

Attribution of Tumor Lethality and Estimation of Time to Onset of Occult Tumors in the Absence of Cause-of-Death Information

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Abstract

A new statistical approach is developed for estimating the carcinogenic potential of drugs and other chemical substances used by humans. Improved statistical methods are developed for rodent tumorigenicity assays that have interval sacrifices but not cause-of-death data. For such experiments, this paper proposes a nonparametric maximum likelihood estimation method for estimating the distributions of time-to-onset-of and the time-to-death-from the tumor. The log-likelihood function is optimized using a constrained direct-search procedure. Using the maximum likelihood estimators, the number of fatal tumors in an experiment can be imputed. By applying the proposed procedure to a real data set, the effect of calorie restriction is investigated. In this study, we found calorie restriction delays the tumor-onset time significantly for pituitary tumors. The present method can result in substantial economic savings by relieving the need for case-by-case assignment of cause of death or context of observation by pathologists. The ultimate goal of the proposed method is to use the imputed number of fatal tumors to modify Peto's IARC test for application to tumorigenicity assays that lack cause-of-death data.

Keywords: Bioassay; Competing risk; Incidental tumor; Interval sacrifice; Likelihood

1 Introduction

In an animal carcinogenicity study, information about onset of a specific disease is confounded with information about the effect of the presence or absence of all diseases on mortality. In order to avoid biases due to differences in intercurrent mortality, Peto (1974) and Peto *et al.* (1980) proposed a method for analyzing tumor data in which tumors are observed in both the fatal and incidental contexts. Tumors that do not alter an animal's risk of death and are observed only as the result of a death from an unrelated cause are classified as incidental, whereas tumors that affect mortality by either directly causing death or indirectly increasing the risk of death from other causes are classified as fatal. The analysis of data on occult tumors using cause of death (context of observation) is performed separately for nonlethal (incidental) tumors and rapidly lethal tumors. This widely used statistical test (Peto *et al.*, 1980) is recommended by the International Agency for Research on Cancer (IARC).

The Peto method assumes that pathologists can determine if a tumor affected an animal's risk of death. In practice, however, pathologists often claim that accurate determinations of the cause of death are impossible, and classification errors can produce biases (Lagakos, 1982; Racine-Poon and Hoel, 1984; Lagakos and Louis, 1988). In many studies, cause of death is not assigned at all. The purpose of this paper is to propose a statistical estimation approach for rodent tumorigenicity assays that have interval sacrifices but not cause-of-death or context-of-observation data. This approach is motivated by the calorie restriction data given later in this section. For experiments that lack cause-of-death data, a nonparametric log-likelihood function is formulated and it is optimized by implementing a constrained direct-search procedure named the Complex Method (Box, 1965). For animals that die with tumors, the tumor lethality is attributed through a lethality function defined in terms of time-to-tumor-onset and tumor-survival functions. A modified IARC cause-of-death test based on the imputed number of fatal tumors is proposed as a future study.

Data from the Project on Calorie Restriction (PCR) conducted at the National Center for Toxicological Research (NCTR) are used for illustration. The PCR study was conducted for investigation of the effects of calorie restriction on tumorigenicity and longevity in Fischer 344 rats (Thurman *et al.*, 1994). For each sex, the study included both *ad libitum* (AL) and calorie restricted (CR) groups of animals. The two tumors of primary interest in the study were mononuclear cell

leukemia (MCL) and pituitary adenoma/carcinoma (PIT). The study involved up to six scheduled sacrifices. A detailed description of interval sacrifices is provided in Section 2. The counts of sacrifices and deaths with cause of death assigned by pathologists in each interval for each sex on MCL and PIT are given in Table 1. These two tumor types have high spontaneous incidence in Fischer rats fed *ad libitum*. The cause-of-death information given by pathologists is provided in these data. However, in unusually long-term studies such as the present one, the assignment of cause-of-death in very old animals is more complicated than in studies of the usual two-year duration. The numbers of fatal tumors will be imputed using the method proposed in this paper and will be compared with the numbers assigned by the pathologists. By applying the proposed procedure to the PCR data, the effect of the calorie restriction will be investigated. The difference in the development of a certain disease between the calorie restriction groups and the *ad libitum* groups will be examined. Although the biological mechanisms of rats and humans are not identical, the results of this study can be used to predict the human dietary effect.

Our proposed method will enhance the statistical analysis and interpretation of rodent tumorigenicity studies in the following ways: First, distributions of time-to-onset-of and time-to-death-from specific tumors of interest can be estimated nonparametrically, to enable visual interpretation of the experimental results. Second, this procedure provides a check on assumptions about the lethality of specific tumors, by attributing the number of fatal tumors among the observed tumors in an experiment. Third, although cause of death is not available, this procedure allows the estimation of a lethality parameter that can be used to implement the test of Peto *et al.* (1980) via a modification similar to the one proposed by Lagakos and Louis (1988). The proposed procedure for attributing cause of death could eliminate the need for pathologists to assign cause of death or context of observation in animal tumorigenicity assays. This could result in substantial economic savings.

