\hat{p}^D(j)$, and replace them with $\hat{\hat{p}}^A(j-1)$, $\hat{\hat{p}}^A(j)$ and $\hat{\hat{p}}^D(j)$, respectively. Then $\hat{\lambda}^T(j)$ becomes zero and thus, $\text{Var}[\hat{\lambda}^T(j)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$ and $\text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)]$ are also zero.

In the second and third cases above, estimation in the j th interval affects estimation in the adjacent intervals. This necessitates an iterative process. We summarize these relationships as follows.

1. If $\hat{p}^A(j) \geq \hat{p}^A(j-1) > \hat{p}^D(j)$, then set (11) in the j th interval. However, the change does not affect estimation of the $(j+1)$ st interval, since $\hat{p}^D(j)$ and $\hat{p}^A(j-1)$ are not used for estimation in that interval.
2. If $\hat{p}^D(j) \geq \hat{p}^A(j-1) > \hat{p}^A(j)$, then set (13) in the j th interval. This change affects estimation of the $(j+1)$ st interval in the following cases:

- (a) If $\hat{p}^A(j+1) \geq \hat{p}^A(j) > \hat{p}^D(j+1)$, then we set $\hat{p}^D(j+1) = \hat{p}^A(j)$ as in (11) and consequently, $\hat{p}^A(j-1) > \hat{p}^A(j) = \hat{p}^D(j+1)$.
- (b) If $\hat{p}^D(j+1) \geq \hat{p}^A(j) > \hat{p}^A(j+1)$, then we set $\hat{p}^A(j+1) = \hat{p}^A(j)$ as in (13) and consequently, $\hat{p}^A(j-1) > \hat{p}^A(j) = \hat{p}^A(j+1)$.
- (c) If $\hat{p}^A(j+1) < \hat{p}^A(j)$ and $\hat{p}^D(j+1) < \hat{p}^A(j)$, then we set $\hat{p}^D(j+1) = \hat{p}^A(j+1) = \hat{p}^A(j)$ as in (15) and thus, $\hat{p}^A(j-1) > \hat{p}^A(j) = \hat{p}^A(j+1) = \hat{p}^D(j+1)$.

In (a), (b) and (c), the change of estimates in the $(j+1)$ st interval makes the equation (13) for the j th interval no longer valid. Hence, we need to set (13) again in the next iteration. Nevertheless, $\hat{p}^A(j-1) - \hat{p}^A(j)$ becomes smaller than before, because we take a weighted average of the two in each step of the iteration.

- 3. If $\hat{p}^A(j) < \hat{p}^A(j-1)$ and $\hat{p}^D(j) < \hat{p}^A(j-1)$, then set (15) in the j th interval. This change affects estimation of the $(j+1)$ st interval in the following cases:

- (a) If $\hat{p}^A(j+1) \geq \hat{p}^A(j) > \hat{p}^D(j+1)$, then we set $\hat{p}^D(j+1) = \hat{p}^A(j)$ and consequently, $\hat{p}^D(j) = \hat{p}^A(j-1) > \hat{p}^A(j) = \hat{p}^D(j+1)$.
- (b) If $\hat{p}^D(j+1) \geq \hat{p}^A(j) > \hat{p}^A(j+1)$, then we set $\hat{p}^A(j+1) = \hat{p}^A(j)$ and consequently, $\hat{p}^D(j) = \hat{p}^A(j-1) > \hat{p}^A(j) = \hat{p}^A(j+1)$.
- (c) If $\hat{p}^A(j+1) < \hat{p}^A(j)$ and $\hat{p}^D(j+1) < \hat{p}^A(j)$, then we set $\hat{p}^A(j) = \hat{p}^A(j+1) = \hat{p}^D(j+1)$ and consequently, $\hat{p}^D(j) = \hat{p}^A(j-1) > \hat{p}^A(j) = \hat{p}^A(j+1) = \hat{p}^D(j+1)$.

In the above three cases, the equation (13) for the j th interval is no longer valid. Therefore, we need to set (13) again in the following iteration. During the iteration, we stop if the estimate of $\lambda^T(j)$ is already nonnegative before the sufficient condition is satisfied.

The next section gives a brief outline of our iterative approach.

4.2 Iterative procedure

We repeat the estimation procedure explained in the previous section until $\hat{p}^A(j) + \delta \geq \hat{p}^A(j-1)$ and $\hat{p}^D(j) + \delta \geq \hat{p}^A(j-1)$ for every j , where δ is a nominal tolerance rate. As we noted in the above section, estimation in the j th interval may change the relationships among $\hat{p}^A(j)$, $\hat{p}^D(j)$ and $\hat{p}^A(j-1)$ and may affect the estimates in the other intervals. Sometimes it causes an infinite loop and does not give the final estimate, even though $\hat{\lambda}^T(j)$ becomes positive for all j . However,

$\hat{p}^A(j-1) - \hat{p}^A(j)$ and $\hat{p}^A(j-1) - \hat{p}^D(j)$ become smaller than the previous iteration. Hence, although $\hat{p}^A(j-1) > \hat{p}^A(j)$ or $\hat{p}^A(j-1) > \hat{p}^D(j)$ in some intervals after many iterations, they become less than the given tolerance rate and the resulting estimates are approximately the maximum likelihood estimates under the nonnegativity constraint.

