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TESTING FOR A POSITIVE DOSE RESPONSE RELATION IN THE PRESENCE OF TWO TYPES OF TUMORS

RAM C. TIWARI and JYOTI N. ZALKIKAR*

ABSTRACT

The experimental data from rodent bioassay, where the occurrence of only one type of tumor is observed, has been analyzed by several authors. However, in a large number of carcinogenesis studies, the subjects are at risk of developing two or more types of tumors. In this paper we develop inference procedures to study the dose response relationship for two different types of tumors, I and II. The suffixes 00, 10, 01 and 11 correspond to neither I nor II, I but not II, II but not I, and both I and II, respectively. We assume that at the dose level \(d_i (i = 0, \ldots, r)\), the random response vector \(x_i = (x_{i00}, x_{i10}, x_{i01}, x_{i11})'\) has a multinomial distribution with parameters \(n_i\) and \(p_i = (p_{i00}, p_{i10}, p_{i01}, p_{i11})'\), where

\[
\begin{align*}
p_{i00} &= (1 - H(a + \xi_1 d_i))(1 - H(a + \xi_2 d_i)), \quad p_{i10} = H(a + \xi_1 d_i)(1 - H(a + \xi_2 d_i)), \\
p_{i01} &= (1 - H(a + \xi_1 d_i))H(a + \xi_2 d_i), \quad p_{i11} = H(a + \xi_1 d_i)H(a + \xi_2 d_i).
\end{align*}
\]

Here, \(H\) is the logistic model, \(d_0 = 0\) (corresponding to the control group), \(d_i < d_{i+1}\) for all \(i\), and \(\xi = (\xi_1, \xi_2)'\).

We derive a Cochran-Armitage type test for testing the null hypothesis \(H_0\) of no dose response relation \((\xi = 0)\) against the alternative hypothesis of positive dose response relation \((\xi > 0)\) based on the current experimental data. In the presence of historical control data, the approach of Tarone (Biometrics, 1982) is used. Under \(H_0\), the probability of occurrence of Type I or Type II tumor is \(H(a)\). To incorporate the historical controls, this probability of spontaneous occurrence of Type I (or Type II)

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tumor is considered to vary across the experiments according to a beta distribution. The asymptotic distributions of the test statistics are derived and the Pitman efficiencies are studied. The test procedures are applied to the ED_{01} data of National Center for Toxicological Research.

KEY WORDS: Linear trend; Historical controls; Carcinogenesis; Pitman asymptotic efficiency.

1. INTRODUCTION

Long term and increased exposure to chemicals, low level radiation and other toxic pollutants in the environment have created a serious public health problem. (In a recent (March 13, 1992) nationally televised program on ABC network, a possibility was raised that prolonged parental exposure to toxic chemicals released by a sunglass manufacturing plant may have caused some forms of autism in children.) The increased risk of cancer due to chronic exposure to toxic substances is one of the many major concerns. Statistical assessment of this risk, in the absence of reliable human data, is inevitably based on animal carcinogenicity experiments. These experiments involve treating tumor free animals with one or more levels of the test agent and an evaluation of the tumor occurrence rates in the treated groups relative to those in the unexposed controls.

Several authors have developed methods suitable for analysis of one type of tumor at a time. In reality, in a large number of carcinogenesis studies, the subjects are at risk of developing several types of tumors. For example, in a large scale experiment initiated in 1974 by National Center for Toxicological Research, called ED_{01} study, animals (mice) were exposed to one of the eight dose levels 0, 30, 35, 45, 60, 75, 100 and 150 ppm. These animals were at risk of developing several tumor types. The literature on multiple tumors includes the works of Clifford(1976), Berlin et. al.(1978), Finkelstein
and Schoenfeld(1989), and Lu(1990). In this article, we extend three widely used inference procedures for one type of tumor to study the dose response relationship for two different types of tumors, using a logistic response model, appropriately adjusted to account for the multiplicity of tumors. We assume that the tumor types are independent. This assumption may be too strong for certain types of tumors, like tumors in the same organ, while being satisfactory for certain other types. For ED_{01} data considered in Section 3, Lu(1990) estimated correlation matrices for marginal tumor occurrence rates under different dose levels and showed most tumor types to be almost independent. It also seems desirable to solicit an expert’s opinion about the independence of tumor types of interest prior to statistical analysis. In Section 2.1, we develop Cochran-Armitage type test procedure to detect a positive dose response trend. This test is based only on the current experimental data, and is a generalization of the test procedure proposed by Cochran(1954) and Armitage(1955).

Although concurrent controls constitute the most appropriate reference group against which to compare the treatment groups, historical controls obtained from other similar studies contain some information regarding the rate of spontaneous tumor occurrence. Hence there is a desire on the part of toxicologists to use this piece of information. For example, as pointed out by Hoel and Yanagawa(1986), for historically rare outcomes among control animals, a toxicologist is quite impressed if the particular outcome is observed among the treated groups of animals. This leads to a criticism of statistical methods such as Cochran-Armitage type test when “statistical significance” is not achieved for these rare events. A second concern arises when toxicologists use historical data informally and at times poorly. For example, the argument is often made that the tumor rate in a current treated group is within the range of tumor rates seen in a set of historical control groups and so the result is likely due to chance. Study
to study variability is incorrectly used to evaluate a within study effect. This leads to a misuse of historical data to discount the significance of Cochran-Armitage type tests (which is based only on the current experimental data). Clearly, there is a need for statistically formal methodology to incorporate historical data into the analysis of the current experiment. The first such procedure was proposed by Tarone (1982), for the analysis of one type of tumor at a time. In this procedure, within the concurrent control group, the distribution of the number of responses was considered to be binomial, and the probability of a response was assumed to vary across experiments according to a beta distribution. This led to a beta-binomial distribution to accommodate the extra-binomial variation among the historical controls. We generalize this procedure in Section 2.2 to study the dose response relationship for two types of tumors. Also a conditional test along the lines of Hoel and Yanagawa (1986) is obtained in Section 2.3. Section 3 contains an illustration of the test procedures using ED\textsubscript{01} data. In Section 4, we briefly address certain issues about our test procedures. The theoretical results in this paper consist of the derivations of the asymptotic distributions of the proposed test statistics, in Theorems 1-5, and a study of the Pitman efficiencies, establishing that the conditional and the Tarone type tests are equivalent (in the sense of the Pitman asymptotic efficiency) and are more efficient than the Cochran-Armitage type test. These results, although obtained using the standard techniques, are the new contributions, particularly in the context of carcinogenesis studies.