2 Estimation of Time to Tumor Onset

In animal carcinogenicity experiments, animals are divided into several groups by randomization. A typical carcinogenicity study involves a control and two to three dose groups of 50 or more animals, usually rats or mice. Typically, a chemical is administered at a constant daily dose rate for a major

Table 1: Counts for fitting the discrete model to data from the PCR Experiment for mononuclear cell leukemia (MCL) and pituitary adenoma/carcinoma (PIT); *ad libitum* (AL) and calorie restricted (CR) groups.

Group	j^a	MCL					PIT				
		death			sacrifice		death			sacrifice	
		with fatal ^b tumor	incid. ^c tumor	w/o tumor	with tumor	w/o tumor	with fatal tumor	incid. tumor	w/o tumor	with tumor	w/o tumor
Male	1	0	0	0	0	12	0	0	0	3	9
AL	2	4	0	8	0	12	3	1	5	6	4
	3	35	2	45	6	6	44	13	16	9	2
	4	36	2	17	5	4	12	19	15	9	0
Male	1	0	0	2	0	12	0	0	2	0	12
CR	2	2	0	6	0	12	3	0	4	2	10
	3	21	1	10	7	5	5	2	19	3	9
	4	50	0	21	6	6	14	11	40	7	5
	5	27	1	24	6	3	12	7	30	7	2
	6	3	0	0	0	0	1	0	2	0	0
Female	1	0	0	0	1	11	0	0	0	1	10
AL	2	2	0	5	0	12	2	0	5	3	7
	3	16	1	38	1	11	25	7	20	11	1
	4	36	10	39	7	5	56	12	14	11	1
	5	6	5	8	0	0	14	2	3	0	0
Female	1	0	0	4	0	12	0	0	4	0	12
CR	2	0	0	10	0	12	1	0	8	0	10
	3	8	0	16	4	8	7	2	13	4	8
	4	30	0	18	3	9	11	6	24	4	8
	5	26	5	23	5	7	22	9	20	5	6
	6	6	1	7	3	1	1	1	8	2	2

^aTime intervals 1-6 represent, respectively, 0-368, 369-555, 556-754, 755-919, 920-1096, 1097-1293 days.

^bNumber of natural deaths from the tumor assigned by pathologists.

^cNumber of incidental tumor deaths assigned by pathologists.

portion of the lifetime of the test animal, for example, for two years. Sometimes, scheduled interim sacrifices are performed during the experiment. At the end of the study, all surviving animals are sacrificed and subjected to necropsy.

For the present, assume a single group of animals in an animal carcinogenicity experiment. Let there be m appropriately spaced sacrifice intervals, with sacrifices at t_1, t_2, \dots, t_m , where t_m denotes the final or terminal sacrifice time. Divide the time scale into discrete intervals, with the j th interval given by $(t_{j-1}, t_j]$, $j = 1, \dots, m$, where $t_0 = 0$.

Define $S(t)$ as the survival function for the distribution of time to tumor onset, i.e., $S(t) = \Pr(T_1 > t)$, where T_1 is the random variable representing time to onset of the tumor of interest. Time to onset is defined as the time when an occult tumor first becomes large enough to be detected histologically. Define $P(t)$ as the survival function with respect to death caused by the tumor of interest, i.e., $P(t) = \Pr(T_D > t)$, where T_D represents the overall time to death from the tumor of interest. Define $Q(t)$ as the survival function with respect to death from competing risks, i.e., $Q(t) = \Pr(X_C > t)$, where X_C is the random variable representing time to death from a cause other than the tumor of interest. Assume that T_1 and T_D are independent of X_C .

For ease of exposition, it is helpful initially to formulate the contributions to the likelihood function in terms of $S(t)$, $P(t)$ and $Q(t)$, as if complete information on cause of death is available for the experimental animals. The probability that each of the d_j animals dying due to the tumor of interest in the j th interval is $\Pr(t_{j-1} < T_D \leq t_j) \Pr(X_C > T_D)$. Because only the interval data are available, $\Pr(X_C > T_D)$ can be expressed only in terms of P , Q and S at the ends of the intervals. Hence the likelihood contribution is given as $Q(t_{j-1})\{P(t_{j-1}) - P(t_j)\}$. The probability that each of the a_{1j} animals dying in the j th interval from unrelated causes but having the tumor of interest is $\Pr(T_D > X_C, T_1 \leq X_C) \Pr(t_{j-1} < X_C \leq t_j)$. Based on the interval data, the likelihood contribution is given as $\{Q(t_{j-1}) - Q(t_j)\}\{P(t_{j-1}) - S(t_j)\}$. The probability that each of the b_{1j} animals dying without the tumor of interest in the j th interval is $\Pr(T_1 > X_C) \Pr(t_{j-1} < X_C \leq t_j)$. Based on the interval data, the likelihood contribution is given as $S(t_{j-1})\{Q(t_{j-1}) - Q(t_j)\}$. For the sacrifice data, the probability that each of the a_{2j} animals sacrificed at t_j and having the tumor of interest is $\Pr(T_1 \leq t_j, T_D > t_j) \Pr(X_C > t_j)$, and it contributes $Q(t_j)\{P(t_j) - S(t_j)\}$ to the likelihood. The probability that each of the b_{2j} animals sacrificed and not having the tumor at t_j is $\Pr(T_1 > t_j) \Pr(X_C > t_j)$, and it contributes $S(t_j)Q(t_j)$. The number of live animals at t_j is

Table 2: Count of each event

Event	Count for interval j
Death from fatal tumor	d_j
Death with incidental tumor	a_{1j}
Death without tumor	b_{1j}
Sacrifice with tumor	a_{2j}
Sacrifice without tumor	b_{2j}

denoted as N_j . Table 2 summarizes the key events and the number of animals for each event.