4.3 Estimation of the variance and covariance of the tumor incidence rates

After estimating $p^A(j)$ and $p^D(j)$ iteratively, we estimate the variance and covariance of the tumor incidence rates. If we consider two adjacent intervals, the estimates of $p^A(j)$ and $p^D(j)$ have the following possible relationships:

0. $\hat{p}^A(j) > \hat{p}^A(j-1)$ and $\hat{p}^D(j) > \hat{p}^A(j-1)$,
1. $\hat{p}^A(j) > \hat{p}^A(j-1) = \hat{p}^D(j)$,
2. $\hat{p}^A(j) = \hat{p}^A(j-1) < \hat{p}^D(j)$,
3. $\hat{p}^A(j) = \hat{p}^D(j) = \hat{p}^A(j-1)$.

In case 0, let $\boldsymbol{\theta}(j) = (p^D(j), p^A(j), \lambda^D(j), p^A(j-1))'$, $j = 1, \dots, m$. Using (2) and some well known results in Rao (1973, p.362), it can easily be shown that when the true value of the parameter belongs to an interior point in the parameter space, the maximum likelihood estimate $\hat{\boldsymbol{\theta}}(j)$ is a consistent estimate of $\boldsymbol{\theta}(j)$ and the asymptotic distribution of $\sqrt{n}(\hat{\boldsymbol{\theta}}(j) - \boldsymbol{\theta}(j))$ is $N(\mathbf{0}, \Sigma(j))$, where the off-diagonal elements of $\Sigma(j)$ are all zero. Let $g(\boldsymbol{\theta}(j)) = 1 - \lambda^T(j)$, then $\nabla g(\boldsymbol{\theta}(j)) = (\partial\{1 - \lambda^T(j)\}/\partial p^D(j), \partial\{1 - \lambda^T(j)\}/\partial p^A(j), \partial\{1 - \lambda^T(j)\}/\partial \lambda^D(j), \partial\{1 - \lambda^T(j)\}/\partial p^A(j-1))'$, and by the delta method, (3) is the asymptotic variance of $\hat{\lambda}^T(j)$. Then the approximate covariance of $\hat{\lambda}^T(j-1)$ and $\hat{\lambda}^T(j)$ is

$$\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] = [\nabla g(\boldsymbol{\theta}(j-1))]'\text{Cov}[\hat{\boldsymbol{\theta}}(j-1), \hat{\boldsymbol{\theta}}(j)][\nabla g(\boldsymbol{\theta}(j))].$$

See Appendix B for the proof. The covariance is the same as (8) in case 0. Thus, the variances and the covariances of the estimated tumor incidence rates using the delta method under no constraint are the same as Malani and Van Ryzin's. In the other cases, the asymptotic variances and covariances under appropriate constraints are found as in Appendices C and D. Further details of the calculations will be provided upon request.

For all cases, the asymptotic variance of $\hat{\Lambda}^T(j)$ is

$$\sigma^2(j) = \text{Var}[\hat{\Lambda}^T(j)] = \sum_{k=1}^j \text{Var}[\hat{\lambda}^T(k)] + 2 \sum_{k=1}^{j-1} \sum_{k'=k+1}^j \text{Cov}[\hat{\lambda}^T(k), \hat{\lambda}^T(k')], \quad j = 2, \dots, m.$$

5 Testing

We can test two treatment groups using (9) by substituting the modified estimates of $\Lambda_i^T(m)$ and $\sigma_i^2(m)$. In addition, we can test for a linear contrast of the tumor incidence rates in various groups. Suppose treatment groups i_1, i_2, \dots, i_r are independent. Since $\hat{\Lambda}_i^T(m)/\hat{\sigma}_i(m)$ converges in distribution to $N(0, 1)$ (See Malani and Van Ryzin, 1988),

$$Z = \{a_1 \hat{\Lambda}_{i_1}^T(m) + \dots + a_r \hat{\Lambda}_{i_r}^T(m)\} / \sqrt{a_1^2 \hat{\sigma}_{i_1}^2(m) + \dots + a_r^2 \hat{\sigma}_{i_r}^2(m)} \quad (16)$$

also converges in distribution to $N(0, 1)$ under $\lambda_{i_1}^T(j) = \dots = \lambda_{i_r}^T(j)$, $j = 1, \dots, m$, when $\sum_{k=1}^r a_k = 0$.

6 Special cases

Malani and Van Ryzin's method assumes that the sacrifices are performed at the ends of the intervals. However, the sacrifice times may be different among the treatment groups, and then we cannot rely on that assumption any more. Also, the sacrifice times cannot be always at the end of the interval in every group if the treatments are compared according to a percent life span time scale. In this case, a sacrifice can be inside an interval or one interval can contain more than one sacrifice or no sacrifice at all, since the intervals should be the same in every treatment group for the purpose of testing. Also, there may be no deaths in some intervals. The existing nonparametric estimation methods cannot solve this problem without further assumption. Dinse (1991) proposed a constant risk difference analysis, which focuses on age-specific incidence rates and does not require times to be grouped into intervals (see also Dinse, 1994). Similar analyses based on a constant ratio between the death rates for animals with and without tumors have been suggested by Lindsey and Ryan (1993).

Now we introduce a new strategy to estimate the parameters in some unusual situations.

1. In the case that sacrifices occur at identical times in every group, and they are at the ends of

intervals:

- (a) If there are no dead animals in an interval, then leave the estimate of $p^D(j)$ undefined. From Section 3, the estimate of $\lambda^D(j)$ is 0 in this case. Hence, from (1), we find that $\lambda^T(j)$ is well-defined and identifiable, and therefore can be both estimated and tested.
- (b) If in any group, there are no sacrificed animals in the last interval for that group (i.e., none survived until the last scheduled sacrifice), then the tumor incidence rate is not identifiable for that group on the interval. Discard that interval from every group for testing the equality of tumor incidence rates.