2. MAIN RESULTS

Long term rodent studies involve groups of animals exposed to different levels of the test substance as well as unexposed controls. More specifically, consider an experiment with \( r \) treatment groups and one control group. At each dose level \( d_i (i = 0, \)
there are \( n_i \) subjects at risk of developing any of the two types of tumors, call them Type I and Type II. The suffices 00, 10, 01 and 11 correspond to neither Type I nor Type II, Type I but not Type II, Type II but not Type I, and both Type I and Type II, respectively. We assume that at dose level \( d_i \), the random vector \( x_i = (x_{i00}, x_{i10}, x_{i01}, x_{i11})' \) of the number of subjects in respective categories has a multinomial distribution with parameters \( n_i \) and \( p_i = (p_{i00}, p_{i10}, p_{i01}, p_{i11})' \). We model the response probabilities by assuming the independence of the two tumor types, as follows:

\[
P_{i00} = (1 - H(a + \xi_1 d_i))(1 - H(a + \xi_2 d_i)),
\]
\[
P_{i10} = H(a + \xi_1 d_i)(1 - H(a + \xi_2 d_i)),
\]
\[
P_{i01} = (1 - H(a + \xi_1 d_i))H(a + \xi_2 d_i),
\]
\[
P_{i11} = H(a + \xi_1 d_i)H(a + \xi_2 d_i),
\]

where \( H \) is the logistic distribution function given by

\[
H(a) = \frac{\exp(a)}{1 + \exp(a)},
\]

(2.1)

\( d_0 = 0 \) (corresponding to the control group) and \( d_i < d_{i+1} \) for all \( i \). Let \( \xi = (\xi_1, \xi_2)' \).

We wish to test the null hypothesis

\[ H_0: \ \xi = 0 \]

of no dose effect against the alternative

\[ H_1: \ \xi > 0 \]

of increasing trend in the occurrences of both tumors. Note that, in the model described here, the probability of spontaneous occurrence of Type I tumor is considered to be equal to the probability of spontaneous occurrence of Type II tumor. This is addressed again in Section 4. In Section 2.1, we develop a Cochran-Armitage type test procedure which uses only the current experimental data. In Sections 2.2 and 2.3, in the presence of historical control data obtained from other similar carcinogen bioassays, the approach of Tarone (1982) and Hoel and Yanagawa (1986) is used.
2.1 Cochran-Armitage Type Test

The traditional Cochran-Armitage test proposed by Cochran(1954) and Armitage(1955) applies only to one type of tumor. In this section, we extend this test to the setup described above accounting for the simultaneous occurrence of two independent tumor types.

The likelihood function of $a$ and $\xi$ given the data $(x_i, i = 0, 1, 2, \ldots, r)$ is

$$L(a, \xi_1, \xi_2) \propto \prod_{i=0}^{r} p_{i00}^{x_{i00}} p_{i10}^{x_{i10}} p_{i01}^{x_{i01}} p_{i11}^{x_{i11}}.$$

Letting, $\ell(a, \xi) = \log L(a, \xi_1, \xi_2)$,

$$\frac{\partial}{\partial \xi} \ell(a, \xi) \bigg|_{\xi = 0} = \begin{bmatrix} \sum_{i=0}^{r} d_{x_{i11}} - H(a) \sum_{i=0}^{r} n_i d_i \\ \sum_{i=0}^{r} d_{x_{i1.1}} - H(a) \sum_{i=0}^{r} n_i d_i \end{bmatrix},$$

where, $x_{i1.} = x_{i00} + x_{i11}$ and $x_{i.1} = x_{i01} + x_{i11}$, $i = 0, \ldots, r$.

Under $H_0$,

$$\ell(a) = \log L(a) \propto \sum_{i=0}^{r} (2x_{i00} + x_{i10} + x_{i01}) \log(1 - H(a))$$

$$+ 2 \sum_{i=0}^{r} (2x_{i11} + x_{i10} + x_{i01}) \log H(a).$$

When $H(a)$ is unknown, the maximum likelihood estimator of $H(a)$, obtained by solving $\frac{d}{dH(a)} \ell(a) = 0$, is

$$\hat{H}(a) = \frac{\sum_{i=0}^{r} (x_{i1.} + x_{i.1})}{2n} + (x_{.1} + x_{..1})/2n,$$

where, $n = \sum_{i=0}^{r} n_i$, $x_{i.1} = \sum_{i=0}^{r} x_{i1.}$ and $x_{..1} = \sum_{i=0}^{r} x_{i..1}$.