Now assume that cause of death is not available. Then the values of b_{1j} , a_{2j} and b_{2j} are known, but not the values of d_j and a_{1j} (i.e., only the total, $d_j + a_{1j}$, is known). The likelihood contribution for an animal dying with tumor is $Q(t_{j-1})\{P(t_{j-1}) - P(t_j)\} + \{Q(t_{j-1}) - Q(t_j)\}\{P(t_{j-1}) - S(t_j)\}$. The multinomial likelihood function based on this mixture of fatal and incidental tumors is

$$L \propto \prod_{j=1}^m [Q(t_{j-1})\{P(t_{j-1}) - P(t_j)\} + \{Q(t_{j-1}) - Q(t_j)\}\{P(t_{j-1}) - S(t_j)\}]^{d_j + a_{1j}} \times [S(t_{j-1})\{Q(t_{j-1}) - Q(t_j)\}]^{b_{1j}} [Q(t_j)\{P(t_j) - S(t_j)\}]^{a_{2j}} [S(t_j)Q(t_j)]^{b_{2j}}. \quad (1)$$

To simplify the formula and make the computation easier, we introduce a reparameterization of the survival functions. For all j , let $\pi_j = S(t_j)/P(t_j)$, $p_j = P(t_j)/P(t_{j-1})$ and $q_j = Q(t_j)/Q(t_{j-1})$. Then the survival functions can be expressed as

$$P(t_j) = \prod_{k=1}^j p_k, \quad Q(t_j) = \prod_{k=1}^j q_k \quad \text{and} \quad S(t_j) = \pi_j P(t_j), \quad j = 1, \dots, m. \quad (2)$$

Since $T_1 < T_D$, $S(t_j)$ should not exceed $P(t_j)$, and thus $0 \leq \pi_j \leq 1$. Note that the monotonicity of $P(t)$ imposes a natural constraint, $0 \leq p_j \leq 1$. That is, $P(t_j) \leq P(t_{j-1})$ if and only if $0 \leq p_j \leq 1$. The monotonicity of $Q(t)$ implies another constraint, $0 \leq q_j \leq 1$. That is, $Q(t_j) \leq Q(t_{j-1})$ if and only if $0 \leq q_j \leq 1$. Since $\pi_j p_j = S(t_j)/P(t_{j-1})$ and $\pi_{j-1} = S(t_{j-1})/P(t_{j-1})$, the monotonicity of $S(t)$ implies $\pi_j p_j \leq \pi_{j-1}$. That is, $S(t_j) \leq S(t_{j-1})$ if and only if $\pi_j p_j \leq \pi_{j-1}$. Satisfying these constraints in the estimation process will insure that the estimates $\hat{P}(t)$, $\hat{Q}(t)$ and $\hat{S}(t)$ are monotone.

After the reparameterization, the log-likelihood function may now be written as

$$\begin{aligned}
l = \sum_{j=1}^m & [(N_{j-1} - N_j) \sum_{k=1}^{j-1} \log(p_k q_k) + (a_{2j} + b_{2j}) \log(p_j q_j) \\
& + (d_j + a_{1j}) \log\{(1 - p_j) + (1 - \pi_j p_j)(1 - q_j)\} \\
& + b_{1j} \log\{(1 - q_j)\pi_{j-1}\} + a_{2j} \log(1 - \pi_j) + b_{2j} \log \pi_j] + c, \tag{3}
\end{aligned}$$

where c is a constant. The maximum likelihood estimators of π_j , p_j and q_j can be obtained by maximizing (3) subject to

$$0 \leq \pi_j \leq 1, 0 \leq p_j \leq 1, 0 \leq q_j \leq 1 \text{ and } \pi_j p_j \leq \pi_{j-1} \text{ for } j = 1, \dots, m. \tag{4}$$

The direct-search algorithm (Complex Method) of Box (1965) is employed to perform the constrained maximization. The proposed method maintains these constraints without losing the statistical properties of maximum likelihood estimators. Given estimates $\hat{\pi}_j$, \hat{p}_j and \hat{q}_j of π_j , p_j and q_j , respectively, the survival functions $P(t)$, $Q(t)$ and $S(t)$ in (2) can be estimated by

$$\hat{P}(t) = \prod_{k=1}^j \hat{p}_k, \quad t_{j-1} < t \leq t_j, \quad j = 1, \dots, m, \tag{5}$$

$$\hat{Q}(t) = \prod_{k=1}^j \hat{q}_k, \quad t_{j-1} < t \leq t_j, \quad j = 1, \dots, m,$$

$$\hat{S}(t) = \hat{\pi}_j \hat{P}(t), \quad t_{j-1} < t \leq t_j, \quad j = 1, \dots, m. \tag{6}$$

Instead of maximising the likelihood given in this section, an EM type algorithm may be considered by separating the probabilities of fatal and incidental tumors in (1). Although the two approaches give identical estimates, the proposed approach is recommended due to a substantial reduction of the computing time.