2. In the case that sacrifices occur anywhere in an interval:

- If necessary, combine some adjacent intervals such that all intervals for each group have at least one sacrifice. For a given group, if the earliest intervals do not contain sacrifices, they are combined with the first interval for the group that does have a sacrifice. Corresponding intervals for the other groups would have to be combined in like manner for testing purposes, even though each interval might already have a sacrifice. Use the rules stated above under 1., but within each interval estimate the functions of interest as follows.
- (a) If there is a single sacrifice in an interval, estimate $p^A(j)$ simply as the proportion of sacrificed animals that have the tumor of interest. If there are additional sacrifices in the interval, then use a weighted average of the individual estimates of $p^A(j)$, with the numbers sacrificed as weights.
 - (b) Estimate $p^D(j)$ separately in the usual way for each sub-interval defined by sacrifice times within the interval. Then use a weighted average of the individual estimates of $p^D(j)$, with numbers dying in each sub-interval as weights.
 - (c) Estimate $\lambda^D(j)$ in the usual way for each sub-interval defined by sacrifice times. Then use a weighted average of the individual estimates of $\lambda^D(j)$, weighted by the numbers alive at the beginning of each sub-interval.
 - (d) Estimate $\lambda^S(j)$ as a weighted average of estimates for sub-intervals, using the numbers alive just prior to sacrifices as weights, where estimates for sub-intervals are done in the usual way.

Table 2: Counts for fitting the discrete model to data from Benzidine Dihydrochloride Experiment (F2-strain female mice).

Dose (ppm)	Interval (Week)	$N^1(j)$	$N^2(j)$	$N^3(j)$	$N^4(j)$	$N(j)$	$S(j)$	$A(j)$
60	0-40	0	2	0	70	2	70	167
	40-60	0	1	10	38	1	48	95
	60-80	7	4*	15	20	11	35	46
120	0-40	0	7	2	44	7	46	143
	40-60	6	4	15	26*	10	41	90
	60-80	16	2	20	1	18	21	39
200	0-40	1	2	4	43	3	47	119
	40-60	17	1	23	12	18	35	69
	60-80	11	2	3	0	13	3	16
400	0-40	0	3	8	14	3	22	71
	40-60	17	4	13	1	21	14	46
	60-80	9	1	1	0	10	1	11

7 Example

The proposed methods were tested on a real data set. We report the results in this section. The value of the tolerance rate δ was chosen to be 10^{-8} in all cases.

A survival/sacrifice experiment with benzidine dihydrochloride was conducted at the National Center for Toxicological Research to study strain and sex differences with regard to chemically-induced liver tumors in mice. The data were previously analyzed by Kodell and Nelson (1980). (See also Kodell *et al*, 1982, and Malani and Van Ryzin, 1988.) Raw counts from the dose groups 60 ppm, 120 ppm, 200 ppm and 400 ppm for fitting the discrete model are given in Table 2. Maximum likelihood estimates of the tumor incidence rate and prevalence functions and estimated standard errors of $\hat{\Lambda}^T(j)$ using Malani and Van Ryzin's method for the data are given in Table 3. The values of $N_1^2(3)$ and $N_2^4(2)$ were reported by mistake as 3 and 16, respectively, in Table 4 of Malani and Van Ryzin (1988). Corrected values are noted with an asterisk in our Table 2. Apparently only the incorrect value of $N_1^2(3)$ was actually used in calculations, since our results for the 120 ppm dose group in Table 3 agree with results in Malani and Van Ryzin (1988). Estimated standard errors in Table 3 for Malani and Van Ryzin's method differ from those in their Table 5, due to our correction of an error in their variance expression.

Maximum likelihood estimates of the two methods were exactly the same in the three lower

Table 3: Maximum likelihood estimates of $p^A(j)$, $p^D(j)$, $\lambda^T(j)$, $\Lambda^T(j)$ using Malani and Van Ryzin's method for the data in Table 2. Standard error estimates of $\Lambda^T(j)$ are in parentheses.

Dose (ppm)	Interval (Week)	$\hat{p}^A(j)$	$\hat{p}^D(j)$	$\hat{\lambda}^T(j)$	$\hat{\Lambda}^T(j)$
60	0-40	.000	.000	.000	.000 (.000)
	40-60	.208	.000	.206	.206 (.058)
	60-80	.429	.636	.341	.547 (.094)
120	0-40	.043	.000	.041	.041 (.029)
	40-60	.366	.600	.364	.406 (.073)
	60-80	.952	.889	.879	1.284 (.090)
200	0-40	.085	.333	.091	.091 (.040)
	40-60	.657	.944	.707	.799 (.074)
	60-80	1.000	.846	.635	1.434 (.245)
400	0-40	.364	.000	.348	.348 (.099)
	40-60	.929	.810	.802	1.151 (.109)
	60-80	1.000	.900	-.273	.878 (1.687)

Table 4: Maximum likelihood estimates of $p^A(j)$, $p^D(j)$, $\lambda^T(j)$, $\Lambda^T(j)$ using our improved estimation method, and the pooled estimates of λ_j by Williams and Portier (1992b) for the 400 ppm dose group of the data in Table 2. Standard error estimates of $\Lambda^T(j)$ are in parentheses. Here, W-P stands for Williams and Portier.