For testing $H_0$: $\xi = 0$ versus $H_1$: $\xi > 0$, we propose the test statistic given by
Throughout this paper \( \sum \) denotes the summation over the dose levels \( i = 0, \ldots, r, \) and \( \text{E}_H^0 \) and \( \text{Cov}_H^0, \) respectively, denote the mean vector and variance-covariance matrix under \( H_0. \) Note that \( \text{E}_H^0(T) = 0 \) and \( \text{Cov}_H^0(T) = nH(a)(1-H(a))V, \) where

\[
V = (\sum \lambda_i d_i^2) I_{2 \times 2} - \left( \frac{1}{2} \right) \left( \sum \lambda_i d_i^2 \right) \mathbf{1}' \mathbf{1}
\]

(2.1.2)

with \( \lambda_i = n_i/n, \) kept constant for each \( i, \) and \( \mathbf{1} = (1, 1)'. \) Here and throughout \( I_{2 \times 2} \) denote the \( 2 \times 2 \) identity matrix. Using standard results, the asymptotic null distribution of \( n^{-1/2} \sqrt{V^{-1}T/(\hat{H}(a))} \) is the bivariate normal (BVN), \( \text{BVN}(0, V), \) distribution. Thus the proposed test based on \( T \) rejects \( H_0 \) in favor of \( H_1 \) if

\[
S = n^{-1/2} \sqrt{V^{-1}T/(\hat{H}(a)(1-\hat{H}(a)))} > \chi^2_{2; \alpha}
\]

(2.1.3)

where \( \chi^2_{2; \alpha} \) denotes the upper \( \alpha \) percentile of the Chi-square distribution with 2 degrees of freedom.

We now consider the sequence of alternatives \( H_{1n}: \xi = n^{-1/2} \xi_0, \) where \( \xi_0 = (\xi_{01}, \xi_{02})' > 0, \) and in particular \( \xi_0 = \xi_0 1', \) with \( \xi_0 > 0. \) Using a Taylor series expansion (see Serfling(1980), Theorem B, p. 44), it can be shown that, under \( H_{1n}, \)

\[
p_{11} = H^2(a) + n^{-1/2} 1' \xi_0 d_1 H'(a) + O(n^{-1}),
\]

\[
p_{10} = H(a)(1-H(a)) - n^{-1/2} 1' \xi_0 d_1 H'(a) + O(n^{-1})
\]

\[
p_{01} = H(a)(1-H(a)) - n^{-1/2} 1' \xi_0 d_1 H'(a) + O(n^{-1}),
\]

and

\[
p_{00} = (1-H(a))^2 - n^{-1/2} 1' \xi_0 d_1 H'(a)(1-H(a)) + O(n^{-1}),
\]

where \( H' \) is the derivative of \( H. \)

After some matrix algebraic computations, we get

\[
\lim_{n \to \infty} \left[ \text{E}_{H_{1n}}(T) - \text{E}_{H_0}(T) \right] = H'(a)V_{\xi_0}.
\]
The proof of the following result is straightforward. (The notation \( \frac{d}{\xi} \) denotes the convergence in distribution.)

**Theorem 1.** Under \( H_{ln} \): \( \xi = n^{-1/2} \xi_0 \), \( n^{-1} T^T V^{-1} T/((H(a))(1-H(a)))^{\frac{d}{\xi}} \chi_2^0(\delta) \) as \( n \to \infty \), where \( \chi_2^0(\delta) \) denotes a noncentral Chi-square random variable with 2 degrees of freedom and the noncentrality parameter \( \delta \) given by

\[
\delta = (H(a)(1-H(a)))^{\frac{d}{\xi}} V \xi_0/(2H(a)(1-H(a))),
\]

where \( V \) is given by (2.1.2).

In case of \( \xi_{01} = \xi_{02} = \xi_0 > 0 \), \( \delta \) reduces to

\[
\delta = \xi_0^2 H(a)(1-H(a)) \left[ \sum \lambda_1^2 \right] - \left( \sum \lambda_1^2 \right)^2.
\]

### 2.2 Tarone Type Test.

The test in (2.1.1) is based only on the current experimental data and completely ignores the historical information. To formally utilize this information in the statistical analysis of the current data, we adopt the approach of Tarone (1982), and assume that \( H(a) \), the probability of spontaneous occurrence of Type I (or Type II) tumor, varies across the experiments according to a beta distribution, Beta \((\alpha, \beta)\), with parameters \( \alpha \) and \( \beta \). Then, the test statistic based on maximum likelihood function, for known \( \alpha \) and \( \beta \), is given by

\[
T_t = \frac{d}{\xi} \int_0^1 L(a, \xi_1, \xi_2) \left( \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} (H(a))^{\alpha-1}(1-H(a))^{\beta-1} \right) dH(a) | \xi = 0
\]

\[
= \sum B_1 x_i - (\alpha/(\alpha+\beta+2n))1,
\]

where \( B_1 \) is the 2 x 4 matrix given by

\[
B_1 = \begin{bmatrix}
    d_1 - \rho_n n^{-1} \sum n_i d_i & d_1 - \rho_n (2n)^{-1} \sum n_i d_i & -\rho_n (2n)^{-1} \sum n_i d_i & 0 \\
    d_1 - \rho_n n^{-1} \sum n_i d_i & -\rho_n (2n)^{-1} \sum n_i d_i & d_1 - \rho_n (2n)^{-1} \sum n_i d_i & 0
\end{bmatrix}.
\]
and \( \rho_n = 2n/((\alpha + \beta + 2)\). 