3 Attribution of Tumor Lethality

A lethality parameter similar to that of Lagakos and Louis (1988) can be estimated for each time interval using the results of the previous section. Define λ_j for an animal naturally dying in the

j th interval by

$$\begin{aligned}
\lambda_j &= \Pr(\text{tumor causes death}|\text{death with tumor}) \\
&= \frac{Q(t_{j-1})\{P(t_{j-1}) - P(t_j)\}}{Q(t_{j-1})\{P(t_{j-1}) - P(t_j)\} + \{Q(t_{j-1}) - Q(t_j)\}\{P(t_{j-1}) - S(t_j)\}} \\
&= \frac{(1 - p_j)}{(1 - p_j) + (1 - \pi_j p_j)(1 - q_j)}, \quad j = 1, \dots, m.
\end{aligned} \tag{7}$$

Then the estimated value of λ_j , $\hat{\lambda}_j$, is calculated simply by substituting values of \hat{p}_j , \hat{q}_j and $\hat{\pi}_j$ into the above expression. The number of deaths from fatal tumor in the interval $(t_{j-1}, t_j]$ is estimated to be

$$\hat{d}_j = \hat{\lambda}_j(d_j + a_{1j}), \tag{8}$$

and the estimated number of nonfatal tumors out of the $d_j + a_{1j}$ tumors found in dead animals in the j th interval is

$$\hat{a}_{1j} = (1 - \hat{\lambda}_j)(d_j + a_{1j}). \tag{9}$$

A built-in function *constr* in a MATLAB optimization toolbox is user friendly for a constrained maximization/minimization problem using a sequential quadratic programming method. However, our problem involves estimation of a number of parameters (3 times the number of intervals) by maximizing a nonlinear likelihood function under inequality constraints consisting of a set of various explicit and implicit constraints (see (4)). In this problem, the result from *constr* heavily depends on the initial conditions. It does not always converge to the same point. Thus the answers are unreliable. However, the Complex Method guarantees a convergence to the global maximum (Box, 1965). It always gave the same answer in our real data analysis and simulations. This is the main reason for using the Complex Method originally introduced by Box. In this study, the Complex Method is efficiently implemented in C in terms of computational time and the size of memory for a broad range of users. The MATLAB function *constr* takes about 9 times more memory than the Complex Method in our example because *constr* is working inside the MATLAB package.

The estimation algorithm has been implemented in C. Computation for the simulation and data analysis were performed on SUN Ultra workstations. Computing time for a data set with 50 animals and 4 intervals took about 20 seconds on a SUN Ultra 60 workstation. The computer

program is obtainable from the authors.

4 Examples

4.1 Calorie Restriction Data

The proposed method is applied to the PCR data discussed in Section 1. Since scheduled sacrifice data were available at six-month intervals in this study, the proposed method can be used to estimate the number of fatal tumors.

Without using the cause-of-death information given by pathologists, the estimated quantities of interest such as $\hat{\pi}_j$, \hat{p}_j and \hat{q}_j were obtained using the proposed constrained nonparametric maximum likelihood estimation method. The estimated number of fatal tumors and the number of incidental tumors in each interval were obtained from (8) and (9) using the estimated values of λ_j in (7). These estimated quantities were used to estimate the distributions of time to onset of a tumor $\{1 - \hat{S}(t_j)\}$ and time to death from the tumor $\{1 - \hat{P}(t_j)\}$ as in (6) and (5), respectively. Table 3 shows the estimated quantities. The standard error estimates of $\hat{\pi}_j$, \hat{p}_j , \hat{q}_j are obtained from the information matrix based on the log-likelihood (3), and the standard error estimate of $\hat{\lambda}_j$ is calculated using the delta method applied to (7). The standard error estimate of \hat{d}_j is obtained from the relationship $\text{Var}(\hat{d}_j) = (d_j + a_{1j})^2 \text{Var}(\hat{\lambda}_j)$ from (8). Note that the standard error estimates are not available at the last interval for some groups. It is due to the lack of sacrifice in that interval. In this case, all the elements of the information matrix becomes 0. We occasionally encounter high standard error estimates, especially in later intervals such as the 6th interval of the female calorie restricted group. It might be due to a small number of animals in those intervals.

For both kinds of tumors, pathologists assigned most of the deaths with tumor as fatal. In contrast, the proposed method classified, on the average, approximately half of the deaths with tumor as fatal. From a Monte Carlo simulation study (Ahn *et al.*, 1999. See also <http://www.ams.sunysb.edu/papers/papers99.html>), our estimation of the number of deaths from the tumor was reasonably accurate for highly lethal and moderately lethal tumors. It implies a possible bias in the cause-of-death assignment by the pathologists. This result confirms the study by Kodell *et al.* (1995) indicating that pathologists were inclined to assign a single lesion as the probable cause of death for most dead/moribund animals. The cause of death assignment by the present

Table 3: Estimated quantities for the PCR data in Table 1. Mononuclear cell leukemia (MCL) and pituitary adenoma/carcinoma (PIT); *ad libitum* (AL) and calorie restricted (CR) groups. The values in the parentheses are standard error estimates.