Interval (Week)	$\hat{p}^A(j)$	$\hat{p}^D(j)$	$\hat{\lambda}^T(j)$	$\hat{\Lambda}^T(j)$	W-P $\hat{\Lambda}_j$
0-40	.364	.000	.348	.348 (.099)	.348
40-60	.917	.810	.792	1.140 (.103)	1.143
60-80	1.000	.917	.091	1.231 (.134)	1.143

dose groups. The estimates for the 400 ppm dose group using our modified method and the pooled estimates by Williams and Portier (1992b) are presented in Table 4. Williams and Portier (1992b) did not provide the standard error estimates. Malani and Van Ryzin's estimate of the tumor incidence rate in the third interval for the 400 ppm dose group was negative, resulting in a decreasing estimate of the cumulative tumor incidence rate. Our estimate of the tumor incidence rate was positive in all the intervals and the corresponding estimate of the cumulative tumor incidence rate was increasing in the 400 ppm dose group. The estimated standard errors of Malani and Van Ryzin's and ours were 1.687 and .134, respectively in the third interval of the 400 ppm dose group, and their estimate seems to be too much inflated.

Results of all pairwise comparisons of treatments with respect to the tumor incidence rate are

Table 5: Standard normal deviates for pairwise comparison of treatments with regard to the cumulative tumor incidence rates using (9).

Dose (ppm)	Malani and Van Ryzin's				Our Estimates			
	60	120	200	400	60	120	200	400
60	-	5.68	3.39	.20	-	5.68	3.39	4.18
120		-	.57	-.24		-	.57	-.33
200			-	-.33			-	-.73
400				-				-

given in Table 5. Malani and Van Ryzin (1988) asserted that the instability of the tumor incidence rate estimate is correctly reflected by the large estimate of standard error, and concluded that using the negative estimate for comparing the tumor incidence rate of two treatments would not affect the size of the critical region. But our results did not agree with their explanation. Malani and Van Ryzin's estimates resulted in significant differences between the 60 ppm and 120 ppm dose groups, and between the 60 ppm and 200 ppm dose groups, but no significant difference between the 60 ppm and 400 ppm groups. In contrast, our methods detected a significant difference whenever we compared the 60 ppm dose group with any other dose group. In particular, using our improved estimates in Malani and Van Ryzin's (1988) approximate normal test (9) (a special case of our test (16)) shows that the 400 ppm dose group has a significantly higher cumulative tumor incidence rate than the 60 ppm group. The P -values of one-sided tests using Malani and Van Ryzin's estimates and ours are .42 and .00001, respectively. Our test results are more reasonable, because we should expect a significant difference between the 60 ppm and 400 ppm dose groups when there is a significant difference between the 60 ppm and 120 (or 200) ppm dose groups. We also see from Table 2 that the tumor rates of the dead and sacrificed animals are much higher in the 400 ppm dose group than in the 60 ppm group in every interval.

8 Discussion

In many animal carcinogenicity experiments, cause of death is not available. Even when cause of death is available, it is subject to error, and the analysis can misrepresent the data. The proposed method utilizes information from serial sacrifices, and therefore, does not need any information on cause of death. It is an extension of the maximum likelihood estimation method of Malani and Van Ryzin (1988). In many real data sets, the tumor incidence rate estimated by the method of Malani and Van Ryzin can be negative even though the ratio of tumors among the dead or

sacrificed animals in the group is quite high. In some cases, even the estimate of the cumulative tumor incidence rate becomes negative.

Williams and Portier (1992a) introduced analytic expressions for maximum likelihood estimates of the tumor incidence rate. Their estimate of the tumor incidence rate is identical to that proposed by Malani and Van Ryzin (1988). Williams and Portier (1992b) modified their analytic expressions by deriving constrained estimators under the imposition of a boundary condition, so that the tumor incidence rate is nonnegative. But their approach is only for a study design with a maximum of three sacrifices. For study designs with more than three sacrifices, they used heuristic estimates of the tumor incidence rate by pooling data together from adjacent intervals.

The method described here was motivated by the twin goals of (i) obtaining an approximate maximum likelihood estimate under the constraint of a nonnegative tumor incidence rate and (ii) providing a procedure to get the estimate even though sacrifices occur anywhere in an interval. These goals can be met by using our improved approximate nonparametric maximum likelihood estimation procedure. Our estimating procedure always gives a nonnegative estimate of the tumor incidence rate and reduces its standard error so that the test of two heterogeneous groups has more power. Furthermore, our methods can handle data having any number of interim sacrifices.

Appendix A

From (1),

$$\begin{aligned}\lambda^T(j) &= P(X_T = j, X_D \geq j) / P(X_T \geq j, X_D \geq j) \\ &= [P(X_T \geq j, X_D \geq j) - P(X_T > j, X_D > j) - P(X_T > j, X_D = j)] / P(X_T \geq j, X_D \geq j).\end{aligned}$$

Now

$$\begin{aligned}P(X_T \geq j, X_D \geq j) &= \{1 - P(X_T < j | X_D \geq j)\} P(X_D \geq j) \\ &= \{1 - P(X_T \leq j - 1 | X_D > j - 1)\} P(X_D \geq j) \\ &= \{1 - p^A(j - 1)\} P(X_D \geq j),\end{aligned}$$

$$P(X_T > j, X_D > j) = P(X_T > j | X_D > j) P(X_D > j)$$

$$= \{1 - P^A(j)\}\{1 - \lambda^D(j)\}P(X_D \geq j),$$

$$\begin{aligned} P(X_T > j, X_D = j) &= P(X_T > j, X_D = j | X_D \geq j)P(X_D \geq j) \\ &= \{1 - P^D(j)\}\lambda^D(j)P(X_D \geq j). \end{aligned}$$

Therefore,

$$\lambda^T(j) = 1 - [\{1 - p^A(j)\}\{1 - \lambda^D(j)\} + \{1 - p^D(j)\}\lambda^D(j)] / \{1 - p^A(j-1)\}.$$