Computations lead to \( E_{H_0}(T_t) = 0 \) and \( \text{Cov}_{H_0}(T_t) = \frac{n\alpha \beta}{(\alpha + \beta + 1)^2} V(\rho_n) \), where

\[
V(\rho_n) = (\sum \lambda_i d_i^2) I_{2 \times 2} - (\rho_n/2) \left( \sum \lambda_i d_i \right)^2 \mathbb{I},
\]

and \( \lambda_i = n_i / n \), kept constant for each \( i \). Note that \( V(\rho_n) \) is positive definite and symmetric and hence it can be factored as \( W(\rho_n) W'(\rho_n) \), where \( W(\rho_n) \) is a nonsingular lower triangular matrix. Thus the change of variables from \( T_t \) to \( n^{-1/2} W^{-1}(\rho_n) T_t \) is permissible. In the following we first study the asymptotic distribution of \( n^{-1/2} W^{-1}(\rho_n) T_t \) assuming \( (\alpha + \beta) \) fixed. Since

\[
E(H(a) \mid x_0) = \omega(\alpha/(\alpha + \beta)) + (1 - \omega)((x_{01} \ldots x_{0r})/2n_0),
\]

where \( \omega = (\alpha + \beta)/(\alpha + \beta + 2n_0) \) is the relative weight of historical information relative to the concurrent control data, we will also consider the case of \( \omega = \text{constant} \) as \( n \to \infty \); that is the case of \( (\alpha + \beta) \to \infty \) as \( n \to \infty \).

**Theorem 2.** Under \( H_0 \), for fixed \( (\alpha + \beta) \), the limiting distribution of \( S_t = (n\alpha \beta)^{-1} (\alpha + \beta)(\alpha + \beta + 1) T_t V^{-1}(\rho_n) T_t \) is given by

\[
\lim_{n \to \infty} P(S_t \leq x) = EP\{\tau_n X_2^2 \leq x\},
\]

where \( \tau_n = H(a)(1 - H(a))(\alpha + \beta)(\alpha + \beta + 1) \), \( \chi_2^2 = \chi_2(0) \), \( V(\rho_n) \) is as defined in (2.2.2), and the expectation is with respect to the marginal distribution of \( H(a) \).

**Proof.** For a given \( H(a) \), the random vectors \( x_0, x_1, \ldots, x_r \) are independent and \( x_i \) has multinomial distribution \( (i = 0, 1, \ldots, r) \). Hence, the conditional mean and variance-covariance matrix of \( T_t \), under \( H_0 \), are given by

\[
E_{H_0}(T_t \mid H(a)) = \frac{(H(a)(\alpha + \beta) - \alpha)}{(\alpha + \beta + 2n)} (\sum n_i d_i) \mathbb{I}
\]

\[
\text{Cov}_{H_0}(T_t \mid H(a)) = n H(a)(1 - H(a)) V_*(\rho_n),
\]
where
\[ V_*(\rho_n) = (\sum \lambda_i d_i^2)I_{2 \times 2} - (1/2)\rho_n(2 - \rho_n)(\sum \lambda_i d_i)^2 I_n. \]

Using standard results, the conditional distribution of \( T_t \), given \( H(a) \), converges to a BVN distribution as \( n \to \infty \). Hence, under \( H_0 \), for any vector \( \xi = (\xi_1, \xi_2)' \),
\[ \mathbb{E}_{H_0}(\xi' T_t | H(a)) = \frac{\xi' T_t - \mathbb{E}_{H_0}(\xi' T_t | H(a))}{\sqrt{V_{H_0}(\xi' T_t | H(a))}} \xrightarrow{d} Z, \quad \text{as } n \to \infty, \]
where \( Z \) a standard normal random variable,
\[ \mathbb{E}_{H_0}(\xi' T_t | H(a)) = \left[ \frac{(H(a)(\alpha + \beta) - \alpha)}{\alpha + \beta + 2n} \sum n_i d_i \right] \xi' \]
and
\[ V_{H_0}(\xi' T_t | H(a)) = nH(a)(1 - H(a))\xi' V_*(\rho_n) \xi \]
are the conditional mean and variance of \( \xi' T_t \), given \( H(a) \), respectively. Define,
\[ C_n = \frac{\alpha \beta}{(\alpha + \beta)(\alpha + \beta + 1)} \xi' V(\rho_n) \xi, \]
\[ R_n = \sqrt{n} (1 - \rho_n)(H(a) - \frac{\alpha}{\alpha + \beta})(\sum \lambda_i d_i)(\xi') C_n^{-1/2}, \]
and \( A_n = H(a)(1 - H(a))C_n^{-1}(\xi' V_*(\rho_n) \xi), \) where \( V(\rho_n) \) is as defined in (2.2.2). Since \( \sqrt{n}(1 - \rho_n) \to 0, R_n \to 0 \) in probability. Also, \( A_n \to r_a \) in probability. Thus, using Slutsky’s theorem,
\[ \lim_{n \to \infty} P((nC_n)^{-1/2} \xi' T_t \leq x) = \mathbb{E} \left\{ \lim_{n \to \infty} P(U_n \leq (x - R_n)/\sqrt{A_n} | H(a)) \right\} = \mathbb{E}(P(Z \leq x/\sqrt{r_a})), \]
where the expectation is with respect to (w.r.t.) the marginal distribution of \( H(a) \) and \( \Phi \) denotes the cumulative distribution function of a standard normal random variable.

Thus, the unconditional asymptotic null distribution of any standardized linear combination of \( T_t \) is a mixture of normal distributions. Hence, under \( H_0 \),
\[ (r_{an})^{-1/2} W^{-1}(\rho_n) T_t | H(a) \xrightarrow{d} \text{BVN}(0, I_{2 \times 2}). \]
ans \( n \to \infty \), and therefore,

\[
\lim_{n \to \infty} \mathbb{P}(S_t \leq x) = \mathbb{E}\{ \lim_{n \to \infty} \mathbb{P}(S_t \leq x|H(a)) \}
\]

\[
= \mathbb{E}\{ \mathbb{P}(\chi^2_2 \leq x/r_n) \}
\]

Remark 1. Note that the limiting distribution of \( S_t \) given in Theorem 2 is a mixture of rescaled chi-square distributions each with two degrees of freedom, which in practice is well approximated by a Chi-square distribution with 2 degrees of freedom. [See Krewski et. al. (1985) for an analogous argument.] The approximation is quite good when \( \alpha, \beta \) are fairly large and it is used in Section 3 for analyzing \( ED_{01} \) data.