Group	j^a	$\hat{\pi}_j$	\hat{p}_j	\hat{q}_j	$\hat{P}(t_j)$	$\hat{S}(t_j)$	$\hat{\lambda}_j$	\hat{d}_j^b	d_j^c	$d_j + a_{1j}$
MCL	1	1.000	1.000	1.000	1.000	1.000	* ^d	0.0	0	0
Male		(0.22)	(0.07)	(0.07)			(*)	(*)		
AL	2	1.000	0.977	0.954	0.977	0.977	0.956	3.8	4	4
		(0.13)	(0.07)	(0.02)			(0.24)	(0.95)		
	3	0.555	0.782	0.538	0.764	0.425	0.454	16.8	35	37
		(0.11)	(0.06)	(0.05)			(0.09)	(3.31)		
	4	0.308	0.277	0.199	0.211	0.065	0.497	18.9	36	38
		(0.14)	(0.09)	(0.06)			(0.04)	(1.33)		
MCL	1	1.000	1.000	0.991	1.000	1.000	*	0.0	0	0
Male		(0.24)	(0.07)	(0.01)			(*)	(*)		
CR	2	1.000	0.990	0.971	0.990	0.990	0.972	1.9	2	2
		(0.21)	(0.07)	(0.01)			(0.58)	(1.16)		
	3	0.705	0.900	0.912	0.891	0.628	0.756	16.6	21	22
		(0.09)	(0.03)	(0.03)			(0.10)	(2.23)		
	4	0.719	0.649	0.668	0.578	0.416	0.665	33.2	50	50
		(0.09)	(0.05)	(0.05)			(0.05)	(2.69)		
	5	0.194	0.462	0.232	0.267	0.052	0.435	12.2	27	28
		(0.11)	(0.12)	(0.06)			(0.06)	(1.64)		
	6	0.041	0.000	0.000	0.000	0.000	0.500	1.5	3	3
		(*)	(*)	(*)			(*)	(*)		
MCL	1	0.984	1.000	1.000	1.000	0.984	*	0.0	0	0
Female		(0.02)	(0.07)	(0.07)			(*)	(*)		
AL	2	0.997	0.987	0.975	0.987	0.984	0.971	1.9	2	2
		(0.14)	(0.01)	(0.01)			(0.26)	(0.53)		
	3	0.962	0.889	0.752	0.878	0.844	0.756	12.8	16	17
		(0.04)	(0.03)	(0.03)			(0.06)	(0.94)		
	4	0.453	0.516	0.331	0.453	0.205	0.486	22.3	36	46
		(0.11)	(0.08)	(0.05)			(0.05)	(2.16)		
	5	0.066	0.000	0.000	0.000	0.000	0.500	5.5	6	11
		(*)	(*)	(0.35)			(0.09)	(0.97)		
MCL	1	1.000	1.000	0.982	1.000	1.000	*	0.0	0	0
Female		(0.21)	(0.07)	(0.07)			(*)	(*)		
CR	2	1.000	1.000	0.950	1.000	1.000	*	0.0	0	0
		(0.19)	(0.07)	(0.02)			(*)	(*)		
	3	0.769	0.974	0.885	0.974	0.750	0.472	3.8	8	8
		(0.10)	(0.02)	(0.03)			(0.28)	(2.26)		
	4	0.854	0.782	0.792	0.762	0.650	0.759	22.8	30	30
		(0.08)	(0.04)	(0.04)			(0.06)	(1.79)		
	5	0.574	0.580	0.444	0.442	0.254	0.531	16.5	26	31
		(0.13)	(0.08)	(0.06)			(0.06)	(1.88)		
	6	0.161	0.565	0.262	0.249	0.040	0.394	2.8	6	7
		(0.16)	(0.23)	(0.12)			(0.14)	(0.96)		

(To be continued)

Table 3: (continued)