Appendix B

Let $\boldsymbol{\beta} = (p^D(j-1), p^A(j-1), \lambda^D(j-1), p^A(j-2), p^D(j), p^A(j), \lambda^D(j))'$ and $k(\boldsymbol{\beta}) = (1 - \lambda^T(j-1), 1 - \lambda^T(j))'$. Then it is easy to see that the asymptotic distribution of $\sqrt{n}(k(\hat{\boldsymbol{\beta}}) - k(\boldsymbol{\beta}))$ is $N(\mathbf{0}, \Sigma)$, where $\Sigma = \text{Diag}(\text{Var}[\hat{p}^D(j-1)], \text{Var}[\hat{p}^A(j-1)], \text{Var}[\hat{\lambda}^D(j-1)], \text{Var}[\hat{p}^A(j-2)], \text{Var}[\hat{p}^D(j)], \text{Var}[\hat{p}^A(j)], \text{Var}[\hat{\lambda}^D(j)])$. By the delta method, $\sqrt{n}(k(\hat{\boldsymbol{\beta}}) - k(\boldsymbol{\beta}))$ converges in distribution to $N(\mathbf{0}, [\nabla k(\boldsymbol{\beta})]'\Sigma[\nabla k(\boldsymbol{\beta})])$, where $\hat{\boldsymbol{\beta}}$ is the maximum likelihood estimate of $\boldsymbol{\beta}$, and

$$[\nabla k(\boldsymbol{\beta})]' = \begin{pmatrix} \frac{\partial[1-\lambda^T(j-1)]}{\partial p^D(j-1)} & \frac{\partial[1-\lambda^T(j-1)]}{\partial p^A(j-1)} & \frac{\partial[1-\lambda^T(j-1)]}{\partial \lambda^D(j-1)} & \frac{\partial[1-\lambda^T(j-1)]}{\partial p^A(j-2)} & 0 & 0 & 0 \\ 0 & \frac{\partial[1-\lambda^T(j)]}{\partial p^A(j-1)} & 0 & 0 & \frac{\partial[1-\lambda^T(j)]}{\partial p^D(j)} & \frac{\partial[1-\lambda^T(j)]}{\partial p^A(j)} & \frac{\partial[1-\lambda^T(j)]}{\partial \lambda^D(j)} \end{pmatrix}.$$

After some calculation, we see that the (1, 2)th element of $[\nabla k(\boldsymbol{\beta})]'\Sigma[\nabla k(\boldsymbol{\beta})]$ is

$$\begin{aligned} \text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] &= [\partial\{1 - \lambda^T(j-1)\}/\partial p^A(j-1)]\text{Var}[\hat{p}^A(j-1)][\partial\{1 - \lambda^T(j)\}/\partial p^A(j-1)] \\ &= [\nabla g(\boldsymbol{\theta}(j-1))]' \text{Cov}[\hat{\boldsymbol{\theta}}(j-1), \hat{\boldsymbol{\theta}}(j)][\nabla g(\boldsymbol{\theta}(j))]. \end{aligned}$$

Appendix C

If equality of prevalence estimates holds within the specified tolerance rate for two intervals and no other equality holds with intervals $(j+1)$ and $(j-2)$, we need to get the following new variance and covariance expressions.

1. In the case that $\hat{p}^A(j) > \hat{p}^A(j-1) = \hat{p}^D(j)$, use (12) to get $\text{Var}[\hat{\lambda}^T(j)]$, use (1) to get $\text{Var}[\hat{\lambda}^T(j-1)]$, and use (1) and (12) to get $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$. Let $\boldsymbol{\theta}^1(j) = \boldsymbol{\theta}(j)|_{p^D(j)=p^A(j-1)} = (p^A(j-$

1), $p^A(j), \lambda^D(j)$ ' ; then $\nabla g(\boldsymbol{\theta}^1(j)) = (\partial\{1 - \lambda^T(j)\}/\partial p^A(j-1), \partial\{1 - \lambda^T(j)\}/\partial p^A(j), \partial\{1 - \lambda^T(j)\}/\partial \lambda^D(j))'$, and by the delta method and a similar argument as in Appendix B,

$$\begin{aligned}\text{Var}[\hat{\lambda}^T(j)] &= [\nabla g(\boldsymbol{\theta}^1(j))]' \text{Cov}[\hat{\boldsymbol{\theta}}^1(j)][\nabla g(\boldsymbol{\theta}^1(j))] \\ &= \{1 - \lambda^D(j)\}[\{1 - p^A(j)\}^2\{1 - \lambda^D(j)\}\{1 - \lambda^S(j-1)\}v(j)/\{1 - p^A(j-1)\} \\ &\quad + b(j) + c(j)/\{1 - \lambda^D(j)\}]/k(j),\end{aligned}$$

where $v(j) = p^A(j-1)/[\{\lambda^D(j)\{1 - \lambda^S(j-1)\} + \lambda^S(j-1)]$ and $b(j), c(j)$ and $k(j)$ are defined in (5), (6) and (7), respectively,

$$\begin{aligned}\text{Var}[\hat{\lambda}^T(j-1)] &= [\nabla g(\boldsymbol{\theta}(j-1))]' \text{Cov}[\hat{\boldsymbol{\theta}}(j-1)][\nabla g(\boldsymbol{\theta}(j-1))] \\ &= [a(j-1) + c(j-1) + \{1 - \lambda^D(j-1)\}\{1 - p^A(j-1)\}v(j) \\ &\quad + \{1 - \lambda^T(j-1)\}^2\{1 - \lambda^S(j-2)\}b(j-2)]/k(j-1),\end{aligned}$$

where $a(j)$ is defined in (4), and

$$\begin{aligned}\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] &= [\nabla g(\boldsymbol{\theta}(j-1))]' \text{Cov}[\hat{\boldsymbol{\theta}}(j-1), \hat{\boldsymbol{\theta}}^1(j)][\nabla g(\boldsymbol{\theta}^1(j))] \\ &= -\{1 - p^A(j)\}\{1 - \lambda^D(j)\}\{1 - p^A(j-2)\}v(j) \\ &\quad /[\{1 - p^A(j-1)\}k(j-1)].\end{aligned}$$