Theorem 3. If \( \rho_n \to 0, 1, \) or \( \rho, \) with \( 0 < \rho < 1, \) as \( n \to \infty \) and \( \alpha/(\alpha + \beta) \to 0, \) as \( (\alpha + \beta) \to \infty, \) then under \( H_0, \)

\[
S_t = (na^2)^{-1}(\alpha + \beta)(\alpha + \beta + 1)T_t^{-1}(\rho_n)T_t^{-1}d \chi^2_2 \text{ as } n \to \infty.
\]

Proof. It is immediate to see that \( H(a) \to 0 \) in probability, and hence \( r_n \) defined in the proof of Theorem 2 converges to 1 in probability and \( U_n \) converges, as \( n \to \infty \) to \( Z \) unconditionally. Now using arguments similar to those of Hoel and Yanagawa (1986) and Theorem 2 above, it can be shown that the unconditional asymptotic null distribution of any standardized linear combination of \( T_t \) is standard normal (and not a mixture of standard normals). Thus, under \( H_0 \) and the conditions in the statement of this theorem,

\[
n^{-1/2}W^{-1}(\rho_n)T_t^{-d}BVN(0, I_{2 \times 2}) \text{ as } n \to \infty,
\]

and the theorem follows. □

Theorem 4. Under \( H_{1n}, \xi = n^{-1/2}\xi_0 \) and the conditions of Theorem 3,

\[
S_t = (na^2)^{-1}(\alpha + \beta)(\alpha + \beta + 1)T_t^{-1}(\rho_n)T_t^{-1}d \chi^2_2(\theta) \text{ as } n \to \infty,
\]
where

\[ \delta(\theta) = \theta(1-\theta) \xi_0 V(\rho) \xi_0, \]  

(2.2.3)

and \( V(\rho) \) is given by (2.2.2) with \( \rho_n \) replaced by \( \rho \).

**Proof.** Proceeding as in Theorem 1 and using the Taylor series expansion for \( p_t \) under \( H_{1n} \), we get

\[ \lim_{n \to \infty} \left[ E_{H_{1n}} (W^{-1}(\rho_n) T_t \mid H(a)) - E_{H_0} (W^{-1}(\rho_n) T_t \mid H(a)) \right] = H(a)(1 - H(a)) W^{-1}(\rho)V(\rho)\xi_0, \]

for the logistic model in (2.1), where \( W(\rho) \) is such that \( V(\rho) = W(\rho) W'(\rho) \). Since \( H(a)(1 - H(a)) \to \theta(1 - \theta) \) in probability as \( (\alpha + \beta) \to \infty \) and \( H(a) \) is bounded, it follows that \( E(H(a)(1 - H(a))) \to \theta(1 - \theta) \) as \( (\alpha + \beta) \to \infty \). Hence,

\[ \left[ E_{H_{1n}} (W^{-1}(\rho_n) T_t) - E_{H_0} (W^{-1}(\rho_n) T_t) \right] \to \theta(1 - \theta) W^{-1}(\rho)V(\rho)\xi_0 = \varphi(\theta) \] say,

as \( n \to \infty \) and \( (\alpha + \beta) \to \infty \). Now, as in the proof of Theorem 2, it can be shown that, under \( H_{1n} \), \( n^{-1/2} W^{-1}(\rho_n) T_t \overset{d}{=} Z \), where \( Z = (Z_1, Z_2)' \) has the BVN(\( \varphi(\theta), I_{2 \times 2} \)) distribution. The result follows since the noncentrality parameter of \( S_t \) is given by

\[ \delta(\theta) = (1/2)\varphi'(\theta) I_{2 \times 2} \varphi(\theta). \]

In case of \( \xi_{01} = \xi_{02} = \xi_0 > 0 \), \( \delta(\theta) \) in (2.2.3) reduces to

\[ \delta(\theta) = \xi_0^2 \theta(1-\theta) \left[ \sum \lambda_i d_i^2 - \rho(\sum \lambda_i d_i)^2 \right]. \]  

(2.2.4)

**Remark 2.** The gain due to the incorporation of historical controls is measured by comparing the Tarone type \( S_t \) test with the Cochran-Armitage type \( S \) test (developed in Section 2.1) through Pitman’s asymptotic relative efficiency. Suppose \( (\alpha + \beta) \to \infty \) as \( n \to \infty \) and consider the \( S \) test in the framework of Section 2.2. Then from Theorems 1 and 4, the gain obtained by the incorporation of historical controls is given by
\[ e(S_t, S) = \frac{\delta(\theta)}{\delta} \]

with \( \delta \) and \( \delta(\theta) \) given by (2.1.4) and (2.2.3), respectively. In case of \( \xi_0^1 = \xi_0^2 = \xi_0 > 0 \), from (2.1.5) and (2.2.4),

\[ e(S_t, S) = \frac{[\sum \lambda_i d_i^2 - \rho(\sum \lambda_i d_i)^2]}{[\sum \lambda_i d_i^2 - \sum \lambda_i d_i^2]} \]

Thus,

\[ 1 \leq e(S_t, S) \leq \frac{\sum \lambda_i d_i^2}{\sum \lambda_i d_i^2} \]

with lower and upper bounds obtained at \( \rho = 1 \) and \( \rho = 0 \), respectively. As expected the expression for \( e(S_t, S) \) in (2.2.5) is same as the one obtained by Hoel and Yanagawa(1986) in the context of one type of tumor. Hence, their observations also hold in the present context of two tumor types, the important one being that the efficiency improves for small values of \( \theta \) and, therefore, a good gain in power, by the incorporation of historical controls, is anticipated for rare tumors.