Group	j^a	$\hat{\pi}_j$	\hat{p}_j	\hat{q}_j	$\hat{P}(t_j)$	$\hat{S}(t_j)$	$\hat{\lambda}_j$	\hat{d}_j^b	d_j^c	$d_j + a_{1j}$
PIT	1	0.824	1.000	1.000	1.000	0.824	*	0.0	0	0
Male		(0.09)	(0.08)	(0.08)			(*)	(*)		
AL	2	0.720	0.986	0.956	0.986	0.709	0.530	2.1	3	4
		(0.10)	(0.01)	(0.02)			(0.35)	(1.41)		
	3	0.451	0.632	0.622	0.623	0.281	0.577	32.9	44	57
		(0.10)	(0.06)	(0.06)			(0.07)	(3.72)		
	4	0.000	0.401	0.216	0.250	0.000	0.433	13.4	12	31
		(0.57)	(0.22)	(0.07)			(0.15)	(4.65)		
PIT	1	1.000	1.000	0.990	1.000	1.000	*	0.0	0	0
Male		(0.25)	(0.07)	(0.01)			(*)	(*)		
CR	2	0.926	0.986	0.977	0.986	0.913	0.877	2.6	3	3
		(0.05)	(0.01)	(0.01)			(0.11)	(0.34)		
	3	0.919	0.964	0.877	0.950	0.873	0.719	5.0	5	7
		(0.04)	(0.02)	(0.03)			(0.13)	(0.92)		
	4	0.722	0.811	0.584	0.771	0.556	0.523	13.1	14	25
		(0.08)	(0.05)	(0.05)			(0.08)	(2.10)		
	5	0.235	0.582	0.223	0.448	0.105	0.384	7.3	12	19
		(0.11)	(0.14)	(0.06)			(0.08)	(1.54)		
	6	0.967	0.000	0.000	0.000	0.000	0.500	0.5	1	1
		(*)	(0.71)	(*)			(0.18)	(0.18)		
PIT	1	0.938	1.000	1.000	1.000	0.938	*	0.0	0	0
Female		(0.06)	(0.07)	(0.07)			(*)	(*)		
AL	2	0.881	0.993	0.971	0.993	0.875	0.662	1.3	2	2
		(0.06)	(0.01)	(0.01)			(0.33)	(0.65)		
	3	0.330	0.920	0.749	0.913	0.302	0.314	10.0	25	32
		(0.09)	(0.05)	(0.04)			(0.16)	(5.16)		
	4	0.132	0.468	0.386	0.427	0.056	0.480	32.7	56	68
		(0.07)	(0.07)	(0.06)			(0.05)	(3.18)		
	5	0.983	0.000	0.000	0.000	0.000	0.500	8.0	14	16
		(*)	(0.58)	(*)			(0.14)	(2.31)		
PIT	1	1.000	1.000	0.980	1.000	1.000	*	0.0	0	0
Female		(0.22)	(0.07)	(0.01)			(*)	(*)		
CR	2	1.000	0.994	0.956	0.994	0.994	0.957	1.0	1	1
		(0.21)	(0.08)	(0.02)			(1.39)	(1.39)		
	3	0.840	0.954	0.900	0.948	0.797	0.699	6.3	7	9
		(0.07)	(0.02)	(0.03)			(0.15)	(1.34)		
	4	0.769	0.880	0.739	0.835	0.642	0.587	10.0	11	17
		(0.09)	(0.04)	(0.04)			(0.12)	(2.11)		
	5	0.602	0.523	0.423	0.436	0.263	0.547	17.0	22	31
		(0.12)	(0.08)	(0.07)			(0.05)	(1.64)		
	6	0.369	0.902	0.293	0.394	0.145	0.172	0.3	1	2
		(0.23)	(0.29)	(0.13)			(0.42)	(0.84)		

^aTime intervals 1-6 represent, respectively, 0-368, 369-555, 556-754, 755-919, 920-1096, 1097-1293 days.

^bEstimated number of fatal tumors using the proposed method.

^cNumber of fatal tumors assigned by pathologists.

^dNot available.

method appears to match with the data. A comparison of the tumor prevalence among the sacrificed animals and the tumor rate among the dead animals indicates that the tumors are not as fatal as the pathologists assigned. Further, since the PCR study included many other tumor types, many of the deaths with the tumor of interest might have been caused by different types of tumors.

Figure 1 compares the time-to-tumor-onset survival functions obtained from the proposed method for the calorie restricted and *ad libitum* groups for each tumor and sex. The two curves are quite different in PIT for both sexes, but they are not much different in MCL. This result coincides with the test result using the two-group Z -test by Ahn and Kodell (1995). For PIT, Ahn and Kodell (1998) report that the p -value for the one-sided Ahn-Kodell test was less than 0.0001 for male and 0.0048 for female. For MCL, the p -values are 0.30 for male and 0.55 for female. That is, calorie restriction delays the tumor-onset time significantly for PIT at level 0.01 for both sexes. However, there is no significant calorie-related effect in MCL at level 0.01. Figure 2 shows the difference between the time-to-tumor-onset survival function and the tumor survival function in each group. From this figure, we can visualize the lag time between the time-to-onset-of and time-to-death-from the tumor. It is the length of time that it takes for the tumor to kill an animal after its onset.

4.2 Simulation Study

A Monte Carlo simulation study was conducted to evaluate the accuracy of the attribution of tumor lethality using the proposed constrained nonparametric maximum likelihood estimation method. The simulation result is not reported in this paper, but it is provided in Ahn *et al.* (1999).

The bias in classifying incidental tumors as fatal tumors by the present method appears to decrease as the tumor lethality increases. Because there are not many deaths with tumor, and the data are highly censored, the percentage bias of the attribution of tumor lethality for low-lethal tumors is higher than that of highly lethal tumors. However, it correctly classifies more tumors as incidental as the (low-lethal) tumor rate increases. For low-lethal tumors, the actual average number of fatal tumors in this simulation is less than a half in every interval. For low-lethal and rare tumors considered in this simulation, it is hard to find even a single fatal tumor in the whole data set. In this case, the bias in misclassification of one or two incidental tumors to a fatal is deceptively large due to the small numbers involved. For highly lethal tumors, the cause-of-death