2. In the case that $\hat{p}^A(j) = \hat{p}^A(j-1) < \hat{p}^D(j)$, use (14) to get $\text{Var}[\hat{\lambda}^T(j)]$, use (1) to get $\text{Var}[\hat{\lambda}^T(j+1)]$, $\text{Var}[\hat{\lambda}^T(j-1)]$ and $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)]$, and use (1) and (14) to get $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$ and $\text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)]$. Let $\boldsymbol{\theta}^2(j) = \boldsymbol{\theta}(j)|_{p^A(j)=p^A(j-1)} = (p^D(j), p^A(j), \lambda^D(j))'$; then $\nabla g(\boldsymbol{\theta}^2(j)) = (\partial\{1 - \lambda^T(j)\}/\partial p^D(j), \partial\{1 - \lambda^T(j)\}/\partial p^A(j), \partial\{1 - \lambda^T(j)\}/\partial \lambda^D(j))'$, and by the delta method and a similar argument as in Appendix B, we get,

$$\begin{aligned}\text{Var}[\hat{\lambda}^T(j+1)] &= [\nabla g(\boldsymbol{\theta}(j+1))]' \text{Cov}[\hat{\boldsymbol{\theta}}(j+1)][\nabla g(\boldsymbol{\theta}(j+1))] \\ &= [a(j+1) + \{1 - \lambda^D(j+1)\}b(j+1) + c(j+1) \\ &\quad + \{1 - \lambda^T(j+1)\}^2\{1 - p^A(j)\}\{1 - \lambda^S(j-1)\}\{1 - \lambda^D(j)\} \\ &\quad \times \{1 - \lambda^S(j)\}e(j)]/k(j+1),\end{aligned}$$

where $e(j) = p^A(j)/[\{1 - \lambda^D(j)\}\lambda^S(j)\{1 - \lambda^S(j-1)\} + \lambda^S(j-1)]$,

$$\begin{aligned}\text{Var}[\hat{\lambda}^T(j)] &= [\nabla g(\boldsymbol{\theta}^2(j))]' \text{Cov}[\hat{\boldsymbol{\theta}}^2(j)][\nabla g(\boldsymbol{\theta}^2(j))] \\ &= \lambda^D(j)\{1 - p^D(j)\}\{1 - \lambda^S(j-1)\}e(j)f(j)/\{1 - p^A(j)\} \\ &\quad + p^D(j)\{1 - p^D(j)\} + c(j)/\lambda^D(j)]/k(j),\end{aligned}$$

where $f(j) = \{1 - p^D(j)\}\lambda^D(j)$,

$$\begin{aligned}\text{Var}[\hat{\lambda}^T(j-1)] &= [\nabla g(\boldsymbol{\theta}(j-1))]' \text{Cov}[\hat{\boldsymbol{\theta}}(j-1)][\nabla g(\boldsymbol{\theta}(j-1))] \\ &= [a(j-1) + \{1 - \lambda^D(j-1)\}\{1 - p^A(j)\}e(j) + c(j-1) \\ &\quad + \{1 - \lambda^T(j-1)\}^2\{1 - \lambda^S(j-2)\}b(j-2)]/k(j-1),\end{aligned}$$

$$\begin{aligned}\text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)] &= [\nabla g(\boldsymbol{\theta}^2(j))]' \text{Cov}[\hat{\boldsymbol{\theta}}^2(j), \hat{\boldsymbol{\theta}}(j+1)][\nabla g(\boldsymbol{\theta}(j+1))] \\ &= \{1 - \lambda^T(j+1)\}\{1 - \lambda^S(j-1)\}e(j)f(j)/k(j),\end{aligned}$$

$$\begin{aligned}\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] &= [\nabla g(\boldsymbol{\theta}(j-1))]' \text{Cov}[\hat{\boldsymbol{\theta}}(j-1), \hat{\boldsymbol{\theta}}^2(j)][\nabla g(\boldsymbol{\theta}^2(j))] \\ &= -\{1 - p^A(j-2)\}e(j)f(j)/[\{1 - p^A(j)\}k(j-1)],\end{aligned}$$

and

$$\begin{aligned}\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)] &= [\nabla g(\boldsymbol{\theta}(j-1))]' \text{Cov}[\hat{\boldsymbol{\theta}}(j-1), \hat{\boldsymbol{\theta}}(j+1)][\nabla g(\boldsymbol{\theta}(j+1))] \\ &= [\partial\{1 - \lambda^T(j-1)\}/\partial p^A(j)][\partial\{1 - \lambda^T(j+1)\}/\partial p^A(j)] \\ &\quad \times \text{Var}[\hat{p}^A(j)] \\ &= -\{1 - \lambda^T(j+1)\}\{1 - p^A(j-2)\}e(j)/k(j-1).\end{aligned}$$