2.3 Hoel and Yanagawa Type Conditional Test.

In this section, following the development of Hoel and Yanagawa(1986), we obtain a locally most powerful test conditional on the response vector \( x_0 \) for the control group. This generalized test is related to the Tarone type test \( S_t \). Consider the setup of Section 2.2, where, given \( H(a), x_i, i = 0, 1, \ldots, r \) are independent multinomial random vectors and \( H(a) \) has a Beta(\( \alpha, \beta \)) distribution. The marginal distribution of \( x_0 \) is independent of \( \xi \) and is given by

\[ f(x_0) = \frac{n_0!}{x_00!x_{01}!x_{01}!x_{01}!} \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} \frac{\Gamma(x_{01} + x_{01} + \alpha)\Gamma(2n_0 - (x_{01} + x_{01}) + \beta)}{\Gamma(2n_0 + \alpha + \beta)} \]

Hence, \( x_0 \) is an ancillary statistic and one can develop a test statistic based on the conditional pdf of \( x \) given \( x_0 \) (see Rao (1973), p. 505). The locally most powerful test
Statistic for $H_0: \xi = 0$ versus $H_1: \xi > 0$ is given by

$$T_t^C = \frac{\partial}{\partial \xi} \log f_\xi(x | x_0) \big| \xi = 0,$$

where $f_\xi(x | x_0)$ is the conditional density function of $x = (x'_0, \ldots, x'_t)'$, given $x_0$. For the logistic response model in (2.1), $T_t^C$ simplifies to $T_t$ of Section 2.2 given by (2.2.1).

Under $H_0$, the conditional mean vector and conditional variance-covariance matrix of $T_t^C$, given $x_0$, are obtained as

$$E_{H_0}(T_t^C | x_0) = \mathbf{0},$$

$$\text{Cov}_{H_0}(T_t^C | x_0) = \frac{n \mu (1 - \mu) (\alpha + \beta + 2n_0)}{\alpha + \beta + 2n_0 + 1} V(\rho_n),$$

where $\mu = \frac{\alpha + x_{01} + x_{01}'}{\alpha + \beta + 2n_0}$ and $V(\rho_n)$ is as defined in (2.2.2). For large $\alpha + \beta + 2n_0$, $(\alpha + \beta + 2n_0) / (\alpha + \beta + 2n_0 + 1) \approx 1$ and thus the statistic defined by $n^{-1}T_t^C V^{-1}(\rho_n)T_t^C / (\mu(1 - \mu))$ is equivalent to

$$S_t^C = n^{-1}T_t^C V^{-1}(\rho_n)T_t^C \left[ \frac{(\alpha + x_{1.} + x_{1.}')(\beta + 2n - x_{1.} - x_{1.})}{(\alpha + \beta + 2n)^2} \right]$$

if $x_{01.}, x_{01.}'$ and $n_0$ are replaced by $x_{1.}, x_{1.}'$ and $n$, respectively.

**Theorem 5.** Under $H_0$, either for fixed $(\alpha + \beta)$ or for $\alpha / (\alpha + \beta) \rightarrow 0$ as $(\alpha + \beta) \rightarrow \infty$, with $\rho_n \rightarrow 0$, 1 or $\rho, 0 < \rho < 1$, as $n \rightarrow \infty$, $S_t^C \rightarrow \chi_2^2$ as $n \rightarrow \infty$.

**Proof.** Consider the case of fixed $(\alpha + \beta)$, and define,

$$C_n^C = \frac{(\alpha + x_{1.} + x_{1.}')(\beta + 2n - x_{1.} - x_{1.})}{(\alpha + \beta + 2n)^2} \xi' V(\rho_n) \xi,$$

for any vector $\xi = (\xi_1, \xi_2)'$, with $V(\rho_n)$ as in (2.2.2). Now, define $U_n, A_n$ and $R_n$ as in the proof of Theorem 2 with $C_n$ replaced by $C_n^C$, and write $(nC_n^C)^{1/2} \xi' T_t = U_n \sqrt{A_n} + R_n$. Then using the arguments similar to those of Theorem 2,
R_n → 0 in probability. Further, since \((\alpha + x_1 + x_2 + \cdots + x_n)/(\alpha + \beta + 2n)\) → H(a) in probability, we have that A_n → 1 in probability. Thus,
\[
\lim_{n \to \infty} P\left(\left(\frac{nC_n^0}{n}\right)^{1/2} T_n \leq x\right) = E\{\lim_{n \to \infty} P(U_n \leq (x - R_n)/\sqrt{A_n} \mid H(a))\} = \Phi(x),
\]
and the theorem follows.

For the cases where \(\alpha/(\alpha + \beta) \to 0\) as \(\alpha + \beta \to \infty\), when \(\rho_n \to 0\), 1 or \(0 < \rho < 1\) as \(n \to \infty\), it is clear that H(a) → 0 and \((\alpha + x_1 + x_2 + \cdots + x_n)/(\alpha + \beta + 2n) \to 0\) in probability. Now, using the arguments similar to those of Theorem 3, the proof can be completed. □

Also, since Theorem 4 holds for \(S^C_t\), it is equivalent to the Tarone Type test \(S^C_t\) in terms of Pitman’s asymptotic relative efficiency. In the applications of \(S_t^C\) and \(S^C_t\), the parameters \(\alpha\) and \(\beta\) are replaced by their consistent estimates obtained by using control groups in the historical data. The illustrations are given in the next section.