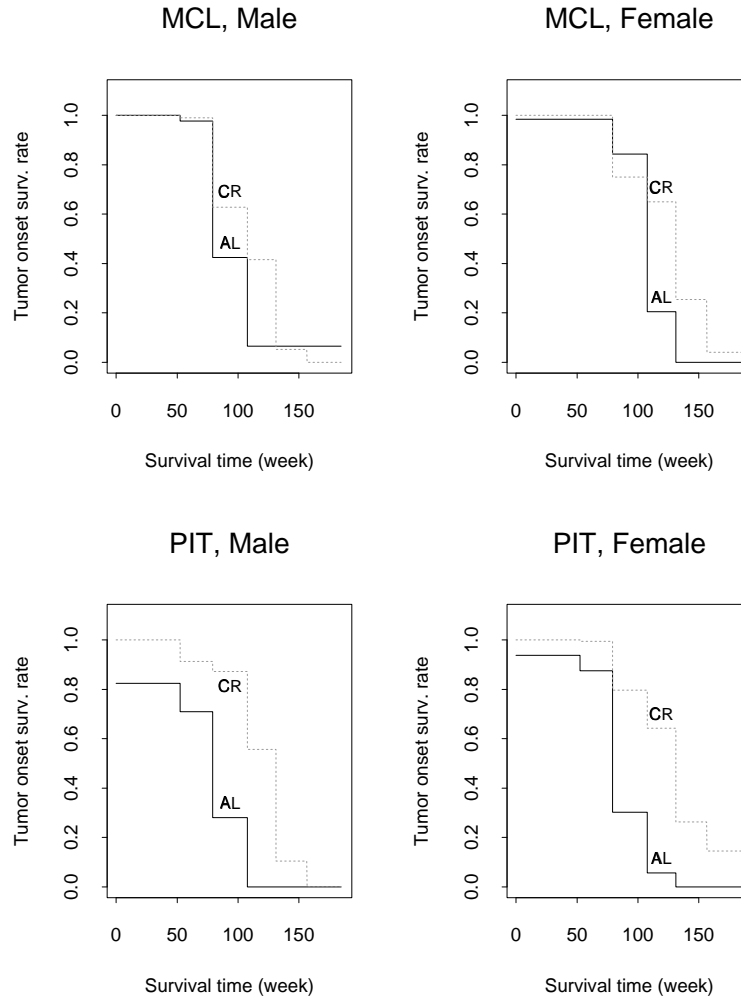


Figure 1: Time-to-tumor-onset survival functions using the proposed method for the calorie restriction data. Here, MCL stands for mononuclear cell leukemia, PIT stands for pituitary adenoma/carcinoma, AL stands for *ad libitum*, and CR stands for calorie restricted.

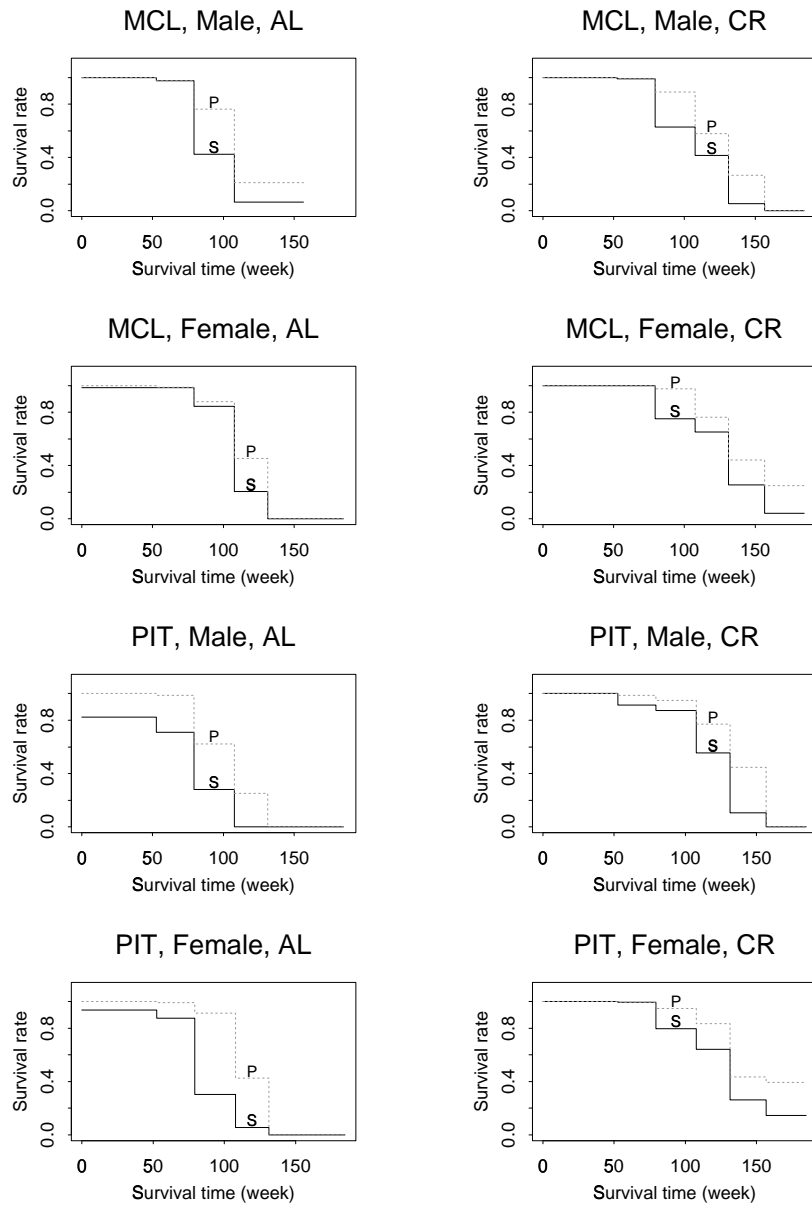


Figure 2: Time-to-tumor-onset survival functions (S) and tumor survival functions (P) using the proposed method for the calorie restriction data. Here, MCL stands for mononuclear cell leukemia, PIT stands for pituitary adenoma/carcinoma, AL stands for *ad libitum*, and CR stands for calorie restricted.

assignment by the present method is quite accurate.