- 3.** In the case that $\hat{p}^A(j) = \hat{p}^A(j-1) = \hat{p}^D(j)$, use (1) to get $\text{Var}[\hat{\lambda}^T(j+1)]$, $\text{Var}[\hat{\lambda}^T(j-1)]$ and $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)]$. Since $\hat{\lambda}^T(j)$ is zero in this case, $\text{Var}[\hat{\lambda}^T(j)]$, $\text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)]$ and $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$ are zero. Let $\boldsymbol{\theta}^3(j) = \boldsymbol{\theta}(j)|_{p^D(j)=p^A(j)=p^A(j-1)} = (p^A(j), \lambda^D(j))'$, then $\nabla g(\boldsymbol{\theta}^3(j)) = (\partial\{1 - \lambda^T(j)\}/\partial p^A(j), \partial\{1 - \lambda^T(j)\}/\partial \lambda^D(j))' = (0, 0)'$, and by the delta

method and a similar argument as in Appendix B, we get,

$$\begin{aligned}
\text{Var}[\hat{\lambda}^T(j+1)] &= [\nabla g(\boldsymbol{\theta}(j+1))]'\text{Cov}[\hat{\boldsymbol{\theta}}(j+1)][\nabla g(\boldsymbol{\theta}(j+1))] \\
&= [a(j+1) + \{1 - \lambda^D(j+1)\}b(j+1) + c(j+1) + \{1 - \lambda^T(j+1)\}^2 \\
&\quad \times \{1 - \lambda^S(j-1)\}\{1 - \lambda^D(j)\}\{1 - \lambda^S(j)\}h(j)]/k(j+1),
\end{aligned}$$

where $h(j) = p^A(j)\{1 - p^A(j)\}/[\lambda^D(j)\{1 - \lambda^S(j-1)\} + \{1 - \lambda^D(j)\}\lambda^S(j)\{1 - \lambda^S(j-1)\} + \lambda^S(j-1)]$,

$$\begin{aligned}
\text{Var}[\hat{\lambda}^T(j-1)] &= [\nabla g(\boldsymbol{\theta}(j-1))]'\text{Cov}[\hat{\boldsymbol{\theta}}(j-1)][\nabla g(\boldsymbol{\theta}(j-1))] \\
&= [a(j-1) + \{1 - \lambda^T(j-1)\}^2\{1 - \lambda^S(j-2)\}b(j-2) \\
&\quad + \{1 - \lambda^D(j-1)\}h(j) + c(j-1)]/k(j-1)
\end{aligned}$$

and

$$\begin{aligned}
\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)] &= [\nabla g(\boldsymbol{\theta}(j-1))]'\text{Cov}[\hat{\boldsymbol{\theta}}(j-1), \hat{\boldsymbol{\theta}}(j+1)][\nabla g(\boldsymbol{\theta}(j+1))] \\
&= -\{1 - \lambda^T(j+1)\}\{1 - \lambda^D(j-1)\}\{1 - \lambda^S(j-1)\} \\
&\quad \{1 - p^A(j)\}h(j)/[\{1 - p^A(j-2)\}k(j)].
\end{aligned}$$

Variance and covariance expressions for cases in which equality among the estimates of $\hat{p}^D(j)$ and $\hat{p}^A(j)$ holds for three time intervals are given in Appendix D.

Appendix D

If equality of prevalence estimates holds within the specified tolerance rate for three intervals and no other equality holds with intervals $(j+1)$ and $(j-3)$, we need to get the following new variance and covariance expressions.

- 1-1.** If $\hat{p}^D(j) = \hat{p}^A(j-1) \neq \hat{p}^D(j-1) = \hat{p}^A(j-2)$, use the maximum likelihood estimate (11) for the j th interval to get $\text{Var}[\hat{\lambda}^T(j)]$ and $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$, for the $(j-1)$ st interval to get $\text{Var}[\hat{\lambda}^T(j-2)]$ and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)]$, and for both the j th and $(j-1)$ st intervals to get $\text{Var}[\hat{\lambda}^T(j-1)]$. In this case, $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j)] = 0$.

1-2. If $\hat{p}^D(j) \doteq \hat{p}^A(j-1) \doteq \hat{p}^A(j-2)$, use the maximum likelihood estimate

$$\{\hat{p}^D(j)N(j) + \hat{p}^A(j-1)S(j-1) + \hat{p}^A(j-2)S(j-2)\}/\{N(j) + S(j-1) + S(j-2)\}$$

to get $\text{Var}[\hat{\lambda}^T(j)]$, $\text{Var}[\hat{\lambda}^T(j-1)]$, $\text{Var}[\hat{\lambda}^T(j-2)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$, $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)]$ and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j)]$.

1-3. If $\hat{p}^D(j) \doteq \hat{p}^D(j-1) \doteq \hat{p}^A(j-1) \doteq \hat{p}^A(j-2)$, use the maximum likelihood estimate

$$\begin{aligned} & \{\hat{p}^D(j)N(j) + \hat{p}^D(j-1)N(j-1) + \hat{p}^A(j-1)S(j-1) + \hat{p}^A(j-2)S(j-2)\} \\ & / \{N(j) + N(j-1) + S(j-1) + S(j-2)\} \end{aligned}$$

to get $\text{Var}[\hat{\lambda}^T(j)]$, $\text{Var}[\hat{\lambda}^T(j-2)]$ and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j)]$. Since $\hat{\lambda}^T(j-1) = 0$, $\text{Var}[\hat{\lambda}^T(j-1)] = \text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] = \text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)] = 0$.