3. EXAMPLES

In 1974, the National Center for Toxicological Research initiated a large scale experiment called \(ED_{01}\) study using 23,429 female BALB/C mice exposed to one of the eight dose levels 0, 30, 35, 45, 60, 75, 100 and 150 ppm of a test substance 2-acetylaminofluorene (2-AAF). The animals were housed in six rooms and were at the risk of developing tumors in any of the 15 sites. A general description of the study can be found in Cairns(1980) and Lu(1990). Even though 34 types of tumors in 15 sites were reported, for achieving independence, we will analyze only the tumor sites making no differentiation between different types at the same site. Throughout this section, we use the term “tumor type” to refer to tumors at a particular “site”. Since a very different scheme was used to observe responses at dose levels 30, 35 and 45 ppm, we exclude them from our analysis. Lu(1990) obtained estimated correlation matrices for
marginal tumor occurrence rates under the dose levels 0, 60, 75, 100 and 150 ppm and showed that most types of tumors in ED_{01} data are almost independent. For an illustration of the test procedures derived in Section 2, we consider only parts of ED_{01} data consisting of dose levels 0, 60, 75, 100 and 150 ppm and moderate (Example 1) and rare (Example 2) tumors. The ED_{01} data on the tumors considered satisfy the assumptions of independence and of the same probability (= H(a)) of spontaneous tumor occurrence. The data are reported in Tables 1 and 2.

The historical data in Table 1 have been generated, for the purpose of illustration, by first generating H(a) from Beta (6, 34) distribution suitable for moderate tumors (see Tamura and Young(1986)) and then generating an observation from the multinomial distribution with parameters n = 1500 and p = ((1 - H(a))^2, H(a)(1 - H(a)), H(a)(1 - H(a)), (H(a))^2)^T.

Table 1. Example 1. Occurrence of Harderian gland and Ovary tumors in mice.

<table>
<thead>
<tr>
<th>Historical controls (computer generated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n_i = 1500, j = 1, 2, ... , 30.</td>
</tr>
<tr>
<td>x_01 :</td>
</tr>
<tr>
<td>197  231  226  279  374  293  65  153  168  385  223</td>
</tr>
<tr>
<td>212  198  226  157  318  460  94  229  182  205  168</td>
</tr>
<tr>
<td>320  174  135  217  161  166  170  161</td>
</tr>
<tr>
<td>x_02 :</td>
</tr>
<tr>
<td>189  212  210  285  424  248  30  140  151  354  231</td>
</tr>
<tr>
<td>213  216  203  183  329  501  103  305  194  174  176</td>
</tr>
<tr>
<td>314  178  154  196  148  138  168  163</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Current experiment (from ED_{01} data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dose (ppm) : 0  60  75  100  150</td>
</tr>
<tr>
<td>n_i : 2397  2846  1983  1276  1282</td>
</tr>
<tr>
<td>Type I Harderian gland percent : 10.35%  10.22%  10.74%  9.4%  11.93%</td>
</tr>
<tr>
<td>Type II Ovary percent : 15.69%  14.76%  16.44%  14.5%  14.12%</td>
</tr>
</tbody>
</table>
Table 2. Example 2. Occurrence of Breast and Pancreas tumors in mice.

### Historical controls (computer generated)

<table>
<thead>
<tr>
<th>dose (ppm)</th>
<th>0</th>
<th>60</th>
<th>75</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>n_j</td>
<td>2397</td>
<td>2846</td>
<td>1983</td>
<td>1276</td>
<td>1282</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type I</th>
<th>Breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>x_{1,2}</td>
<td>15</td>
</tr>
<tr>
<td>percent</td>
<td>0.63%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type II</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>x_{1,4}</td>
<td>13</td>
</tr>
<tr>
<td>percent</td>
<td>0.54%</td>
</tr>
</tbody>
</table>

The current experimental data have been analyzed previously (see Lu(1990)) using three different test procedures. Two procedures called HW test (Hoel and Walburg (1972)) and log-rank (LR) test (Peto(1974)) are based on, respectively, the assumptions of non-lethality and rapid lethality of tumors. The third procedure called MV test (Malani and VanRyzin(1988)) requires serial sacrifice of animals during the experiment. All the three methods are suitable for the analysis of one type of tumor at a time and need Bonferroni adjustment for multiplicity of tests in the presence of more than one tumor. For both harderian gland and ovary (after the adjustment), the LR test picks up a trend but the HW and the MV tests fail to give significant results.

Example 2 in Table 2 deals with breast and pancreas tumors which are rare tumors (for ED_{01} data). The historical data have been generated using Beta(1.5, 200) distribution which has 50th percentile of 0.0059 and 95th percentile of 0.019. For the
current experimental data in Table 2, all the three HG, LR and MV tests showed that there is no significant positive trend in the occurrences of breast as well as pancreas tumors.

In Table 3, we report the p-values of the Cochran-Armitage type S test, the Tarone type $S_t$ test and the Hoel and Yanagawa type $S_t^C$ test developed in this paper for Examples 1 and 2. The calculations for $S_t$ and $S_t^C$ are based on the moment estimators of $\alpha$ and $\beta$, obtained by using the $M$ historical control groups through the reparameterization as $k = \alpha + \beta$ and $\eta = \alpha/\beta$. Assume that $(x_0^1, p_0^1), \ldots, (x_M^1, p_M^1)$ are independent random vectors such that conditional on $p_0^1$, $x_0^1$ has multinomial distribution with parameters $n_0^1$ and $p_0^1 = ((1-p_j)^2, p_j(1-p_j), p_j^2)$, and $p_j = H_j(a)$ are i.i.d. according to Beta $k(1-\eta)$. Then the moment estimator of $\eta$ is given by