5 Concluding Remarks

In many animal bioassays for carcinogenicity, cause-of-death information is not available. Even when cause of death is available, it is subject to error, potentially leading to misrepresentative estimates and tests of tumor rates. The present paper develops procedures for estimating distributions of time-to-onset-of and time-to-death-from tumors, without the need for cause-of-death data. These estimated distributions can be used to attribute the number of fatal tumors among the observed tumors in an experiment, and to calculate a representative estimate of the length of time that it takes for a tumor to kill an animal after its onset. The new procedure will enhance the statistical analysis and interpretation of rodent tumorigenicity studies.

In this paper, evaluation of cause-of-death assignment was conducted using a data set from a rodent bioassay. Our study indicates that pathologists tended to assign too many tumors as fatal. This confirms the analysis of cause-of-death assignment by Kodell *et al.* (1982). Perhaps it is natural for a pathologist to err in the direction of classifying a tumorigenic lesion as fatal, if it could have contributed in any way to an animal's death. In addition, the fact that cause of death or context of observation is assigned to moribund as well as dead animals is considered to be a source of bias. Not only are the moribund animals not dead, but also their lesions often resemble lesions observed in sacrificed animals of comparable age. Perhaps it is unreasonable and unfair to require pathologists to assign cause of death. According to the Monte Carlo simulations conducted in conjunction with this study (Ahn *et al.*, 1999. See also [http:// www.ams.sunysb.edu/ papers/papers99.html](http://www.ams.sunysb.edu/papers/papers99.html)), the proposed method estimates the number of deaths from fatal tumor closely to the real value. The proposed procedure could eliminate the need for assigning cause of death or context of observation in animal tumorigenicity assays. This could result in substantial economic savings.

McKnight and Crowley (1984) showed that, in general, the tumor onset rate could not be estimated without cause of death information or data from a large number of interim sacrifices in the absence of parametric assumptions. To avoid routine interim sacrifices various authors have proposed either semi-parametric or fully parametric methods (see, for example, Dinse, 1988a, b;

Dewanji *et al.*, 1993; Lindsey and Ryan, 1993; Omar *et al.*, 1995). However, the primary purpose of the current study is to attribute the tumor lethality from data without cause of death information rather than estimating the tumor onset rate, and make it available for a modification of the Peto cause-of-death test. It is different from the main purpose of the other studies mentioned above. Indeed, most of the animal carcinogenicity studies are designed with a single terminal sacrifice. However, although not frequent, bioassay designs with multiple sacrifices occur. The proposed method was motivated by analyzing the PCR data which includes up to 6 sacrifices. We plan to extend the proposed multiple-sacrifice method to the case of experiments that have only a terminal sacrifice in a future study. As proposed in Dinse (1991), Lindsey and Ryan (1994), and Kodell *et al.* (1997), several intervals with only natural deaths can be created for data with a single terminal sacrifice for the purpose of analyzing the data. The proposed method can be used for analysis of these data with some modification.

A typical carcinogenicity study involves a control and two to three dose group of 50 or more animals. Although the result is not reported in this paper, in our simulation study, six animals were randomly selected to be sacrificed at the end of each interval. All the remaining live animals were sacrificed at the end of the experiment. This design is close to a typical bioassay design. The number of intervals normally ranges from three to eight. As discussed in Section 1, the National Toxicology Program uses four intervals with three interim sacrifices. For data sets with a different number of sacrifice times, the present method can be applied without any modification.

This paper focused on the maximum likelihood estimation of the time to tumor onset and tumor survival functions and attribution of tumor lethality. The attributed number of fatal tumors among the observed tumors in an experiment can be used to compute a modified cause-of-death test (Lagakos and Louis, 1988) like the widely used IARC test (Peto *et al.*, 1980). Development of a modified IARC cause-of-death test is deferred as a future study. Ultimately, the feasibility of eliminating the need for pathologists to assign cause of death in animal tumorigenicity studies, whether with respect to statistical estimation or statistical testing, will depend on the development of a suitable statistical procedure for attributing tumor lethality in studies that have only a single (terminal) sacrifice.

Acknowledgements

Hongshik Ahn and Hojin Moon's work was supported by NIH grant 1 R29 CA77289-01. Hongshik Ahn's research was partially supported by the Faculty Research Participation Program at the NCTR administered by the Oak Ridge Institute for Science and Education through an interagency agreement between USDOE and USFDA. We would like to thank Dr. Yunlei Zhang for helpful discussions. Conversations regarding cause of death and context of observation with Drs. Dale Thurman, Thomas Bucci and Angelo Turturro were most helpful.

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