2-1. If $\hat{p}^A(j) = \hat{p}^A(j-1) \neq \hat{p}^D(j-1) = \hat{p}^A(j-2)$, use the maximum likelihood estimate (13) for the j th interval to get $\text{Var}[\hat{\lambda}^T(j)]$, $\text{Var}[\hat{\lambda}^T(j+1)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)]$ and $\text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)]$. Use (11) for the $(j-1)$ st interval to get $\text{Var}[\hat{\lambda}^T(j-2)]$ and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)]$, and use both (13) for the j th interval and (11) for the $(j-1)$ st interval to get $\text{Var}[\hat{\lambda}^T(j-1)]$. In this case, $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j)] = \text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j+1)] = 0$.

2-2. If $\hat{p}^A(j) \doteq \hat{p}^A(j-1) \doteq \hat{p}^A(j-2)$, use the maximum likelihood estimate

$$\{\hat{p}^A(j)S(j) + \hat{p}^A(j-1)S(j-1) + \hat{p}^A(j-2)S(j-2)\}/\{S(j) + S(j-1) + S(j-2)\}$$

to get $\text{Var}[\hat{\lambda}^T(j+1)]$, $\text{Var}[\hat{\lambda}^T(j)]$, $\text{Var}[\hat{\lambda}^T(j-1)]$, $\text{Var}[\hat{\lambda}^T(j-2)]$, $\text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$, $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)]$, $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j)]$, and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j+1)]$.

2-3. If $\hat{p}^A(j) \doteq \hat{p}^D(j-1) \doteq \hat{p}^A(j-1) \doteq \hat{p}^A(j-2)$, use the maximum likelihood estimate

$$\begin{aligned} & \{\hat{p}^A(j)S(j) + \hat{p}^D(j-1)N(j-1) + \hat{p}^A(j-1)S(j-1) + \hat{p}^A(j-2)S(j-2)\} \\ & / \{S(j) + N(j-1) + S(j-1) + S(j-2)\} \end{aligned}$$

to get $\text{Var}[\hat{\lambda}^T(j+1)]$, $\text{Var}[\hat{\lambda}^T(j)]$, $\text{Var}[\hat{\lambda}^T(j-2)]$, $\text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)]$, $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j)]$, and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j+1)]$. Since $\hat{\lambda}^T(j-1) = 0$, $\text{Var}[\hat{\lambda}^T(j-1)] = \text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] = \text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)] = \text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)] = 0$.

3-1. If $\hat{p}^D(j) = \hat{p}^A(j) = \hat{p}^A(j-1) \neq \hat{p}^D(j-1) = \hat{p}^A(j-2)$, use the maximum likelihood estimates (15) for the j th interval to get $\text{Var}[\hat{\lambda}^T(j)]$, $\text{Var}[\hat{\lambda}^T(j+1)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)]$ and $\text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)]$. Use (11) for the $(j-1)$ st interval to get $\text{Var}[\hat{\lambda}^T(j-2)]$ and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)]$, and use both (15) for the j th interval and (11) for the $(j-1)$ st interval to get $\text{Var}[\hat{\lambda}^T(j-1)]$. Here, $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j)] = \text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j+1)] = 0$.

3-2. If $\hat{p}^D(j) \doteq \hat{p}^A(j) \doteq \hat{p}^A(j-1) \doteq \hat{p}^A(j-2)$, use the maximum likelihood estimate

$$\frac{\{\hat{p}^D(j)N(j) + \hat{p}^A(j)S(j) + \hat{p}^A(j-1)S(j-1) + \hat{p}^A(j-2)S(j-2)\}}{\{N(j) + S(j) + S(j-1) + S(j-2)\}}$$

to get $\text{Var}[\hat{\lambda}^T(j+1)]$, $\text{Var}[\hat{\lambda}^T(j-1)]$, $\text{Var}[\hat{\lambda}^T(j-2)]$, $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)]$, $\text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)]$ and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j+1)]$. Since $\hat{\lambda}^T(j) = 0$, $\text{Var}[\hat{\lambda}^T(j)] = \text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)] = \text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] = 0$.

3-3. If $\hat{p}^D(j) \doteq \hat{p}^A(j) \doteq \hat{p}^D(j-1) \doteq \hat{p}^A(j-1) \doteq \hat{p}^A(j-2)$, use the maximum likelihood estimate

$$\frac{\{\hat{p}^D(j)N(j) + \hat{p}^A(j)S(j) + \hat{p}^D(j-1)N(j-1) + \hat{p}^A(j-1)S(j-1) + \hat{p}^A(j-2)S(j-2)\}}{\{N(j) + S(j) + N(j-1) + S(j-1) + S(j-2)\}}$$

to get $\text{Var}[\hat{\lambda}^T(j+1)]$, $\text{Var}[\hat{\lambda}^T(j-2)]$ and $\text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j+1)]$. Since $\hat{\lambda}^T(j) = \hat{\lambda}^T(j-1) = 0$, $\text{Var}[\hat{\lambda}^T(j)] = \text{Var}[\hat{\lambda}^T(j-1)] = \text{Cov}[\hat{\lambda}^T(j), \hat{\lambda}^T(j+1)] = \text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j)] = \text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j-1)] = \text{Cov}[\hat{\lambda}^T(j-1), \hat{\lambda}^T(j+1)] = \text{Cov}[\hat{\lambda}^T(j-2), \hat{\lambda}^T(j)] = 0$.

In the case that the equality holds within the specified tolerance rate for more than three intervals, estimate the tumor prevalence rate among live animals and the tumor prevalence rate among dying animals under the nonnegativity constraint and get the asymptotic variance and covariance of the tumor incidence rates using the constrained estimates the same way as above.

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