$$\hat{\eta} = \frac{\sum_{j=1}^M \hat{p}_j/M}{\sum_{j=1}^M 1/n_0^j}, \quad (3.1)$$

where

$$\hat{p}_j = (x_{01}^j + x_{M1}^j)/2n_0^j, \quad (3.2)$$

and the moment estimator of $k$ is given by

$$\hat{k} = \{\hat{\eta}(1-\hat{\eta})[1-\frac{1}{2M} \sum_{j=1}^M \frac{1}{n_0^j}] (\frac{s^2}{2M} - \sum_{j=1}^M \frac{1}{n_0^j}) \hat{\eta}(1-\hat{\eta})^{-1}\} - 1 \quad (3.3)$$

where $s^2$ is the variance of $\hat{p}_j$, $j = 1, \ldots, M$ given by $s^2 = \frac{1}{M} \sum_{j=1}^M (\hat{p}_j^2 - \frac{1}{M} \sum_{j=1}^M \hat{p}_j)^2$, with $\hat{p}_j$ as defined in (3.2). The estimates, (3.1) and (3.3), for the data in Tables 1 and 2 are also reported in Table 3.
Table 3. Test for a trend in Examples 1 and 2.

<table>
<thead>
<tr>
<th>Example</th>
<th>Test Statistic</th>
<th>Moment Estimates</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>–</td>
<td>&lt; 0.001 (51.42)</td>
</tr>
<tr>
<td>1</td>
<td>S_t</td>
<td>34.93</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>S_t^c</td>
<td>34.93</td>
<td>0.148</td>
</tr>
<tr>
<td>2</td>
<td>S</td>
<td>–</td>
<td>0.94 (0.13)</td>
</tr>
<tr>
<td></td>
<td>S_t</td>
<td>293.93</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>S_t^c</td>
<td>293.93</td>
<td>0.007</td>
</tr>
</tbody>
</table>

The numbers in brackets in the last column are the values of the corresponding test statistics. In both Examples 1 and 2 all the three tests give similar results. The moment estimates of \( \eta \) in both examples are reasonable, whereas as the moment estimates of \( k \) appear to be biased, resulting in over estimation of \( k \) in case of rare tumors (Example 2) and underestimation of \( k \) in case of moderate tumors. However, these do not seem to have an effect on the test statistics \( S_t \) and \( S_t^c \). This is due to the fact that the large concurrent experiment size (\( n = 9784 \)) dominates the contribution of historical controls in both the examples. This is also evident from the efficiency \( e(S_t, S) \) of \( S_t \) compared to \( S \). For moderate tumors (Table 1), \( e(S_t, S) \) takes value 1.00, whereas as for rare tumors (Table 2), \( e(S_t, S) \) is 1.03. Thus for ED01 data the gain obtained by incorporating historical controls is marginal.
4. DISCUSSION

All the methods derived in this paper can be extended to the case of more than two tumor types in a straightforward manner. However, as the number of tumor types under consideration increases, it becomes more difficult to work within the framework of (1) independence between tumors, and (2) equal probability of spontaneous occurrence for all the tumors. Within the setup of independent tumors, assumption (2) can be relaxed by modelling the response probabilities through different logistic models \( H(a_1) \) and \( H(a_2) \). However, in that case, the two sample test procedure developed here is equivalent to the one sample test procedure for each of the tumors.

Note that, the \( S_t \) and \( S^C_t \) tests developed in Section 2.2 and 2.3 are based on the unstated assumption that the historical controls used in the analysis come from the experiments which are similar to the current experiment in factors known to affect the values of spontaneous tumor occurrence rates. Such factors include animal body weight, housing conditions, age of the animals, type of food and pathologists. Hence by preliminary investigations, the appropriateness of the historical data for inclusion in the analysis of a particular experiment should be determined. If such investigations are not feasible or arrive at a conclusion that the historical data are subject to uncontrolled variability due factors such as age, body weight and pathologists, the approach of Zalkikar and Tiwari (1992) may be followed to appropriately model the variation among the historical controls, and to develop the test procedures based on mixtures of beta distributions in the presence of several independent tumors.

It is much more difficult to model dependency between different tumor types and derive trend tests. One approach may be to use a bivariate logistic distribution introduced by Gumbel (1961). Let

\[
p_{111} = H(a + \xi_1d_1)H(a + \xi_2d_1)\left[1 + \lambda H(a + \xi_1d_1)H(a + \xi_2d_1)\right]
\]
\[ p_{i10} = H(a + \varepsilon_1 d_i) - p_{i11}, \quad p_{i01} = H(a + \varepsilon_2 d_i) - p_{i11}, \quad p_{i00} = 1 - p_{i10} - p_{i01} - p_{i11}, \]

where \( H = 1 - H \) and \( H \) is as defined in (2.1). Note that, under \( H_0: \xi = 0 \), \( P(\text{Type II tumor occurs} \mid \text{Type I tumor has developed}) = H(a)(1 + \lambda H^2(a)) > P(\text{Type II tumor occurs}), \) if \( \lambda > 0 \) and \( < P(\text{Type II tumor occurs}), \) if \( \lambda < 0 \).

Thus depending on whether \( \lambda \) is positive or negative the probability of occurrence of one type of tumor increases or decreases when the other type of tumor has already occurred.

Under this set up, one can obtain the test statistic \( T(\lambda) = \frac{\partial}{\partial \xi} \log L(a, \xi_1, \xi_2) \big| \xi = 0 \) and calculate the mean and variance-covariance matrix of \( T(\lambda) \) and obtain the standardized test statistic for testing \( H_0: \xi = 0 \) versus \( H_1: \xi > 0 \). This approach is not explored here and is a topic of further investigation.

**REFERENCES**


